

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project
licences granted during 2019

Volume 2 (N to Z)

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Project	Natural killer cell therapy for cancer
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
What's the aim of this project?	The aim is to develop new ways of activating natural killer cells to kill cancer.
Why is it important to undertake this work?	Cancer is one of the biggest causes of morbidity and mortality. There are over 350,000 cases of cancer each year in the UK alone, with over 160,000 deaths (Source: https://www.cancerresearchuk.org/health-professional/cancer-statistics-for-the-uk). Many cancers are thus difficult to treat. Natural killer cells are cells of the immune system that are known to recognise and directly kill cancer cells. However, currently they are still emerging as a therapeutic entity. Therefore it is important to perform research on natural killer cells in order to learn how they can be best used to treat cancer.
What outputs do you	Cancer is one of the major killers worldwide. Immunotherapy

<p>think you will see at the end of this project?</p>	<p>is an area of growing interest that offers an alternative treatment to conventional cancer chemotherapy. Currently immunotherapy is focussed on activating T lymphocytes, however there is a growing body of work suggesting that natural killer cells may be an alternative target for immunotherapy as they work in different ways from T lymphocytes and appear to have fewer side-effects. The overarching aim of the project is to develop a new type of cancer therapy based upon natural killer cells, which is based upon a fundamental discovery that we have made. The project will develop and test the parameters required for a successful natural killer cell targeting vaccine. To do this we need to take a stepwise approach by developing and testing our therapeutic strategy. During this programme of work we will:</p> <ol style="list-style-type: none"> 1. develop new tumour models for understanding natural killer cell therapy 2. develop new methods for activating natural killer cells 3. investigate different ways to activate natural killer for immunotherapeutic benefit 4. test the effectiveness of our strategies for targeting cancer 5. identify which cancers are susceptible to our therapeutic strategy <p>We will thus gain new knowledge in natural killer cell activation and the protocols by which they can be stimulated. We will also identify how this activation relates to an anti-cancer immune response and a method by which it can be translated to the clinic for patient benefit.</p>
<p>Who or what will benefit from these outputs, and how?</p>	<p>In the short-term the scientific community will benefit from our research. In particular the immunology and cancer immunology specialities will gain new knowledge relating to how natural killer cells can be activated and how this can be used for immunotherapy. Our strategy is unique at present as it is based upon a recent scientific discovery that my research team have made. As we will identify new methods for natural killer cell activation we will present these findings at academic meetings and also publish them in the scientific press. We anticipate that these initial outputs will be realised in the next 3 years. Understanding the best methods for using natural killer cells to treat cancer will be the next goal and this may take 3-5 years. However we have a strong desire to translate these findings to the clinic, and this is a primary medium term</p>

	<p>goal for our approach, so that over the next 5-10 years we hope to perform clinical trials in patients using our vaccination strategy, so that ultimately in the longer term we will have developed a new type of cancer immunotherapy that can be used to treat cancer.</p>
<p>Will this work be offered as a service to others?</p>	<p>No</p>
<p>How will you look to maximise the outputs of this work?</p>	<p>The works will be presented to the academic community at national and international meetings and also through the scientific literature to disseminate the findings. We will also disseminate the work locally through academic meetings and presentations. Significant findings can be disseminated through the REDACTED press office and also public engagement activities such as "Pint of Science".</p>
<p>Explain why you are using these types of animals and your choice of life stages.</p>	<p>We are using mice as these are the least sentient animal that we can perform these experiments on. The mouse has a well studied immune system to allow parallels to be drawn with humans. As a well studied animal model, sufficient reagents are available to analyse responses within the murine immune system to the level required to make these experiments insightful. Furthermore mice can be genetically modified to express the human genes that we are targeting with our vaccination strategy. We are using adult mice as these are the most resilient life stage and allow the experiments to be standardised. In general they will be studied between weeks 12-16, well before age-related effects will be observed.</p>
<p>Typically, what will be done to an animal used in your project?</p>	<p>In a typical experiment adult KIR-transgenic mice will receive two DNA vaccine injections into the thigh muscle. These injections will be given one week apart and injections will be into alternate thighs. The mice will be killed humanely one week later, using a Schedule method and their spleens removed. Natural killer cells will be isolated from the spleen and transferred into a second mouse strain (NOD/SCID/gamma chain knockout) through intravenous injection into the tail vein. These mice will have been injected subcutaneously the day before with a tumour cell line. Mice will then be monitored daily for growth of the tumour. Seven to fourteen days after the initial sub-cutaneous injection of tumour cells mice will receive a further intravenous injection of natural killer cells.</p>
<p>What are the expected impacts</p>	<p>Animals may experience local irritation to injections. This is usually mild and passes within 24 hours. Some of the</p>

<p>and/or adverse effects for the animals during your project?</p>	<p>injections may have more generalised side effects of longer duration such as shivering, erection of the fur, reduced motor activity, hunched position, lack of responsiveness and shallow breathing. Animals exhibiting these signs will be warmed and monitored closely. These effects usually pass within 1-2 hours. This may affect 10-20% of animals. If the symptoms are prolonged the animals will be humanely killed to alleviate suffering.</p> <p>We anticipate that by operating within the guidelines for blood drawing and tumour challenge, and with careful monitoring then animals are not expected to experience significant adverse effects related to these procedures.</p>
<p>What are the expected severities and the proportion of animals in each category (per animal type)?</p>	<p>We anticipate the 90% of animals will have severity scores of mild, and that up to 10% will have a severity score of moderate.</p>
<p>What will happen to animals at the end of this project?</p>	<p>killed</p>
<p>Why do you need to use animals to achieve the aim of your project?</p>	<p>We need to use animals to develop and test our vaccine strategy. The ultimate goal of this project licence is to device a new way to treat human cancer, but this needs development and refinement in animals before we can move to human studies.</p>
<p>Which non-animal alternatives did you consider for use in this project?</p>	<p>We are gaining as much as information as possible in vitro cell culture experiments using cell lines, prior to evaluating the use of the vaccine in humans. Our vaccine is a novel first in class therapeutic that targets natural killer cells. We have performed a literature search to identify alternatives to this, such as in silico modelling or organoid culture, and have not found one which can test the effect of a vaccine on natural killer cells.</p>
<p>Why were they not suitable?</p>	<p>We have not identified a system that can faithfully recapitulate the response to a vaccine that targets natural killer cells. We have been unable to identify a suitable in silico or in vitro model for this work. In general these types of model systems</p>

	do not recapitulate a whole animal experiment that is required prior to using a potential therapeutic in humans. This is because generating an immune response requires multiple steps and the interplay of many different immune cells in a co-ordinated fashion within a localised environment. This interplay cannot be readily or faithfully reproduced in tissue culture conditions to the standards required to inform a clinical trial. Furthermore, unanticipated toxicities cannot be readily identified in in vitro model systems
Enter the estimated number of animals of each type used in this project.	mice: 2000
How have you estimated the numbers of animals you will use?	<p>We have used our current experimental data to inform a power calculation to estimate the number of animals required to give a statistically valid experiment. When comparing two groups of animals for tumour growth we use ANOVA with a post test for each timepoint. This allows an overall comparison of tumour growth over the course of an experiment as well as at discrete times. The power calculation was made using the p values from the ANOVA, rather than the post-test as this gives a greater power for the experiment and so uses less animals. For other experiments where we wish to measure phenotypic effects of the vaccine strategies on NK cells, we have used t-tests as the source for our p values to input into the power calculation.</p> <p>We have then calculated the approximate number of experiments that we wish to perform to test our hypotheses, in combination with these power calculations, to calculate the number of mice required over the period of the project license.</p>
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	In the design phase we have used inbred mice to reduce intra-group variability and allowing reduced mouse numbers for experiments. We have designed experiments using the fewest animals consistent with obtaining statistically valid results as determined from our power calculations. We will also store material from our experiments so that they can be used to interrogate our research questions in more detail and inform experiments prior to using further animals.
What measures, apart from good experimental design, will you use to optimise the number	We will carry out small pilot experiments to assess simple factors such as dose or route of administration prior to performing larger experiments. Where multiple inter-relating parameters are to be evaluated, we will use a factorial design for experiments in order to prevent use of excess mice as

<p>of animals you plan to use in your project?</p>	<p>controls. Furthermore we will may optimal use of multi parameter analysis eg flow cytometry or RNAseq so that as many different parameters as possible can be analysed within a single sample. We will make full use of stored samples from our vaccination experiments to answer our research questions prior to performing further experiments in live animals.</p>
<p>Which animal models and methods will you use during this project?</p>	<p>We will use genetically altered immunocompromised mice as our animal model. We will use the following methods:</p> <ol style="list-style-type: none"> 1. injections of DNA/cytokines/antibodies/toll-like receptor agonists delivered by subcutaneous, intramuscular and intravenous route 2. injections of tumours by the subcutaneous route 3. injections of cells delivered by the intravenous route
<p>Why can't you use animals that are less sentient?</p>	<p>Mice are the least sentient mammal species with an immune system similar to humans. Mice represent a relevant animal model for these studies and the clinical successes now being reported using immunomodulatory drugs against cancer were dependent on data arising from such murine studies. Mouse cancers are well characterised and the widespread availability of commercial reagents allows direct comparisons between mouse and human immune systems.</p>
<p>How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?</p>	<p>We will stay informed through the regular meetings of the animal facility and the user groups which occur every 3 months. Additionally, we receive e-mails to update us about changes in policy or practice. We will also check on-line databases to identify any changes such as the NC3Rs, Altweb and Norecoba web pages. Any changes will be implemented directly through the experimental design, and if necessary through a project license amendment.</p>
<p>How will you refine the procedures you're using to minimise the welfare costs (harms)</p>	<p>Environmental enrichment, good husbandry and frequent monitoring ensure high welfare standards. Few adverse effects are anticipated but, should any occur, rapid steps will be taken to ameliorate them or humanely kill humanely affected animals. All animals will be maintained by qualified</p>

<p>for the animals?</p>	<p>and experienced animal technicians who are familiar with the models. Mice will be handled using non-aversive methods eg not picking up by the tail, but moved using a tunnel or a cupped hand.</p> <p>Any animals which are anticipated to be nearing a defined end-point, or for which a defined end-point is not yet established, will be monitored more closely. Should a technician find an animal that has reached an end-point the animal is either immediately humanely killed or the PIL holder is informed that the animal is required to be humanely killed immediately.</p> <p>We have established end-points for humane killing before pain/distress occurs, based on accepted guidelines. Our tumour models are subcutaneous in nature, allowing easy monitoring of tumour size. Experiments will therefore be terminated before tumour size limits behaviours (feeding, drinking, movement) using the guidance of 12mm diameter for Protocol 1 in which we are defining tumour biology and growth characteristics, or 15mm diameter in immunotherapy experiments (Protocols 2 and 3). Occasionally, following therapy a subcutaneous tumour resolves from the inside out giving the appearance of ulceration; we have adopted a scoring system to ensure that these are managed with minimum adverse effects to the mice. While the maximum severity limit for much of the work to be conducted under this PPL is set as 'moderate', through experience and good management of the mice, we have found under our existing PPL that the actual severity of most experiments is 'mild'.</p>
<p>What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?</p>	<p>We will use the NC3Rs as a resource for our animal studies and experimental design. We will use information available in the NC3Rs website (https://www.nc3rs.org.uk/3rs-resources). Guidelines contained there include "Responsibility in the use of animals in bioscience research" and the "ARRIVE" guidelines for reporting the use of research using animals. We will also use information from Norecopa and will follow PREPARE guidelines (https://norecopa.no/prepare) for all animal experiments.</p>

Project	Nervous System Injury and Repair	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Both brain and spinal cord injury are crippling conditions due to the severance of nerve fibres that connect the brain with the spinal cord and body. Injury to the brain or spinal cord can be acute, as in stroke or traumatic brain and spinal cord injury, or chronic as in neurodegenerative diseases, leading to loss of motor and sensory functions, potentially resulting in paralysis and/or loss of sensation. The number of patients living with paralysis due to brain and spinal cord injury is growing in both the developing and developed world.</p> <p>Repairing nervous system damage in a rodent model involves inducing cut nerve fibres to regenerate across the injury and to make connections below it.</p>	

	<p>Alternatively, or in addition, undamaged nerve fibres remaining after injury can be made to return some function through stimulation of plasticity (fibre sprouting), bypassing the lesion.</p> <p>The goal of this project is to develop and test treatments to repair damage to the nervous system by promoting nerve fibre regrowth from injured fibres (regeneration) and/or fibre sprouting from existing or uninjured fibres (plasticity). In addition, we will evaluate the robust growth response which occurs in the visual and peripheral nervous systems (PNS) as well as the immature/developing central nervous system (CNS) relative to the minimal growth response occurring after adult CNS injury to further our understanding of these differences and determine how they may be utilised to enhance CNS repair.</p> <p>Our treatments for nervous system damage aim:</p> <p>(1) to block the degeneration process around the lesion and/or to inhibit the gradual loss of cellular function in chronic neurodegeneration,</p> <p>(2) to repair the lesion, by inducing nerve fibre regeneration or reactivating plasticity/sprouting in the brain and/or spinal cord.</p> <p>This project will explore the normal injury responses anatomically within the growth-poor CNS and growth-rich PNS to better understand endogenous malfunctions contributing to the lack of repair. This project will also examine different strategies for repair and protection of the brain and spinal cord after injury focusing on modification of endogenous cells through gene therapy (through non-toxic viral vectors) and cell replacement therapies (including stem cells).</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Results from the proposed experiments described in this project will have direct benefits for scientists in the near term but will also have potential benefits for human patients living with brain or spinal cord injury in the future as we work toward viable treatments for repairing the damaged nervous system. As such, these experiments will add to our fundamental knowledge of nervous system injury and impaired regeneration as well as the basic biological processes and connections in the nervous system. Furthermore, we will publish our findings in high</p>

	<p>impact peer reviewed journals to inform other scientists working in similar fields. The studies included in this proposal will provide prospective treatments which one day may be suitable for patients suffering from nervous system damage such as spinal cord injury, traumatic brain injury or stroke. In addition to validating our novel CNS repair treatments, we will combine these with therapies already used in the clinic such as rehabilitation and therapies close to clinical trials in order to move forward translation of viable and novel therapies towards application in human patients.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The proposed experiments will be performed in rats and mice, in which the biology of the nervous system is similar to humans. Up to 500-600 rats and 500-600 mice will be used yearly during the 5 year duration of the project for experimental studies. Additionally, up to 300-400 rats and 400-500 mice will be used yearly for the support procedures (breeding and obtaining tissue) required for the project.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The overall goal of this project is to investigate the molecular and cellular mechanisms regarding why some parts of the nervous system do or do not regenerate while also implementing strategies to enhance the regenerative capacity of the nervous system specifically after injury and within areas that do not regenerate. The types of injury that we perform to the brain, spinal cord, or peripheral nerve will be in the form of direct, physical lesions (cutting or crushing of the nervous tissue) or chemically-induced (injection of a chemical) lesions. The majority of lesions used in this licence (brain and spinal cord) are non-paralysing lesions. Our lesions are of moderate severity, whereas within a week post-surgery, animals will have returned to a near normal condition similar to pre-surgery condition with very mild deficits only being apparent through specific behavioural and anatomical analysis. In lesions that affect dorsal roots or peripheral nerve, there may be dragging of the affected hindlimb which may be associated with hypoesthesia (dorsal root injury or peripheral nerve injury) or paralysis (peripheral nerve injury). The animal will still be able to use the paw for certain movements including mobility. Likewise, gross feeding and drinking ability should not be compromised. For animals</p>

	<p>undergoing surgical procedures, our models of brain and spinal cord injury will be performed under general anaesthesia, with additional analgesia being given peri-operatively (pain relief administered at the time of surgery) to minimise suffering during and in the days and weeks following the surgical procedure. Specifically following surgical procedures, post-operative observations will be performed continually until the animal regains consciousness, following that, the animals will be observed several times in the first few days following surgery as well as at any other stages in the experiments that pose a higher risk of adverse effects. Once the animals have stabilised, post-operative care and observations will be performed daily at a minimum. The animals will be humanely killed at the end of the experimental procedures, and tissues will be collected for analysis. Specific humane endpoints will be used to ensure that adverse effects do not go beyond the minimum required to achieve the scientific objectives and the numbers of animals will be minimised by careful experimental design. On the rare occasion of post-surgical complications, such as animals which exhibit signs of pain, distress, or have difficulty eating, drinking, or moving about as normal will be humanely killed. In addition, approximately 100-120 animals per year will be used for tissues only and not undergo surgical procedures. In these cases, animals will be humanely killed to obtain the necessary tissues.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Much of the development of our treatments is performed with extensive tissue culture analysis (on cells) prior to moving to an animal model. Once we have interrogated these treatments fully in vitro and in order to determine whether these treatments are likely to help human patients, it is vital that they are then evaluated in animal models. For this aim, we will carefully design our experiments so as to use the fewest numbers of animals possible to achieve significance in our results.</p> <p>The basic concepts and treatments for nervous system injury repair are worked out using tissue culture models. Concepts developed in tissue culture have to be tested and refined in an animal model where the complex environment of the adult nervous</p>

	<p>system is present, and where functional recovery can be measured. No treatment for nervous system injury repair could be tested in human patients without extensive prior validation in animal models.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of animals used in these experiments will be kept to a minimum whilst ensuring power in our experimental design and ensuring that we can adequately address and answer the questions we propose. We will obtain behavioural and anatomical (and in some experiments electrophysiological) data from animals following our injury and repair procedures in the nervous system. Our experiments allow for multiple types of analysis on one animal (behavioural and anatomical) which effectively reduce the total numbers of animals required to reach our outcomes whilst not compromising animal welfare.</p> <p>No animal experiments are performed until a well-developed treatment concept has been developed using tissue cultures. By making very repeatable lesions we achieve minimal variation between animals, making it possible to use smaller experimental groups. Animal group size is determined based on previous experience as well as reference to statistical readouts, so that the number of animals is sufficient to achieve statistically significant results. For example, in studies using neurohistology (tract tracing) as a readout, 3-4 animals per group is required as we and others have found that there is extremely low variability in these types of experiments. On the other end of the spectrum is in studies using behavioural testing as a readout. In these cases, because there is larger variability amongst animals, 8-12 animals per group are required.</p> <p>Small pilot studies will be used for new studies to assess feasibility and outcome measures of the experimental paradigm, mainly regarding new treatments. The number of animals included in these pilot studies will be kept to a minimum (usually 6-10 per experimental treatment group) followed by analysis through statistical tests. Prediction of numbers of animals needed for experimental design will be based on our 17 years of experience with these surgical models and results from the pilot studies. When possible, we will attempt to further optimise these pilot studies and reduce the number</p>

	<p>of animals used, in addition to seeking statistical advice from experts within the applicant's research establishment.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Project Goals and choice of animal models</p> <p>The overall goal of this project is to investigate the molecular and cellular mechanisms regarding why some parts of the nervous system do or do not regenerate while also implementing strategies to enhance the regenerative capacity of the nervous system specifically after injury and within areas that do not regenerate. We use rats and mice in our studies as both have beneficial characteristics vital for nervous system research and both are accepted animal models in nervous system research.</p> <p>When rats are used in experiments, it is because they have a nervous system that is sufficiently similar to that of humans and the biology of axon regeneration and plasticity is almost the same. Rats are also capable of complex behaviour and skilled paw use, making it possible to achieve good behavioural outcomes with only subtle changes in behaviour stemming from the small circumscribed lesions that we use in our experiments.</p> <p>When mice are used in experiments, it is because they can be genetically manipulated, allowing molecular hypotheses to be tested. Their behaviour is almost as good as that of rats however in certain behavioural tasks such as skilled forepaw reaching (one of our main behavioural assays), mice do not perform this task well enough to obtain usable data. Likewise, some of the molecular and cellular responses to tissue injury in mice differs substantially to the human response, whereas rats have a very similar molecular and cellular injury response to that of humans.</p> <p>Surgery, post-operative care and humane endpoints</p> <p>Animal suffering will be kept to an absolute minimum by ensuring necessary post-operative monitoring and care including the administration of peri-operative analgesia for all surgical procedures. We minimise suffering by developing and/or using behavioural outcome tests of high resolution that pick up deficits in fine movement control. Therefore, it is not necessary to make large and disabling nervous system injuries, and although we study</p>

nervous system injury, the majority of our lesions do not paralyse the animals. In these cases, animals recover sufficiently to show normal behaviour within their home cage within the first week post-surgery. In certain cases, including peripheral nerve or dorsal root injury, animals may experience dragging of the affected paw due to decreased sensation (dorsal root injury) and/or reduction in motor function (peripheral nerve injury). In these cases, only one limb will be affected and it will not gross affect mobility in terms of the animal's ability to move around their cage nor will adversely affect eating or drinking behaviour.

Post-operative observations will be performed continually until the animal regains consciousness, following that, the animals will be observed several times in the first few days following surgery as well as at any other stages in the experiments that pose a higher risk of adverse effects. Once the animals have stabilised, post-operative care and observations will be performed daily at a minimum. Our monitoring of post-operative animals will include analyses of mobility, body condition (piloerection, hunching), facial expression (<https://www.nc3rs.org.uk/using-facial-expressions-pain-animals>) and weight. Loss of up to 20% of body weight will be taken as a humane endpoint. This monitoring protocol will ensure that any animal showing signs of paralysis or other adverse effects will be picked up immediately so as to limit suffering as much as possible. Any animals exhibiting signs of distress will be closely monitored and advice from the local NVS will be sought.

In our experience, by choosing well-established lesion models that have been extensively used in my previous studies, we are able to ensure a high rate of reproducibility with our lesions (consistent size and outcome), leading to less adverse effects in animals and overall lower numbers. Likewise, our behavioural tests build upon well-established protocols for which the adverse effects are known and preventative measures will be taken to avoid them. Furthermore, we continue to refine our surgical methods to ensure reproducible results.

If we observe evidence of distress, measures will be taken to alleviate these symptoms as described in the adverse events sections for the Protocols. If animals show signs of distress for which a cause

	<p>cannot be identified, we will seek advice of the local NVS. If animals are anticipated to be close to a defined endpoint, they will be monitored more closely. From experience, very few (<5%) animals experience or show signs such that humane endpoints may be reached.</p>
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Project	Neural bases of action
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	With our work we expect to gain new insights into how ensembles (networks) of neurons work together to control movements. Understanding the nature of these circuits, the genetics of them, how they are assembled and how they function is fundamental for the understanding of how we produce purposeful movements and why we fail to do so in a number of neurodegenerative disorders.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We believe that the approach of measuring the activity of neurons within the living animal we will allow us to understand novel aspects of how the brain controls movements and identify neuronal elements (or brain regions) that are crucial for the production of movements. There

	<p>is an ever-increasing incidence of neurodegenerative disorders that affect motor function to various degrees. These have an enormous impact on the life of millions of patients, with motor defects ranging from dyskinesia to complete loss of voluntary movements. We believe that our findings will be useful to identify and target more precisely those neuronal populations whose impairment leads to these severe motor defects.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use 3000 mice for the tracing and electrophysiological recordings and to breed a total of 17000 transgenic mice over 5 years to maintain the stocks and provide animals for the experimental procedures.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The advantage of using mice for our project is that we can selectively mutate genes of interest. To this aim we generate mutant animals by injections, for example, of genetic material in eggs followed by in vitro fertilization. To make sure that the animals born from these procedures do indeed carry the mutant gene, we take a very tiny piece of tissue from the outer ear and test the expression of the genes of interest. This causes minimal distress or pain to the mice. Animals undergo surgical procedures and for this reason we expect them to show clinical signs of a moderate severity as a result of electrodes, fibers or cannulae implantation. Surgeries last about 1-3 hours, during which we will make a very small window in the skull to gain access to the brain. After which, we implant tiny screws and a probe no more than 5 mm long. We finally seal everything with dental cement. Very rarely the severity of these signs may be such that the humane end points may be reached. Animals are expected to reach moderate level of severity exclusively during surgery and during the period immediately following the surgery, which represents <2% of the time spent by the animal in this protocol. They are expected to recover very quickly from the surgery, typically they are already walking around the recovery cage 15-30 minutes after the surgery. They will be given painkillers and post-operative care just like people recovering in hospital. One day after</p>

	<p>surgery animals normally show no signs of discernable discomfort for the presence of implanted devices or as result of injected tracers during the recording sessions and/or the behavioural routines. Therefore, apart from the surgery period, animals are expected to reach only mild level of severity for the rest and longest part of this protocol. To study the visual system, we also perform injection in the eye, the capillary we use for the injection is very small, about 2-3 times the size of a hair. Mice recover quickly and normally show no signs of sight loss. Unless otherwise specified, the administration of substances and withdrawal of body fluids will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm. To assess the behaviour of the animal, mice are kept on a diet so to increase their propensity to perform specific tasks in order to obtain food reward. These diets only take down the weight of the animal of about one tenth of their initial weight. At the end of the experiments mice will be killed using a large dose of anaesthetic followed by cardiac perfusion, which allows preserving the tissue for the successive analysis. At no point during the procedure the animal is conscious or feels any pain.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The role of neural networks activity in motor performance can only be studied in the intact, freely moving animal. Implantation of chronic indwelling electrodes in humans is only permissible in a very small number of clinical situations and thus is impractical for research purposes. We have collaborated (and will continue to do so) with colleagues who devise computational models of motor networks and have on occasion used these to design experiments and predict their outcomes, but the models are extraordinarily simple in comparison to the complexity of the brain, and cannot substitute for experiments themselves.</p>
<p>2. Reduction</p>	<p>We intend to use the minimum number of animals consistent with achieving our</p>

<p>Explain how you will assure the use of minimum numbers of animals</p>	<p>experimental aims. The animals are often tested for long periods and thus considerable information is obtained from each animal, minimising the total number used. With an appropriate use of statistical methods and the use of inbred strains we keep the use of the animal at a minimal required level. Whenever possible we make use ex vivo recordings. This will reduce the instances in which we have to perform in vivo acute or chronic recordings which greatly decreases the number of animals used under these protocols.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the experimental species of choice because it is possible to generate and acquire genetically modified strains, which allow the visualization and manipulation of selected neuronal populations. In order to trace neuronal circuits and record neuronal activity we implant microelectrodes chronically or acutely. The implantation of electrodes in defined brain regions might seem intrusive at first but the presence of the implants is completely painless. The surgical approaches used are the least severe available, involving the smallest amount of tissue damage. Animals are given extensive post-operative care including antibiotics and analgesics. Animals are closely monitored throughout the experiments and any signs of problems with implants or other aspects of surgery are immediately dealt with, or, if this is not possible, the animal will be killed. Similarly, animals are closely observed and monitored during the recording experiments and during interactions with other animals.</p>

Project	Neural basis of cognitive impairment in neurodegenerative disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Although our understanding of the causes of Alzheimer's disease has greatly advanced, there is still no cure for this brain disease which affects memory and thinking. Previous research suggests that very early in the disease process, the connections between brain cells and the way they use energy are affected. We will use mouse models of Alzheimer's disease to understand the very first events that lead to memory loss. These mice contain genes with mutations known to cause Alzheimer's disease in humans. We will study the emergence and progression of this disease in the mice to analyse the earliest signs of memory loss	

	<p>and symptoms. We are interested in finding out if a normal diet can protect against dementia-like symptoms (compared to a high fat diet), or whether an enriched environment within the cage (running-wheels, novel objects) can also have this effect. Finally, when dementia-like symptoms arise we want to test new drugs to tackle the aspects of brain function that are being affected, to see if these drugs can stop or slow down the disease process.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Diagnostic methods for dementia, in particular Alzheimer's, are rapidly advancing. It is predicted that in the next couple of decades, routine clinical analyses using spinal taps or even blood tests will identify high risk patients up to a decade before any symptoms appear. It is at this stage that it would be ideal to be able to provide a drug that can stop dementia from progressing. Whilst this particular work would primary advance our basic understanding of how dementia starts, the insights gained highlight the positive or negative effects of specific drugs that target Alzheimer's disease molecules and mechanisms.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The results of studies in mice are highly relevant to studying human disease. The brain areas involved in memory have conserved anatomy and functions. Nowadays, the scientific tools available to study mice and rats allow unprecedented access to modification of genes and neuronal circuits, which can help us to answer these questions. We expect to use approximately 20-100 mice for each of our tests. The result from these tests will become a peer-review scientific publication that will advance our knowledge in the understanding of how dementia. In the lifetime of this project license we expect to complete 20-40 tests to establish the utility of drugs and clinical targets in preventing brain network dysfunction in the brain of Alzheimer's disease mouse models. We have requested the use of 4250 mice in total.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the</p>	<p>In protocol one, we will induce of terminal anaesthesia to collect brain tissue. The levels of anaesthesia will be carefully monitored to ensure the animals feel no pain at all. In a second protocol we will be able to manipulate the conditions in which the animals live to test whether dementia-like symptoms advance at a slower pace in animals in</p>

end?

an enriched environment or with a normal diet (in contrast to a high fat diet). We will monitor the animal's skin, teeth and levels of energy to ensure that the high fat diet does not affect these. Here we will also be able to give the animals different drugs either in the diet, by gavage or with nasal drops to prevent the development of the dementia-like symptoms. The drug delivery should cause only minor discomfort and not exceed moderate severity. At the end of these experiments animals will be terminally anaesthetised and the tissues will be collected for further analysis. Or they will be killed via a Schedule 1 method. In a third protocol in addition to the procedures for protocol 2, we will observe and analyse the animal behaviour to observe whether their natural behaviour (e.g. exploration of new environments) is normal, and to test whether their anxiety levels are comparable to normal mice (do they prefer an enclosed space to an open space). We will also test the animals memory by using a water maze after which, animals will be thoroughly dried and warmed up to avoid hypothermia. We will constantly monitor the mice weight and appearance. In a small set of experiments, we need to carry out surgery to either inject molecules in the brain that will help us map exactly where in the brain the deficits are coming from. To prevent the main adverse effects of pain, distress and infection, these surgeries will be performed using strict aseptic technique, excellent surgical practice, peri-operative pain management, and constant monitoring during recovery. An optional final step in this protocol will allow us to perform physiological recordings in vivo in anaesthetised mice for up to 5 hrs. At the end of this experiment, animals will be killed without recovery from anaesthetic. The tissues will be collected and all the information gained will allow us to advance our understanding of how memory loss and dementia starts and progresses and whether it can be stopped with drugs targeted to protect brain circuits. In our fourth and final protocol we will be able to add specific molecules into developing brain cells. To do this we will need to perform a laparotomy in the pregnant female mouse and inject very small volumes of substances into the embryos brains through the uterine wall. We will analyse these cells once the mice are born and after they mature, to interrogate brain function under normal and dementia conditions. These analyses will allow us to

	compare normal cells and cells that express specific molecules within the same animal to dissect their role in maintenance and plasticity of neural networks relevant to memory.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>To advance our knowledge on how dementia starts in the brain, the best available systems are mouse of neurodegenerative disease. This is because they allow analysis from molecules to function of brain cells and their connections, to their impact in behaviour and memory. We will complement this work with collaborations and investigations using human tissue discarded from biopsies or post-mortem and invertebrate systems, in vitro and computational models. However, investigations in these alternative systems alone, would not answer the proposed questions. Only a live mammalian preparation would permit the levels of analysis required for this work.</p> <p>The number of animals required for this work will be kept at the minimum possible by ensuring adequate experimental design consistent with collection of statistically robust and reproducible data. We will use the latest technology to obtain and analyse high levels of information that can be obtained from brain tissue from a single mouse.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Animals use for both in vivo and in vitro preparations, will have the assessment of behaviour, brain electrical activity, and pathology-relevant molecular expression analysis. In this manner, we will ensure the maximum data obtained from a single animal that will reduce the number of animals used for this study without compromising the welfare of the animals.</p> <p>Appropriate experimental design will help us plan ahead and avoid unnecessary use of animals.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general</p>	<p>Pain and distress will be kept at a minimum by using anaesthesia and analgesia. High welfare standards will be maintained with good husbandry and environmental enrichment. We aim to identify subtle changes in behaviour that indicate the start of memory problems in mice. For this we are using the most refined mouse model with a genetic</p>

<p>measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>modification that causes AD-like symptoms in adult age (the production of AD molecules can be turned on/off). Although it may be necessary to allow some progression of the behavioural impairment to relate it back to the disease, the vast majority of experiments will involve young mice with minor behavioural abnormalities (for example poor memory). Our licence will allow a staged approach to first analyse brain networks and test drug effects using ex-vivo work in isolated brain tissue. We will then be able to test the effects of drugs delivered in vivo and their effects on brain networks. Finally we will be able to test animal's memory and the function of brain networks in vivo in anaesthetised mice. The results from this integrative work will advance our understanding of Alzheimer's disease and contribute towards the generation of a cure.</p>
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Project	Neural basis of memory
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	At present, the majority of research into memory focuses on a single brain structure, the hippocampus. While this region is clearly important for memory, there are wider networks of brain regions that are also critical for memory, but our knowledge of these areas is much more limited. While we know that damage to these regions causes severe memory impairments in both humans and animals, we are not sure why memory is affected and how these regions support processes necessary for effective memory. The aims of this project are to uncover how these extended memory systems contribute to memory processes. There are two main questions: 1. How do these networks support memory formation in intact (“normal”) animals?

	<p>and 2. What are the widespread effects when these systems are disrupted? Answering these questions will enable us to develop neural models that underpin memory formation as well as understand how the brain is affected when the neural systems are disrupted.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Learning and memory are critical cognitive skills that are vital for every day existence and interaction with the world. More than that, our memories are a key component of our personalities, fundamentally making us who we are. Understanding how our brains enable us to learn and remember events continues to be a crucial goal within neuroscience. Furthermore, by fully understanding how the brain supports memory in “normal” systems we can far better understand how memory can be disrupted in numerous neurological disorders. Only by better understanding how these systems function in normal situations, and what happens when these systems break down, can we start to fully develop effective targets for treatment. By the end of the project the expectation is to have identified mechanisms that can be targeted in animal models to improve aspects of memory. The next step would be to be to robustly test these treatments in an array of animal models before determining whether similar approaches can be used to benefit human memory.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Over the 5 year period we will need: 1650 rats 1050 mice</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>To understand how systems in the rodent brain support memory it is necessary to carry out behavioural tests that can demonstrate the learning and remembering of information. To be able to interpret the data it is important that animals do not suffer additional sensory, motor or motivational problems. As such, manipulations will be mild or moderate so that behavioural data can be understood and related to specific damage to memory systems. To determine the contribution of specific brain regions and networks of brain regions it is necessary to either measure or modify their activity. As such, techniques will be used that</p>

	<p>typically target the specific regions. While there are some genetically modified animals where specific brain regions are preferentially affected, the most common approach will be to use intracranial surgery to carry out permanent or temporary lesions or to implant devices for long-term monitoring of activity. To analyse memory, it is necessary to apply a variety of behavioural tests that address different forms of learning. Food and/or water restriction may be used to ensure reliable responding over multiple trials. Mildly aversive conditions such as swimming in a water tank to assess specific aspects of spatial memory. At the completion of a study, rodents will typically be killed by overdose of a general anaesthetic so that their brains can be removed and processed further.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The goal is to understand the interplay between multiple brain sites and how they support learning and memory in the mammalian brain. The complexity of these interactions, embedded within the many unknown structural details of the brain, means that it is necessary to derive information from intact organisms. Invertebrates do not possess homologous CNS structures so it is not possible to use non-protected species. It is also necessary to validate learning, e.g., through behaviour. Specific issues concern the anatomical resolution of the proposed procedures and the fact that some of the key target areas, e.g., individual thalamic nuclei, are not prone to selective pathologies in humans and cannot be distinguished using non-invasive imaging techniques for humans, e.g., fMRI/MEG.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>1. By refining behavioural tasks to improve performance/reduce variance in control animals so impairments can become apparent with fewer animals.</p> <p>2. A number of approaches support a within-subject design, with repeated measures therefore reducing numbers required. e.g. MR imaging, in vivo imaging, electrophysiology, temporary lesions.</p>

	<p>3. Using techniques that enable the examination of multiple regions within the same animal instead of different animals for different brain regions. This includes in vivo recording from multiple brain regions using multichannel electrodes, the use of MRI and/or immunohistochemical imaging which are appropriate for whole brain analyses.</p> <p>4. By using anatomical tracers with different wavelengths so parallel tracing experiments can be carried out.</p> <p>5. The in vivo imaging studies naturally reduce the number of animals needed as extensive information can be acquired from even a single animal.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>For the research to produce findings which can be applicable to the human brain it is necessary to use a model with the same anatomy, i.e., the same brain structures and connectivity, which is why rodents are required. Furthermore, these animals are able to perform a number of tasks that have similarities to those that are used with patients with amnesia, in particular spatial memory tasks and object/location tasks, which increases the relevance of the findings.</p> <p>By using sophisticated viral vectors it is possible to selectively disconnect specific pathways rather than damaging the whole brain region under investigation. The use of temporary inactivation means that again, the brain region under investigation is only disrupted for short periods rather than permanently, i.e., the animals will not be rendered with a permanent memory impairment when using this methodology.</p> <p>Where possible we will look into alternatives for repeated injections, which are needed for experiments using chemogenetics for example. Possible options may be oral or optic administration of substances if considered less invasive.</p> <p>All procedures are inherently designed to be the least disruptive for the animals due to the overall projects aims and need for animals to perform behavioural tasks. Animals in chronic discomfort</p>

	or with gross sensory-motor impairments would be uninformative for these types of studies.
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Project	Neural basis of spatial cognition and memory in the hippocampus	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We will determine how particular brain cells that act like an internal Global Positioning System [GPS] system connect and communicate with each other to allow us to navigate and to remember places. Importantly, we will study the relationship between the hippocampus (the area of the brain which acts as an internal GPS system and is crucial for remembering new events and navigating) and Alzheimer's disease, the most common type of dementia. Alzheimer's disease is associated with an ongoing decline of brain function which severely affects memory, thinking skills and other mental abilities. The	

	hippocampus is one of the first areas affected by the disease.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	An understanding of how different parts of our GPS system communicate to create internal maps of the environment will provide fundamental insights into the relationship between mind and brain. The ability to artificially manipulate these brain GPS cells to remember and imagine previously unvisited places would be an important breakthrough in our attempt to build devices which would help to restore and maintain one's ability to remember places and events associated with these places. Finally, the brain GPS is one of the first areas impaired during Alzheimer's disease. Identifying what goes wrong and how this is reflected in our ability to navigate will help identify methods for early diagnosis and effective treatment.
What species and approximate numbers of animals do you expect to use over what period of time?	250 rats & 3000 mice, 2000 of which used for breeding. Over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will measure and manipulate brain cell activity in navigating rats and mice in order to understand how different activity patterns enable an animal to perceive space, learn and remember environments and navigate to a goal location. Rodents will have tiny electrodes or optical lenses implanted and, in some cases, drugs injected into their brains or damage made to small areas of the brain. All surgical interventions are made under deep anaesthesia and animals are given painkillers before, during and after the surgery to minimise pain and discomfort. Mice usually show signs of full recovery within a few hours; to assess this we look at whether they are eating normally and displaying their usual behaviours (e.g. running on their toy wheels, building nests in their cages). It takes around 1 to 5 days for the rats to reach a comparable level of recovery, during which animals mostly rest and sleep. After recovery, animals will explore real and virtual environments. They will be freely foraging in differently sized and shaped enclosures looking for sweet rice, or will navigate to a reward location in different mazes and corridors. Some

	<p>environments will be presented in virtual reality, using screens, projectors and other sensory stimuli. In these cases, an animal will have a small head post permanently fixed to its skull. It will be head-fixed via this post to stationary metal bars while it is running on a cylinder or an air-suspended ball in front of two screens displaying the virtual enclosures (or similar). Head fixation allows us to use light to measure and manipulate the activity of the specific identified brain cells, which is essential for studying memory-related processes. Before we carry out the actual experiment, the animals are given two to three days to get used to running on the ball to lower their stress levels. When animals are first head-fixed, they produce more urine/faeces, indicating that they dislike it; however, they stop responding this way several minutes after their first exposure to head fixation. Food rewards are then used to encourage navigation; the animals usually perform the task adequately after two to five days of experience. Once familiar with head-fixation, mice willingly explore virtual environments, similar to what they do on running wheels in their home cages. Behavioural experiments usually involve training the animals to seek sweetened food rewards (e.g. soya milk, sweet rice) and are carried out in such a way as to minimise harm to the animal by habituating the animal to the experimental room as well as to Experimenter. In order for the food reward experiments to be effective, animals have to be given a restricted amount of food during the experiment. The food restriction is always closely monitored and kept to a minimum. During the exploration experiments, we record the activity of brain cells in both the hippocampus and connected brain areas to determine how these cells interact and activate each other, and how this lets the animal navigate and perceive space. In some cases, these neurons are manipulated via lesion, drug, electrophysiological or optical methods to reveal what activates these cells and how. Also, in some cases, we use these methods to try and mimic the damage observed in hippocampus-related dementia such as Alzheimer's disease.</p>
Application of the 3Rs	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We inform our study design by describing brain activity using computers whenever possible. We use rats and mice because it is not possible to study the role of the hippocampus in real-world navigation without using behaving animals. Moreover, mice present one of the best animal models for studying the mechanism of Alzheimer’s disease. Namely, it is possible to genetically modify mice to express substances that we find produced in human brains with Alzheimer’s disease; this lets us investigate their effect on brain cells and an animal’s ability to use its GPS system and remember places.</p> <p>Finally, we will share our data with other researchers to reduce the risk that experiments are repeated.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We minimise the number of animals used in these experiments wherever possible. We used computer-based mathematical descriptions of brain activity to make specific predictions that require fewer animals to test. Furthermore, continual technical advances allow us to monitor more and more brain cells within each given animal, allowing us to use fewer animals overall. Almost all procedures involve long term experimentation with the same animals, which significantly reduces the number of animals needed to reach reliable conclusions.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>In these studies, we use rats and mice since they are very good at navigating in familiar environments and remembering what has happened to them there. We know a lot about the structure and basic working principles of their brains and, in particular, about the parts of the brain to be studied in this project.</p> <p>Optimal results in behavioural experiments require that the animals are healthy, in good spirits, and motivated to perform well. For this reason, the majority of our behavioural tests involve positive reward rather than punishment, in order to encourage animals to navigate. Before animals are used in experiments, they will be acclimatised to their home cages as well as experimental environments, if this does not interfere with experimental design, e.g. when</p>

responses to novel environments are investigated.

We are also using the minimum level of food restriction required to make sure the animals perform adequately on each experimental task. Professional surgical procedures and pre- and post-surgical care including administration of pain relief drugs ensure a minimum of adverse effects and the minimum level of suffering caused by any surgical or other intervention.

All animals live in enriched environments with a lot of space and toys such as wooden balls, play tunnels and exercise wheels. Because both rats and mice are highly sociable animals, the animals are housed in groups in large home cages wherever possible.

Project	Neural basis of tactile behaviour
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The general aim of the project is basic research into the mechanisms of how the nervous system represents tactile information and makes decisions based on touch. The project addresses two important gaps in our knowledge. First, most past experiments on this topic have been undertaken in anaesthetised animals. It is unclear how tactile perception operates in the conscious brain. Second, despite the fact that the sensory regions of the brain contain millions of brain cells, most past experiments measured the activity of only one or a handful of neurons at a time. Coordinated activity amongst large numbers of brain cells is likely to be crucial to perception, but is poorly understood. The

	<p>specific objectives of the project are: (1) to determine how touch-related parts of the brain respond to touch; (2) to develop a method for studying the activity of brain cells by measuring signals related to cell Calcium; (3) to determine the coordinated response of many brain cells to touch.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>There are short-term and long-term benefits: (1) The project will advance our understanding of one of the great mysteries of science – how the activity of brain cells allows us to perceive the nature of the outside world. (2) Basic science such as this project is fundamental for brain health, since it will provide the clinicians of the future with a richer and more useful scientific base, from which to develop improved therapies for neurological disease. (3) The powerful new methods that we develop during the course of the project can be applied to animal disease models and thereby get more insight into disease mechanisms than was previously possible.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use not more than 4600 mice over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We will measure the activity of cells from the brains either of behaving animals, trained to perform a task for fluid reward, or of anaesthetised animals in response to touch. Recovery surgery may cause post-operative pain and/or infection: these will be prevented by delivery of analgesics/antibiotics and by use of aseptic techniques. Restraint may cause stress: this will be minimised by habituation and training. No more than moderate severity is expected. Animals will be killed at the end.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Our knowledge of how sensory pathways process sensory information is incomplete. Hence a pure computer modelling approach cannot be used. In order to determine how neurons respond to sensory stimuli, the full circuitry from sensory receptors, including the</p>

	sensory organ (here the whiskers), must be intact. This precludes in vitro approaches.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>By using advanced techniques for measuring neuronal activity (multimicroelectrode arrays and imaging), we will maximise the number of observations measured per animal. This will reduce the required number of animals. The minimum number of animals will be determined by a combination of statistical analysis and pilot study.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rodent species are of low neurophysiological sensitivity. We will use the most refined techniques possible that minimise adverse effects or discomfort to the animals. To minimise harms, the project includes the development of a refined, non-invasive imaging technique for measuring the activity of brain cells.</p>

Project	Neural Circuitry Assembly	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
3 Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Neuronal circuitries underlying the function of the mammalian cerebral cortex collectively constitute one of the most complex biological systems. As such, unravelling the mechanisms that control their development represents one of the most challenging questions in science. Understanding this process is also an imperative need in biomedicine, because abnormal connectivity among neurons is thought to cause severe neuropsychiatric disorders. Thus, while there is growing awareness of the urgency for translation from basic findings to the clinic, it is also becoming clear that the translational bridge must be built on the solid footing of fundamental neuroscience. In other words, we need a better understanding of how the brain works in both	

	<p>health and disease.</p> <p>The function of neural networks in the cerebral cortex of vertebrates relies on the interaction between 2 classes of neurons, excitatory projection neurons and inhibitory interneurons. In these circuits, the output of excitatory neurons is fine-tuned and synchronized by the function of inhibitory neurons. Interneurons play similar role than the conductors of an orchestra that pace the rhythms of the musicians to compose a beautiful symphony. For example, now we know that the function of these neuronal conductors (interneurons) is key for any sensory discrimination including visual stimulus and for cognitive function. The general aim of my research is to understand how the connectivity of these interneurons is formed and matured- for example how the axons of these neurons (cables of these networks) find their partner to make synapses (the connection between neurons)- and what is the consequence when during development they fail to establish their connections. The understanding of how the brain works in both health and disease, will represents a major opportunity to expand the search for novel targets to treat disorders in which cognitive deficits are at the core of the disease, such as autism and schizophrenia.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Neuropsychiatric disorders represent the leading source of disease burden in the developed world for people between ages 15 and 49. In contrast to heart disease or most forms of cancer, many neuropsychiatric disorders such as autism or schizophrenia begin early in life and contribute to lifelong incapacity or reduced longevity. Consequently, brain disorders will become an even greater public health challenge in the coming decades. Existing medications for most neuropsychiatric disorders are merely palliative, largely because our limited understanding of their causes. In this context, the development of new animal models with impaired cognition represents a major opportunity to expand the search for novel targets to treat disorders in which cognitive deficits are at the core of the disease, such as autism and schizophrenia. Mice are excellent animal models to investigate brain development. Given our ability to manipulate their genome and</p>

	<p>their susceptibility to some of the same genetic defects that cause disease in humans-we share 95% of the genes- mice are the gold standard for the type of experiments presented in this Project.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice 12000</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>To reach our goals, we will breed and maintain genetically altered mice. We will use of cell-specific neuronal mutants (mutations only in a population of selected neurons), which will lead us to obtain more accurate results to implement refinement, although it will require a more extensive breeding to reach the appropriated mouse. We will also perform experiments both in vitro (neuronal cultures) and in vivo. This will include post-mortem analyses (e.g. immunohistochemistry, biochemistry), in utero and neonatal manipulations of mouse, behavioural analyses and electrophysiological recordings in adult animals under terminal anaesthesia. These experiments are either mild or moderate in severity. Animals will be killed at the end of our experiments.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Animal behaviour relies in the very precise connectivity among different neurons across diverse brain areas. In particular, this connectivity reaches its highest complexity in the mammalian cerebral cortex. Our understanding on how the wiring of cortical neurons emerges during development is still very limited. Therefore, it is difficult to built computer models based on what is still unknown. In addition, the architecture and function of cortical networks are very complex and can hardly be reproduced in vitro. Nevertheless, whenever is possible we will use ex vivo and in vitro alternatives to improve our methodology before taking any in vivo approach.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of</p>	<p>In addition, the project has been designed with the goal of reducing the number of animals used. For example, we have developed a database to register every piece of tissue obtained from these</p>

<p>animals</p>	<p>animals, which will be efficiency stored and used for future studies. I have more than 20 years of experience in using mice as a model system and in my laboratory experiments are always designed to use the minimum number of animals required to generate statistically significant data. All members of my lab will visit the web site of Dr. Michael F.W. Festing http://www.3rs-reduction.co.uk/ for any experimental design. They will use the http://www.biomath.info/ web site to estimate the sample size. Additionally, I have established a mandatory induction for all new staff that will join my lab to improve the management of the colonies and the experimental design and I discuss with them monthly the management of their colonies. This induction will be complemented by the use of databases like http://www.nc3rs.org.uk. Finally, to implement our standards, we will use factorial experimental design to maximize the data collected from each animal. We will also seek for statistic advice to improve the quality of our design and reduce the number of animals used.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are excellent animal models to investigate brain development. Given our ability to manipulate their genome and their susceptibility to some of the same genetic defects that cause disease in humans, mice are the gold standard for the type of experiments presented in this Project. In this context, we plan to use cell-specific neuronal mutants by using the Cre- loxP strategy; this will lead us to obtain more accurate results to implement refinement. Also, only personal that has been thoroughly trained first with animal corpses and then under strict supervision of senior researchers with in vivo animals will perform any procedure. In all procedures, we have reduced the duration time of the experiment and shorten the time the pups are without the mother.</p> <p>To assure the welfare of the animals, anaesthesia, analgesia and general protection will be provided to the mice to avoid any suffering prior to manipulation or sacrifice for the experimental procedures, using approved methods. In particular, additional local anesthetize will be use for surgeries.</p>

Project	Neural circuits of flexible behaviour	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	In order to survive, an animal's behaviour must be flexible. How the brain selects different actions in response to the same stimulus remains a mystery. One of the key elements underlying flexible behaviour is the filtering of sensory information. Animals receive a constant barrage of sensory information all the time. In general, for animals to successfully navigate the world, it is essential to focus on relevant stimuli while filtering out stimuli that are irrelevant. Indeed, selectively attending to relevant stimuli is one of the most fundamental of cognitive processes, yet its underlying neural mechanisms remain poorly understood.	

	<p>In this project we will combine modern neuroscientific tools to study the brains of mice and rats as they perform flexible behavioural tasks. We will record and manipulate the activity of neurons in the brain and determine what is special about their activity patterns that makes the animal capable of performing context-dependent flexible behaviours.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The primary potential benefits of this project will be the generation of new knowledge. Our research will advance our understanding of how the brain processes information, and will explain how multiple brain structures enable flexible behaviour. By understanding the detailed neural basis of a simple cognitive phenomenon, we will be able to gain insight into the basic building block of complex intelligent thought. The data obtained through this project will consolidate existing scientific research and bridge various disciplines. We will provide a framework for the key determinants of cognitive behaviour. We will continue to publish this work in academic journals and disseminate the results in national and international meetings. In addition, a secondary potential benefit relates to the value of our results to clinicians. For example, cognitive deficits underlie several neurological and neuropsychiatric diseases including epilepsy, autism, ADHD and schizophrenia. Elucidating the neural mechanisms that produce complex cognitive behaviours might shed light into the pathophysiology of these disorders.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to breed and maintain 10,000 mice and 300 rats. Of these mice a large number will not carry the appropriate genes required due to the nature of genetic inheritance. Thus we will use about 6000 of these mice over the next five years for performing the experiments on.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Part of the project will involve the breeding of genetically altered mice to allow us to investigate the functions of particular cell-types in flexible behaviour. These animals are very similar to wild-type animals and we do not expect any suffering caused by their breeding. In some animals we will inject substances into</p>

	<p>the brain to deduce brain function. This will be carried out under general anaesthesia, in aseptic conditions, with animals recovering from the surgery with appropriate post-operative care and analgesia. This will involve a moderate amount of suffering for the animals for a brief period of time, specifically during the post-operative period. Post-operative analgesia and care will be of the highest standard and approved by a veterinarian. After full recovery, animals will be trained to perform behavioural tasks such as discriminating between visual patterns. This will involve restriction of food or water in order to motivate the mice to perform the task for food or water reward. This will result in some weight loss, and mice weights will be monitored routinely and maintained within the approved range. This is expected to result in a mild to moderate amount of suffering, and any mice showing clinical signs of increased suffering will be immediately given unlimited food and the experiment stopped. Animals will be killed humanely at the end of the experiments.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>This research program aims to understand cognition, which is inherently a mental phenomenon, existing only in awake, behaving animals. For this reason it is not possible to perform this research without using animals. While computer models are capable of running algorithms that can perform various computations such as recognising patterns in data, these algorithms are far from achieving the performance of real brains in even the most basic of tasks such as object detection. Although computer simulations do have a role in advancing our understanding of cognition, they can only meaningfully contribute after incorporating extensive experimental findings from animals. This research program will include such efforts in developing computer simulations to test hypothesis suggested by the experimental findings. Examination of the website www.frame.org.uk confirms that there are no current alternatives to animal</p>

	experiments for this research.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The proposed experiments will involve recording neural activity on multiple days from the same animal and will thus provide a large amount of data per mouse. This will therefore limit animal use by design. This is because for most experiments we will record neural activity from individual mice over multiple days, and from multiple brain locations, maximising the amount of information obtained per mouse and reducing by many fold the number of animals required for each experiment. Thus, we will ensure that the use of animals in this research is kept to the minimal by maximising the amount of data collected from each animal and furthermore by efficient data analysis and computational modelling.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse has emerged in recent years as an ideal species to uncover the mysteries of the brain. The mouse brain circuitry is relatively similar to human, allowing us to address many fundamental issues of function and dysfunction in the brain without having to make use of higher mammals such as monkeys. Another advantage of mice as an experimental organism is transgenic technology, which is used to express genes to assess the activity, function or structural properties of neurons. These genetic tools will facilitate our understanding of the brain at the different levels of brain hierarchy.</p> <p>To minimise animal suffering we will continually employ refinements to our techniques and procedures. For example, we will incorporate the use of motorised robotic equipment for performing precise drilling of the skull during surgery to minimise damage. We will also continuously refine the procedure for behavioural training, by incorporating automatic algorithms which modify the training parameters for each mouse individually to enable easier learning.</p>

Project	Neural codes for perception
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We wish to understand how brain cells store, process, and transmit information via electrical impulses. How does the pattern of electrical impulses in the brain drive perception or enable performance of an action? Such crucial questions have yet to be answered at the level of electrical impulses in groups of neurons despite substantial recent progress in neuroscience. We propose to use novel approaches to answer these fundamental questions in order to reveal how our brains work at a greater level of detail than revealed previously.
What are the potential benefits likely to derive from this project (how science could be advanced or	Improving our understanding of the neural basis for perception and action at the level of electrical impulses in individual neurons is in the first

<p>humans or animals could benefit from the project)?</p>	<p>instance a matter of considerable, fundamental scientific interest. In the longer term, this may also open up a variety of practical applications, from improved diagnosis and treatment of patients to the development of better brain machine interfaces. Such understanding may give us deep insights into the workings of the cerebral cortex, and will aid the fight against debilitating disorders of the brain.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Over the 5 year period of the project, we will expect to use 3000 mice in procedures other than breeding and maintenance. We may breed and/or maintain up to 7000 mice, some of which will be the same ones as in the other procedures.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Part of the project will involve the generation of genetically altered mice to allow us to investigate the functions of particular cell types in sensory processing. These animals are expected to be not fundamentally different in the way they behave from wild-type controls and thus expected levels of severity will be mild. In some animals, we will need to trim some of the whiskers of the animal to be able to relate neural signals to touch sensation in specific whiskers. This has no effect on animal well-being. In some animals it will be necessary to inject substances into the brain to deduce anatomical structures and function. This will be carried out under general anaesthesia in aseptic conditions with some animals being recovered with appropriate post-operative care and only causing moderate amounts of discomfort to the animals in the study. The behavioural tasks we will use to record conscious, sensory perceptions are painless. In some cases, it will be necessary to motivate the animals to perform these tasks by rationing their food or water during testing. This may result in a temporary weight loss, but this will always be monitored carefully and extra food or water provided if this occurs. The availability of modern techniques for monitoring or altering neural activity in particular regions of the brain make it possible to carry out almost all of this work in a manner that should cause only moderate amounts of discomfort to the animals in the study. For example, surgical operations for implantation of ultrafine microelectrodes or for</p>

	<p>inserting genes into the brain will be carried out under general anaesthesia, in aseptic conditions, and with appropriate post-operative care. The adverse effects that may occur following surgery include transient pain and bleeding, but their incidence is likely to be very low. Chronic implants for recording neural activity or for delivering flashes of light to the brain for the purpose of altering that activity are small and lightweight, and do not materially affect the animal's quality of life. Some animals will be used in tests where the head needs to be fixed to enable stable recordings of brain activity. In these tests, the animal is supported on a moveable platform that allows the animal to perform behavioural tasks, for example, navigating through a virtual maze projected onto screens. This method is now very established and well tolerated by mice displaying the same behaviour as when walking freely and should cause only moderate amounts of discomfort to the animals in the study. Animals will be killed humanely at the end of the experiment.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Our project investigates the neural basis for sensory perception. Currently, this can only be studied by using the brains of animals or humans, as our understanding of brain function is too rudimentary to generate realistic mathematical models for testing. Brain imaging measures in humans lack the sensitivity to observe changes in the properties of individual brain cells in response to sensory stimuli. Moreover, a key aim of this project is to manipulate brain activity with single-cell resolution using non-invasive optical stimulation, which is not available in humans. Additionally, we aim to relate brain cell activity to the underlying neural circuitry at a microscopic level. This requires the use of post-mortem histological measurements, which would not be ethical or practical to carry out in humans.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of</p>	<p>Calculations are carried out to determine the necessary number of animals for each experiment, ensuring significance of our results but also minimising the number of animals used.</p>

animals	We are additionally able to keep animal numbers to a minimum by using cutting edge methods that yield large amounts of data and experimental designs that allow multiple measurements to be made from each animal.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice will be used because they are the lowest vertebrates with a sensory system that is comparable to that in humans. The ability to use genetically altered mice is particularly crucial to our studies as it enables us genetic access to specific neuronal cell types.</p> <p>Animal welfare costs will be minimised by carrying our procedures in state-of-the-art facilities and using best practice methods. Breeding and colony maintenance, including genetically altered mice, will follow the Home Office assessment framework for efficient breeding and maintenance. We will only use genetically altered mice that exhibit a mild phenotype (e.g. with no effects on feeding or welfare) or no measurable behavioural phenotype (e.g. mice producing a fluorescent marker in certain brain cells). Surgical operations are carried out very carefully under anaesthesia and aseptic conditions, and the animals are given painkillers and will be closely monitored until they have fully recovered.</p> <p>Sometimes it will be necessary to regulate the food or water intake in mice in order to motivate them to perform behavioural tasks for a food or water reward. We have very strict guidelines in place to mitigate any harm from this food or water regulation, as well as for the behavioural tasks used.</p> <p>The use of state-of-the-art methods, such as optogenetics and recording/manipulation of brain activity in behaving animals aimed at reducing the impact on animal welfare, while, at the same time, increasing the amount of scientific insight that can be obtained from each experiment. The earliest endpoints consistent with the scientific aims are applied.</p> <p>The data obtained from these experiments will be used to refine computer models of the brain that will help to guide subsequent experiments and contribute to a reduction in the number of</p>

	animals needed.
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Project	Neural Control of Sensorimotor and Autonomic Function in Health and Neurological Conditions	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim to study the mechanisms and effects of different interventions capable of promoting repair of injured nerves in the brain and spinal cord to produce functional recovery. We will specifically investigate changes in stepping behavioural and other functions such as bladder control following injury and recovery through different interventions.	
What are the potential benefits likely to derive from this project	The ultimate goal of our project is to provide interventions that can facilitate recovery of	

<p>(how science could be advanced or humans or animals could benefit from the project)?</p>	<p>function following injuries to the brain and spinal cord. Some of the interventions included in this project, such as epidural electrical stimulation and rehabilitation have already shown promise in clinical application. However, further refinements are required to improve their effectiveness. Other newer potential interventions are in earlier development and require to be tested in animals.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Rats = 2500 Mice = 1800</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Some animals will only reach a mild level in the severity scale. Others, will never wake up from the anaesthetic. For the nervous system injury procedures, most animals will be expected to be in the severe level of severity. This is mostly due to disruption of movement and sensation in some parts of the body. The level of disruption will also vary depending on severity of the injury. Control of bladder function is often compromised in these animals. Therefore, we care for each animal at least twice daily for the duration of the experiment to manual express their bladders and check for any abnormal behaviours. For example, persistent weight loss will be treated with supplemental diet, wet food and if necessary saline injections. However, we do not expect to induce pain in these animals. In severe lesions, the communication between the brain and spinal cord is completely severed and pain sensation cannot be processed by the brain. Because our primary aim is to facilitate recovery of function after lesions, the wellbeing and health of animals is of paramount importance. Ill health and pain are not conducive to such recovery. In the rare occasion when adverse effects exceed expected changes (for example, persistent excessive weight loss, inability to eat or drink, etc.) the animal will be humanely killed using an approved method. Therefore, we individually care for each animal at least twice daily for the duration of the experiments. At the end of all experiments, animals receive an overdose of anaesthetic for collection of tissues or are killed using an approved Schedule 1 procedure.</p>

Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The complexity of the events leading to and as a consequence of lesions to the nervous system involves several physiological systems in the body. Unfortunately, reduced preparations such as cell in a dish, parts of brain kept in a dish, cannot provide enough information from all of the systems simultaneously. Our primary objective is to investigate functional recovery, which can only be measured in alive behaving animals.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Our experimental design has been optimized to use the lowest number of animals required to produce reliable statistical inferences. We combine several different techniques, including behavioural, physiological, anatomical tests and computational modelling. This allows us to reduce the number of animals required by eliminating the need to repeat experiments to collect different types of data. In addition, our experiments can provide very powerful results when looking at different types of measurements (behaviour, physiology, anatomy) from the same animal.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rodents (rats and mice) are the preferred animal model for these projects for the following reasons. First, given the stage of our understanding a certain number of animals need to be studied, which precludes use of larger animals (pigs, cats, monkeys). Second, the nervous system of rodents have several similarities to humans, including the processes involved in responses to injury. Third, rodent models of nervous system lesions have been prevalent in the last years, and much is known about the disease process of the lesion in those species. All surgical procedures are done under anaesthesia and under aseptic conditions, which minimizes the need to deliver medication such as antibiotics. As mentioned before, each animal is individually taken care of at least twice daily. Also, pain and distress are unwanted outcomes because they are detrimental to functional recovery. Therefore even the smallest changes, for example in skin condition, such as a small</p>

	sore, are immediately treated.
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Project	Neural correlates of vitamin B supplementation	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project will investigate the effect of dietary supplementation of B-vitamins on the brain neural activity as well as on cognitive behaviour using a rodent model. It will also aim to establish a mathematical model linking neural signals recorded invasively in the brain to non-invasive neural signals recorded on the scalp.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefit of B vitamins supplementation for people with moderate deficiency in B vitamins or those at risk of deficiency, such as older people or pregnant women, has not been established. This project will investigate the effect of such supplementation in a systematic way across	

	<p>different age groups using an animal model. Results from this programme may have important implications on the prevention of cardiovascular diseases and dementia, thus enhancing the well-being of older people and generate considerable economic as well as public health benefits. In parallel, the mathematical model will allow non-invasive neural recordings to be better interpreted in terms of neural activities normally collected using invasive methods. The potential benefit of having such a model is to reduce the need for invasive methods, which, in term, reduces the need for animal research and facilitates human brain research.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>A rat model will be used, and the number of animals required under this project is estimated at 700 (maximum) over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>During the feeding programme, animals will be monitored routinely to ensure their well-being. Concentrations of B vitamins will be carefully chosen based on published literature to ensure no neurotoxicity will be induced. Although dietary supplementation of B vitamins is expected to enhance cognitive abilities of animals, if markers of terminal decline are observed, the animal will be humanely killed. At the end of the feeding programme, aseptic surgery will be performed to collect neural data. The animal will be anaesthetised while physiological variables, such as heart rate and breathing rate, will be monitored closely to ensure they are within appropriate ranges. At the end of the procedure the animals will be humanely killed while under deep anaesthesia without recovery.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>To achieve the objectives of the project, we need to record signals from specific areas of the brain to see how these are affected by vitamin supplements. If possible, signals from multiple locations will be recorded concurrently. Such procedures cannot be conducted safely in healthy humans.</p>

<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>To minimise animal usage, we will make multiple recordings from a single location so that we can take the average of signals to reduce noise level. Whenever appropriate, we will record neural signals from multiple locations at the same time. The more information we can record from a single animal within a limited time, the less number of animals will be needed. At all stages of the project, we will consult a professional statistician, when required, to ensure an optimal statistical design and the number of animals required is minimised, yet sufficient precision and power are maintained.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rats will be used because of their physiological similarity to humans. Rats are also more suited to cognitive studies because they are more capable of learning tasks than other species. Furthermore there exists a wealth of research and data which we can use to compare our results with. All animals will be under terminal anaesthesia which will be carefully monitored throughout the experiment to ensure that all physiological parameters (e.g., body temperature, heart rate, respiration rate) are within appropriate ranges and are stable to minimise animal suffering.</p>

Project	Neural mechanisms in health and disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>To understand how networks of nerve cells in the brain give rise to physiological functions and to identify abnormalities in these networks that cause dysfunction associated with cognitive/emotional, motor or metabolic disorders.</p> <p>There are many diseases rooted in the brain such as cognitive, degenerative or metabolic. A lot of research and many scientific discoveries have been made in the past years with the aim to understand causes, prevention and cure.</p> <p>However, what has become clear is that due to the high complexity of the brain not only in terms</p>	

	<p>of structures but also because of the enormous heterogeneity of types of nerve cells, it is necessary to tackle these issues using the most precise model organisms and up-to-date and sophisticated technologies.</p> <p>This is mainly because to restore neuronal functions requires that we first understand how nerve cells function at the cellular and molecular level, and how they connect and form complex networks that underlie specific behaviours and functions.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This Project will provide new insights in the molecular mechanisms that control the activity of nerve cells, and will contribute to the understanding of high order functions such as learning and memory, emotion, as well as motor behaviour and metabolism. It will also advance our understanding about the contribution of specific molecules to these functions. Ultimately, advancing knowledge into these mechanisms may well lead to new or improved treatments.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice, based on our experience with the use of genetically modified mouse models we expect to use approximately 11,000 animals over the 5 years. Most of these will be in the breeding programmes that will generate the genetically modified mice that we need.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Most of the animals will be used in a protocol with a mild severity limit such as Protocol 1, breeding and maintenance of genetically modified animals. Animals produced under this protocol are not expected to exhibit any harmful phenotype. In the case of unexpected and unwanted harmful phenotypes the animal will be humanely killed, or in the case of animals of particular scientific interest with an unexpected harmful phenotype, advice will be sought from the NVS/NACWO. Four protocols have a moderate severity limit; one involves aging and the other surgery. A smaller proportion of the animals will be used in these protocols and we envisage that for many of the animals the actual severity will be mild. In particular, mice on Protocol 2 will be behaviourally tested at different stages and so mice will be aged. These</p>

animals are not expected to show specific problems as they age. In some, after 12 months of age learning will deteriorate quicker than normal mice. However, ageing animals will be monitored closely including weighing, clinical examination and body condition scoring in consultation with the NVS, and any adverse effect due to a particular behavioural test will be dealt with accordingly. Mice on Protocol 3, instead, could present adverse effect due to surgery; these will be treated according to the effect presented. Mice on Protocol 4 will be analysed for metabolic dysfunctions. The severity limit of this protocol is mild, and there are no major adverse effects expected. Protocol 5 deals with breeding and maintenance of genetically modified mice with a moderate severity limit. Therefore, animals produced under this protocol are expected to exhibit harmful phenotypes. These animals will be inspected carefully and closely monitored to ensure they do not exceed a moderate severity limit. Animals exhibiting any unexpected unwanted harmful phenotypes or in case the severity is likely to exceed moderate will be killed, or in the case of animals of particular scientific interest with an unexpected harmful phenotype, advice will be sought from the NVS/NACWO. Protocol 6, newly added protocol, combines ageing/behaviour and surgeries with the administration of substances, therefore, mice on this protocol will be monitored closely including weighing, clinical examination and body condition scoring in consultation with the NVS, and any adverse effect due to a particular behavioural test will be dealt with accordingly. Seemingly, for those undergoing surgeries any adverse effect due to surgery will be treated according to the effect presented. The work has been organized in stages, first of all a precise analysis of the genetically altered mice will be obtained from mice humanely killed which will suffer only transient pain and distress. Once preliminary information are obtained we will then proceed with in vivo functional validation of the identified gene(s)/cell type involving stereotaxic injections into the brain. Good animal care, husbandry, health checks based on veterinary advice will be applied to all animals that go through surgery.

	This will help identifying any adverse effect as soon as it appears. In this event appropriate steps will be taken to minimize it. Clear end points have been established.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The type of investigation outlined in this project cannot be carried in cell cultures as these lack information about the networks of neurons that are found in an intact brain. Furthermore, cell cultures do not replicate disease progression.</p> <p>Manipulation of the mouse genetics provides a unique opportunity to study the molecular basis of complex cell-cell interactions <i>in vivo</i>. The increasing availability of cell-type specific promoters gives particular power to the generation and analysis of precise mouse models that undoubtedly will help dissecting the complexity of the brain.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Generation of very precise and specific genetically altered mice results in more defined, less variable and hence more relevant data. This correspondingly reduces the number of animals necessary to obtain significant results as well as improves the quality of life of the animal.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice remain one of the best species to study brain mechanisms in health and disease. Moreover, being a mammal, the similarities in the brain between mouse and man will allow the transfer knowledge to human more easily.</p> <p>We will provide an environment that will meet the animals' specific needs, such as enriched cage environments with as little stress to the animals as possible. This, of course, will allow us to obtain more reliable results. Finally, understanding these molecular mechanisms will undoubtedly impact on the development of therapies to improve high order functions such as learning, memory, emotion, motor behaviour and brain metabolism.</p>

Project	Neural mechanisms of appetitive learning	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Exposure to information or ‘signals’ (e.g. sound of ice cream truck) that are linked to palatable foods (e.g. ice cream) shape our urge and motivation to eat. For example, when we see a fast-food sign, we might be reminded about snacks and experience food cravings. We do not fully understand how brain cells actually store and retrieve these types of linked associations. Without understanding this, development of therapies to treat conditions such as excessive food cravings and overeating would be difficult. Using rodents, the aim of this project is to determine how a tiny minority of brain cells called ‘neuronal ensembles’ stores and retrieves memories about food. We will	

	<p>study the cells in brain regions that are important for motivation to satisfy our basic needs (e.g. drinking and eating). We will also study at a molecular and cellular level, what is special about the brain cells that enables memories to be formed and be retained. This research can be applied to humans and is important not just for better understanding food-related memory, but also conditions such as overeating and obesity, which are a major burden to health, society and the economy.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This project has many benefits largely at the basic science level because when this project is completed, we would have identified the contents of brain cells and their cellular environment that help store and retrieve memories about food. For example, we may find out whether these brain cells contain specialised proteins that make them behave differently from other brain cells that do not participate in food memory retrieval and storage. Indirectly, the results from this research may be useful in the distant future (e.g. 10-15 years), as such information may be useful for creating better medicines for people that suffer from conditions such as excessive appetite, overeating, and obesity. These types of conditions are suffered by millions of people in the UK and are associated with diseases such as diabetes. We hope that these medicines will specifically target brain cells that control conditions such as excessive appetite, without affecting the brain cells that control normal mental functions such as remembering to buy groceries. Since we are studying learned associations, this research will also provide clues on normal and abnormal mental functioning such as how the brain links and remembers other types of important information that shape our behaviours (e.g. the smell of smoke signaling fire). Such clues may also reveal more about conditions that affect our health such as drug abuse, since the development of drug addiction involves linking information about drugs and the environment where drugs are used (e.g. the sight of smokers creating a cigarette craving).</p>

<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice 14,000, Rats 3,500</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In most of the behavioural tests, the animals are free to move around. These tests may involve a rodent reacting to a tone that predicts food availability (similar to how a pet dog might react before being fed), and/or a rodent performing a response such as a lever press to obtain a food reward. Usually these tests are conducted in an experimental chamber (often called 'Skinner Box') that is equipped with levers, lights, speakers, and food dispensers. The animals usually go into this chamber twice a day for 30 minutes at a time, and the training will last from several days to weeks. The animals get used to these chambers very quickly because they may receive rewards in there, and do not exhibit signs of discomfort, akin to a pet rat or mouse going into a new home cage. Where food restriction is required to maintain an animal's motivation, animals will be monitored for excessive weight loss every day (i.e. we ensure that their weights do not drop by more than 15% compared to animals which have unlimited access to food) and their diet is adjusted accordingly. A minority of animals will have to be slightly restrained during brain imaging in order to clearly see the very fine structures of brain cells. These animals will still be able to move on a ball or flat surface similar to a treadmill. They will be gradually introduced to the restraining and imaging procedures, and wherever possible be given rewards to minimise stress during the imaging process. Surgical work will be carried out under sterile conditions similar to a hospital operation room to reduce the risk of infection. Where possible, gas anaesthetics will be used for rapid recovery, and analgesics or 'painkillers' to manage pain. Some animals will be surgically implanted with a thin tube called a 'guide cannulae' to deliver substances into the brain to control brain cell activity, or an imaging probe to observe brain cell activity. This will allow us to study the relationship between brain cell activity and behaviour. This procedure is of moderate severity, but has been shown to be well-</p>

	<p>tolerated in many peer-reviewed studies. Also, painkillers will be provided during and after the surgery to minimise discomfort. After completing these experiments, all animals are humanely killed using approved euthanasia methods and usually their brain cells will be further analysed using laboratory tools such as a microscope.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We would like to study how food-related memories are stored and retrieved. For these reasons, the use of animals that possess the ability to learn and remember information is essential. It is not possible to investigate such complex processes using cell culture systems, computer modelling, or brain imaging methods in humans. In this project, we will use genetically altered animals because they allow us to answer scientific questions that cannot be answered in normal animals. For example, we can identify the precise brain cells that are involved in learning and memory by using animals which have certain types of brain cells genetically marked, which we can then identify under a microscope. This means we can gain more information, and produce higher quality scientific reports, than if we only studied normal animals. Although mice and rats do not behave completely like humans, their brain circuits thought to control the behaviours of interest here are largely similar (e.g. learning about food). Hence, rodents are a useful experimental tool to study the brain mechanisms of how we store and retrieve food-related memories.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The numbers of animals to be tested will be the minimum number required to obtain reliable experimental results, based on previous experience in the laboratory, and from published studies. Where appropriate, we will use mathematical formulas called 'power calculations' to estimate the minimum number of animals we would need to be confident in our results. Also, where possible we test the same animal many times using an approach called 'within-subject comparisons' which means we need to test fewer animals overall. Both of these methods allow more reliable experimental</p>

	results to be obtained and limits the numbers of animals used.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>This project involves complex behavioural tasks (e.g. learning to make a particular response such as pressing a lever to obtain food) and rodents perform these tasks very accurately. During behavioural training, we carefully observe the animals using a live camera feed, and by examining their training data. These observations are useful for detecting if animals might be unwell. In addition, many aspects of the rodent brain are similar to humans, including which brain areas are important in producing the behaviours that we study. We reduce stress to these animals through use of food rewards in training, rather than punishment. Training using food rewards can be very successful and is widely used to train service animals (e.g. police dogs). To improve their well-being, animals will be group housed as rats and mice are social species which naturally choose to live in groups. Also, they will usually undergo behavioural tests in the same familiar location in a dark and quiet room that minimizes stress. In general, we pay lots of attention to the well-being of the animals (especially after surgeries), including trained and qualified personnel examining a wide variety of behavioural and physical signs. If necessary, animals that are unwell would be humanely euthanised to prevent excessive suffering.</p>

Project	Neural mechanisms of feeding and reward	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project is designed to understand how the brain controls what we eat. We are especially interested in how the nutrients in food - such as the sugar, fat and protein – affect the brain. When we are hungry or when we don't have enough of a certain nutrient, this can make the brain tell us to eat certain foods but we don't know why this is. Also, if we eat a lot of junk food this can also change what we want to eat in the future.</p> <p>In our experiments, we can measure when brain cells are active to work out which areas of the brain are important for eating. We will make these measurements in animals that are given</p>	

	<p>different types of food so that we can work out how the brain is controlling this. In some experiments, we will put nutrients directly into the stomach with a tube so that we can find out if it is the taste or the effects in the stomach that is most important.</p> <p>We are also interested in how certain foods might change the way we react to drugs. This is because the same parts of the brain that are active when we eat junk food are also involved in drug addiction.</p>
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By conducting these experiments, we will learn more about how the brain controls what we eat especially when faced with foods containing different nutrients. We will learn more about why we want to eat certain foods more than others and how the brain is involved in this. This work will contribute to our understanding about obesity and, in the future, could help to come up with ways of treating people.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use rats and mice. Over the five years, we expect to use 1200 rats and 1200 mice.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For some experiments, animals will have surgery to either put a device in the brain (so that we can measure brain cells) or to put a tube in the stomach (so we can inject nutrients). This will be done under general anaesthesia and we will reduce the pain caused as much as possible by using pain-relieving medications before and after surgery until animals recover. These are classed under moderate severity limit. At the end of experiment animals will be humanely killed. Brain and other tissues will be taken as we can examine them in the lab to gain even more knowledge.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal</p>	We need to use animals to understand how the brain is involved in feeding. To measure what is going on in the brain, we need to insert devices and so this cannot be done in humans. Also, it is impossible to work out how the stomach and

alternatives	other organs talk to the brain without studying it in a whole animal. Although some of our work now involves other animals like snails, as these animals are not that similar to humans, these new experiments cannot completely replace the use of mammals, like mice and rats.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We design our experiments so that they use the smallest number of animals possible. One way of doing this is by using maths to work out exactly how many should be needed to see an effect. Also, by looking at different measurements in the same animal, instead of using lots of different animals, we can reduce the numbers that we need.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We will use rats and mice in this project because their brains and other organs are similar to humans so what we find out will be useful in understanding feeding in people and possibly coming up with treatments for diseases such as obesity. We will try to keep animals comfortable during experiments by housing them in groups and providing things in the cage to play with and make nests.</p>

Project	Neural regulation of fertility	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Up to one quarter of human couples suffer from infertility and require medical help to have children. For most of these couples, the reason they are unable to have children is not known.</p> <p>The project aims to understand how a specific group of brain cells work together to control fertility. These cells regulate hormone levels in the blood that then control the activity of the ovary and testis.</p> <p>Previous research in animals and humans suggest that a population of brain cells making a chemical called "REDACTED" are important for fertility. The studies undertaken in this project aim to determine exactly how two different groups of REDACTED cells work to</p>	

	control hormone levels necessary for fertility in both male and female mice.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The studies in this project are expected to give us a better understanding of how REDACTED cells in the brain function to control fertility. This information is essential for us to understand how the brain may go wrong when individuals are infertile. The immediate benefit will be increased knowledge of how the brain works to control fertility. Longer-term benefits will be opportunities for making new treatments for controlling fertility in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will use adult mice, including genetically-modified animals. We expect to use approximately 8,000 animals over five years for breeding and up to 1,850 for experiments.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the animals used in this project will be used for breeding and experience no adverse effects. The next biggest group of animals will receive minor, non-harmful manipulations such as monitoring reproductive cycles (by examining vaginal secretions) and hormone levels (by blood sampling from the tail tip) and being given minor stresses such as being held still for periods of time. These manipulations have no long-lasting effect on the normal behaviour of the animals. Some animals in this group will have surgery to remove their ovaries or testes under anaesthesia followed by injections or implants of sex hormones. A smaller group of animals will have surgery under anaesthesia involving the placement of thin fibres into the brain with full recovery. These surgeries last 1-2 hours and require small holes to be drilled in the skull to allow very thin fibres (less than 0.5 mm in diameter) and microinfusion tubes to be placed into specific brain areas before everything is sealed. Pain relief medicine is given to all mice undergoing surgery. The fibers allow the activity of brain cells to be monitored while the tubes allow neurochemicals to be given into the brain of awake mice housed by themselves in their normal home conditions. The smallest group of mice in addition have a

	<p>tiny camera placed on their head for short periods of time to monitor cell activity in great detail. Although complications to such surgery are rare, the animals will be followed closely for signs of ill health, and if such complications were to occur and could not be promptly remedied, the animals would be humanely killed and their tissues collected for analysis. All animals will be humanely killed at the end of the experiment.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Animals are necessary for these experiments as we want to understand how the REDACTED brain cells work to control hormone levels and fertility in living animals. It is not ethically justified to use humans for these studies. Also, no computer model is currently available that can mirror the complexity of brain function or hormone control in the living animal. Previous attempts to use cell cultures to investigate how the brain controls fertility have failed but further developments in computer modelling and special culture conditions may hold promise.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will ensure that the minimum number of animals will be used by making sure that we are using the best experimental design and statistical methods.</p> <p>Wherever possible, we do experiments on cell lines or brain sections in dishes to reduce the number of experiments to be undertaken on animals.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are an excellent model to study how the brain controls fertility. The hormonal regulation of fertility appears to be similar between mice and humans but it is not understood how this actually works in any species. Working with mice also allows us to build upon research carried out over the last 40 years. Importantly, studies on REDACTED brain cells can only realistically be done in genetically-modified mice as this allows their DNA to be manipulated to enable the REDACTED cells to</p>

	<p>be seen and modified.</p> <p>Most experimental animals receive minor procedures such as monitoring of reproductive cycles and blood sampling before being anaesthetised and humanely killed.</p> <p>For animal experiments requiring surgery, all surgeries are undertaken with great care to avoid infection and complications such as prolonged bleeding and are followed by post-operative monitoring by scientists and animal technicians and given pain relief as recommended by the vet. These mice are accustomed to humans by regular handling with results being collected from mice living in their normal enriched home environment going about their daily activities.</p>
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Project	Neural stem cell mechanisms in health and disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>We are carrying out this work because it will advance scientific knowledge of how the brain develops in general. There are large gaps in our understanding of how stem cell self-renewal, progenitor cell proliferation, cell migration and functional cell maturation are controlled by various molecules. We will use this knowledge to lead us to the best molecular interventions for repair. It is also likely that knowledge gained from these studies will be important in other stem cell fields.</p> <p>We are investigating the molecular regulation of brain stem cell proliferation and differentiation into specialised brain cells. We are also</p>	

	<p>studying how brain stem cells and their progeny migrate to normal destinations during health and to ectopic areas of injury and disease. We seek to determine how to manipulate brain stem cells to improve repair of brain damage in models of injury and disease.</p> <p>The brain injury and disease models we plan to use include: neurological injury (traumatic brain injury), neurodegenerative disease (Alzheimer's), and</p> <p>neuropsychiatric disease (depression & schizophrenia).</p> <p>These diseases are devastating to personal lives causing loss of mobility, cognition, memory and emotional stability. They are long-term illnesses and incur enormous financial burdens on families and on the NHS. There are no current cures, thus we are carrying out this work because brain stem cells are one of the most promising avenues for treating brain disease and injury. Our goal is to augment stem cell mediated repair and functional recovery by discovering molecules that regulate stem cells.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>There is a pressing need for novel treatments for neurological disorders. Stem cells and regenerative medicine are one of the most promising avenues of work. However we must not rush into the clinic without a deep understanding of how they function. This programme of research will carry out fundamental studies to advance our scientific understanding of how our brain stem cells can be harnessed for repair.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>During the next 5 year period we have predicted use of up to 17,750 mice and 1,275 rats.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>A number of the proposed procedures are likely to cause some adverse effects in the animals. Firstly, induction of disease-like states in animals is likely to cause some harms. As such, other research groups have described seizures and increased mortality rate (up to 10%) in the J20 line of genetically modified mice, however</p>

	<p>we have not observed such effects in our experiments. Some of our protocols involve surgical procedures on the brain, such as models of traumatic brain injury in which small portions of the brain are removed, or injection of substances into the brain tissue. All such procedures will be performed under deep anaesthesia, accompanied by pre- and post-surgery provision of painkillers. We have not observed any long-lasting harms induced by these models. However, some short-term harms such as transient mild motor disturbances resulting in difficulty in feeding or watering could occur. This will be mitigated by providing easy access to food and water after the surgeries. We will terminate the animals when and if serious symptoms arise. We will also inject animals in the abdomen and under the skin with various molecules to study their function, which could cause some mild immediate distress and pain in the animal but does not cause any long-lasting effects. Some of the behavioural tests used in the protocols are likely to be stressful to animals and cause some levels of anxiety and fear. This is an unavoidable part of behavioral studies as aversive cues are needed to motivate learning and measure memory and cognitive functions. The most stressful cue we are proposing to use is the footshock, which can cause immediate discomfort to the animals but unlikely to induce any long-lasting harm. The number of exposures to aversive cues for each individual animal will be strictly controlled to avoid inducing any long-lasting stress. Some animals will be aged to study how stem cells can be beneficial during ageing. Aged mice may develop dermatitis, tumours, cataracts or dental problems. We will terminate the animals when and if serious symptoms arise.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>There are no computer models currently available that adequately mimic the complexity of brain anatomy, molecular regulation and development. We obviously cannot do functional experiments on human brain stem cells in vivo. However we can, and are, carrying out studies of human brain stem cells in post-mortem</p>

	<p>sections. These data are directly compared with our animal work, to help validate the latter.</p> <p>We are also working on human skin cell-derived human induced pluripotential stem cells. Pluripotential stem cells can give rise to a wide variety of mature cells. We use them to generate human nerve cells and they are used to understand human neuronal development and also to understand the molecular mechanisms of disease. This powerful approach will allow us to reduce the number of animals needed in our research.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We use mice because the number and variety of genetically altered (GA) strains far surpasses other mammalian species. GA mice allow us to discover molecular mechanisms that regulate stem cells. The complexity of human brain anatomy and pathology is such that we cannot use lower organisms (e.g. fruit flies) to adequately model it. We use statistical approaches to make sure the number of animals will be sufficient to reach significance. We have determined the minimum number of animals needed to obtain these scientific end points. Sound scientific technique is used to ensure we do not have to repeat experiments.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Our models of depression, schizophrenia and Alzheimer's disease are refined since they involve simple injections, or administration in the drinking water, of molecules that induce symptoms associated with the disease. To model AD we will also use genetically altered mice (J20's) that carry the same specific mutations in genes as some humans with the disease.</p> <p>We ensure that all animals receive optimal anaesthetics and post-surgery analgesia. We have endeavoured to reduce the suffering in each of the models of brain injury and disease.</p> <p>At least 48 hours will elapse between any steps involving general anesthesia and recovery. We will limit the maximum number of neurosurgical interventions to a maximum of 2 per animal, not including the final terminal procedure. A small subset of animals might undergo the maximum</p>

	amount of 5 periods of general anaesthesia.
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Project	Neurobehavioural Mechanisms of Mental Health	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our research aims to understand how and why mental health disorders occur, and to develop new treatments for people suffering from these disorders. Our work focuses on the psychological processes (e.g. attention, memory, compulsion) that are dysfunctional in a number of different mental health disorders, meaning that our work goes beyond individual disorders (i.e. it is “transdiagnostic”). Some aspects of our work, however, have greatest relevance to specific mental health disorders, including drug addiction, obsessive-compulsive disorder, schizophrenia and post-traumatic stress disorder. We use animal models that allow us to investigate dysfunctional psychological processing – developing new models	

	<p>if necessary – to understand the neurobiological causes and consequences of mental health disorders. Building on our previous work, our research aims to identify new drug targets and new forms of behavioural therapy that could treat mental health disorders. Some of our previous work is now beginning to be translated to humans.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Mental health problems cost the UK an estimated £105 billion per year, with 50% of these costs reflecting decreased quality of life for those affected. Thus, mental health disorders place a considerable burden on not only the affected individual, but also social and economic burdens on society. The case for new treatments is strong, as currently available therapies are not effective for all patients; for example, only 50% of those with post-traumatic stress disorder show a reduction in fear with cue exposure therapy. Our research aims to understand the bases of these disorders, and develop new treatments for them. We aim to develop new rodent models for mental health disorders that give us a better understanding into why certain behavioural or drug therapies work, to investigate why certain subpopulations are vulnerable to mental health disorders, and why they respond differently to treatment. We also aim to use these models to develop new and better treatments for mental health disorders.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We use the minimum numbers of animals possible to achieve biologically and statistically meaningful data. We anticipate that we will use approximately 8700 rats in 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The specific research questions that each experiment aims to address will determine the types of procedures that are experienced by the animals. Many of our research questions can be addressed by testing animals' memory and decision-making in sophisticated behavioural tasks rewarded with palatable food. Where we are attempting to modulate the psychological processes we are studying, we may give injections of specific types of drugs that affect activity in the brain; sometimes, we will give multiple injections, either with different doses of the same drug, or with drugs having different effects on the same chemical system (e.g. increasing or decreasing activity in</p>

that system). We only give multiple injections to the same animal where we need to be able to compare an individual animal's behaviour across these different conditions. Some of our research questions investigate the parts of the brain that are involved in these psychological processes. We target the parts of the brain that constitute the 'limbic corticostriatal circuitry'. For these experiments, we have to manipulate the brain directly by intracranial surgery (by e.g. surgically damaging specific parts or implanting recording devices). For our research into drug addiction, we have to implant the animals with intravenous catheters so that they can later self-administer drugs of abuse. This is critical for our experiments, as our addiction research studies the psychological processes that allows drug use to become compulsive (rather than dependence, which could be induced by experimenter-delivered injections of drugs). We need animals to be able to initiate their drug use in order to address our scientific questions, and to produce translational models that will be of maximum benefit to addicted patients. Whenever our animals undergo surgical procedures, they receive appropriate anaesthetics and painkillers around the time of the operation, and are very carefully monitored at the time of surgery and throughout the experiments for any signs of pain or distress. If the animals show signs of suffering and we are not able to ameliorate these in consultation with the Named Veterinary Surgeon, then we euthanise the animal. Fortunately, such instances are very rare. Some aspects of our research address disorders in which aversive learning plays a major role (e.g. phobia, or post-traumatic stress disorder). These disorders can only be studied by exposing animals to inescapable (uncontrollable) stressors, and in our experiments we use mild electric shocks as the aversive outcome. Many of our animals experience no more than three mild inescapable electric shocks in their lifetimes, and this is sufficient to allow us to study the psychological processes that underlie learning about stressful events. Electric shock is the most useful aversive outcome for our scientific purposes, because it allows us to precisely control the timing of cues predictive of an aversive outcome and the outcome itself (unlike more general stressors, such as exposure to the scent of predators) and because it engages the same brain circuitry as the mental

health disorders that we are studying (unlike, for example, air puffs to the eye, which engages reflexive circuitry with little relevance to our scientific questions). Animals that experience these inescapable stress conditions do not show changes in behaviour outside the environment in which the shock is delivered (e.g. changes in body weight or interaction with other animals). For some of our research addressing post-traumatic stress disorder, the animals are required to experience stronger stressors, and in these experiments they are exposed to up to 15 mild inescapable (uncontrollable) electric shocks in a single training session, which acts as a trauma analogue. This procedure leads to changes in the brains of animals that are relevant to post-traumatic stress disorder, and are necessary for us to understand changes in psychological processing that are relevant to the development of the disorder, and to developing new treatments. This procedure is also not effective for all animals (approximately 13% do not show changes in behaviour or brain changes), which allows us to study this 'resilient' population with the hope of identifying why they are effectively protected against post-traumatic stress disorder following stressful conditions. This is a well-established model of post-traumatic stress disorder and represents a refinement over some other models, but we will investigate further refinements to this procedure in parallel with our behavioural studies. Animals may experience more than one of these broad types of procedure in the course of an experiment, depending on the specific question that the research is addressing. Where we are specifically investigating vulnerability to mental health disorders, animals may undergo early life stressors (e.g. repeated intermittent maternal separation or social isolation) in addition to the procedures listed above. For questions relating to comorbidity of mental health disorders (e.g. between post-traumatic stress and addiction, for which comorbidity is estimated to be as high as 60% of patients) animals may undergo the more stressful aversive conditioning and later be implanted with catheters so that they can self-administer drugs of abuse. Thus, although animals may experience more than one type of procedure, for each animal we perform the minimum number of procedures that will allow us to address the specific scientific question we are investigating with

	that experiment. At the end of experiments, all animals are humanely killed, and wherever possible and appropriate we collect brain tissue for further in vitro analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This research is only possible with the use of animals. Human studies (e.g. brain imaging studies) are useful, but can only provide correlative data that do not address causation. Furthermore, it is not ethically possible to study the genetic and/or environmental factors that underlie predisposition to, and the development of, neuropsychiatric disorders in humans. Similarly, it would not be possible to develop new treatments for brain disorders without testing them in animal models first. In vitro models (e.g. brain slice preparations) or computer simulations cannot be used because the modelling of behaviour in these systems is not yet sufficiently advanced.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are fully committed to using the minimum number of animals required to obtain data that are statistically and biologically meaningful. We carefully design our experiments to maximise the behavioural data collected from each animal, and to minimise distress. We take replicability of our data very seriously, and routinely calculate effect sizes from pilot studies or previous literature to determine the minimum number of animals required for reliable data. We randomly allocate rats to experimental groups wherever possible, though sometimes rats are 'pseudorandomly' allocated (e.g. if we testing the effects of a particular treatment on a specific behaviour, rats are assigned to groups to ensure that their pre-treatment behaviour is the same). We also make use of automated software to collect behavioural data wherever possible, and where this is not the case (e.g. when behaviour has to be quantified by a person) we take great care to ensure that the person scoring is unaware of the experimental group allocations. We design our statistical analyses of the data in advance, and have extensive experience of this; additionally, when we are designing new studies that might require new analysis methods, we can refer to experts within

	our establishment to advise on this.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use rats because they are the least sentient species that can model neuropsychiatric disorders. The brain circuitry implicated in many mental health disorders is very similar between rodents and humans, and the behavioural tasks that we use are widely recognised as modelling specific aspects of these disorders. (Some of these tasks can be used in both humans and animals.) As stated above, one aspect of our work involves developing animal models of mental health disorders, and this includes refinement work where necessary. We also validate our manipulations in unconscious animals where necessary and possible. We take the welfare of the animals very seriously. Most of our animals are trained to perform sophisticated behavioural tasks, and any type of illness or pain would compromise their behaviour. Thus, we have scientific as well as ethical reasons to ensure high standards of welfare. When an experiment requires that animals undergo surgery, we conduct surgery to aseptic standards and provide pain relief during and after surgery. Animals are monitored frequently (often undergoing daily testing) and any adverse effects are observed by scientific and animal care staff, recorded and discussed with the Named Veterinary Surgeon. If these cannot be quickly ameliorated then animals are euthanised to prevent suffering. We have extensive experience of working with rats and we are well-trained in the clinical signs that mean an animal is unwell. If any animals show any signs consistent with brain damage following surgery, they are immediately killed to avoid suffering. If animals show other clinical signs such as subdued behaviour, piloerection or hunching, they are monitored closely and the Named Veterinary Surgeon will be consulted. If no improvement was shown within 24 hours, then the animal would be humanely killed.</p>

Project	Neurobiological basis of learned fear and its inhibition	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Anxiety-related disorders are serious psychiatric diseases associated with a huge economic and social burden. Although there are treatments available for these disorders, they can be limited, temporary, and/or have adverse side effects, highlighting the need for a better understanding of how such treatments work.</p> <p>Disorders such as phobias and post-traumatic stress are often characterized by persistent fear memory, even after treatment. Therefore understanding how fear memories influence behaviour may provide novel insight on the link between fear memory and certain symptoms of anxiety. Key to this is understanding how such</p>	

	<p>memories are encoded, retrieved, and inhibited by the brain. Sufferers of these disorders show abnormal cognition and emotional regulation. This is associated with dysfunction in certain inter-connected brain areas that normally perform these functions.</p> <p>This project will determine how these brain areas, along with certain brain chemicals important for regulating brain function, are involved in learned fear and its inhibition using rodent models that are translationally relevant to anxiety-related disorders. It will also determine if selected novel anti-anxiety or cognitive enhancing drugs reduce learned fear and/or enhance its inhibition by regulating the function of these brain areas and chemicals.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Determining the brain basis of learned fear and its inhibition will lead to a better understanding of the mechanistic links between persistent fear memory, dysfunction in the brain underlying cognition and emotional regulation, and anxiety-related disorders and their treatment. Determining if certain potential anti-anxiety drugs can reduce learned fear or enhance its inhibition will help to identify new leads for the development of novel or the repurposing of existing drugs for treating anxiety-related disorders.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Up to 2000 rats will be used over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Some animals will undergo recovery surgery to (1) implant electrodes into the brain to record neural activity in certain areas, (2) implant cannulae into the brain to infuse drugs directly into certain areas, and/or (3) infuse harmless viruses into the brain encoding Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) that inactivate neurons in certain areas when the animals are given the designer drug. Such animals will be anesthetized during surgery but will feel some pain afterwards for a limited time, which will be mitigated by peri- and post-operative analgesia. After recovery from surgery, animals will be trained to fear a specific cue or context by</p>

	<p>pairing them with mild electric shock. The shock does cause some transient pain but is necessary for the animals to acquire learned fear. The shock parameters (number, duration, intensity) will be the least severe that we can use while still ensuring that fear memory is demonstrable and replicable after training. High standards of animal health and welfare will be maintained during the experiments as minimizing pain and distress to the animals is a requirement not only due to ethical reasons but also for ensuring that the behavioural data obtained is consistent and reliable. All protocols used will be of moderate severity and all animals will be humanely culled after completing the experiments.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We will examine brain function directly by recording neural activity in relation to learned fear and its inhibition. We will also determine the effects of manipulating neural activity and the role of certain brain chemicals and potential new anti-anxiety drugs on behaviour and brain function. As this type of invasive work cannot be conducted in humans, we will use animals instead. We need to use live animals to investigate brain function and chemistry in relation to behaviour, therefore we cannot use <i>in vitro</i> methods. The use of computational modelling to understand brain function underlying behaviour is feasible and we collaborate with local colleagues using such <i>in silico</i> methods. This contributes to replacement, although these computational models cannot fully replace the use of animals until we better understand the underlying neurobiological processes involved. We will also use complementary <i>ex vivo</i> methods to measure the levels of certain brain chemicals in relation to learned fear and its inhibition in this work.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>Power analysis and statistical power from our previous studies will be used to design the experiments and the data will be analyzed using the appropriate statistical tests to ensure that the minimum numbers of animals are used in these studies. We will use newer behavioural analysis methods (i.e. automated scoring) to reduce potential bias. We will also use newer</p>

	<p>electrophysiology methods of assessing activity in more than one brain area. Both of these aspects will further reduce the number of animals used.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rats will be used given the wealth of evidence showing that homologous brain areas in this species and humans mediate learned fear and its inhibition. Surgical procedures will be conducted aseptically under general anesthesia using peri- and post-operative analgesia to minimize pain and the risk of acquiring infection after surgery. We need to use mild electric shock for the animals to acquire fear memory but the least severe shock parameters will be used that support fear memory in a consistent and replicable manner. We have recently refined various procedures in our work to improve animal welfare (e.g. use of IVC cages, group housing animals after brain implant surgery) and to reduce potential bias with analyzing behavioural data (i.e. automated scoring). We also plan to record brain activity under anesthesia in some animals, which is a refinement on conducting such recordings in conscious animals. We will continue to implement such refinements in our work.</p>

Project	Neurobiology of emotional and cognitive impairments in psychiatric disorders
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	Regulatory use and routine production
	Protection of the natural environment in the interests of the health or welfare of humans or animals
	Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project aims to further our knowledge about the way the brain functions to control emotional behaviour and cognition, so that new improved treatments for mood disorders can be developed. Our project uses animals to model specific aspects of human psychiatric disorders and their symptoms.</p> <p>Specifically, this project will:</p> <ol style="list-style-type: none"> 1. Investigate how mood disorders develop and the underlying biology. This work will help to explain why some people get depressed and

	<p>how treatments might be improved in the future.</p> <p>2. Investigate the co-morbidity of emotional and cognitive symptoms in chronic illnesses. We will try to find out why so many patients with long term health issues also suffer with diseases like depression.</p> <p>3. Develop better methods for studying psychiatric disorders in animals and check that these can accurately mirror aspects of the human condition.</p> <p>4. Assess the welfare of animals in response to laboratory stress to develop more refined methods.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The immediate benefit of the work will be to the wider scientific community, whilst in the longer term the work is expected to lead to improvements in clinical treatment. The majority of studies will use a behavioural measure where the animal is trained to perform a task to obtain a food reward. These behaviours are then shaped so that the animal has to follow a specific rule and use cognitive and/or emotional processes which we can relate to similar experiments in humans. This will provide information which can be used to advance our understanding of how the brain processes emotional and cognitive information, and the mechanisms which regulate these processes under normal conditions and when they go wrong in a disease. As so little is currently known about the relationship between the biological processes in the brain and what these mean in terms of psychological effects i.e feeling sad, the work will provide important scientific advances. In the longer term, the knowledge gained from this work should provide novel drug targets and methods to better treat psychiatric symptoms. Specific benefits and beneficiaries include: Neuroscientists (academia and industry, short term) through knowledge gain Animal behaviour scientists and animal welfare researchers (short term) through validation of improved methodologies and refined techniques Patients, psychiatrists and health care workers providing patient care (medium and long term) through improved understanding of disease</p>

	<p>biology and better treatments. Public understanding of depressive illness and mental wellbeing by raising public perception that psychiatric symptoms are part of a biological illness.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Rats ~ 2200 over 5 years, Mice ~1600 over the 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The animals used in the licence will primarily be used in reward-based behavioural tasks for which a licence is not needed but, they are used in combination with method which can cause pain suffering or lasting harm. For most animals, their experience will involve mild procedures such as injection and exposure to short term stressors. None of the procedures used in the awake animal will cause more than transient pain however, animals may be exposed to multiple stressors or repeated drug administration which can contribute to cumulative suffering. To monitor the impact of the treatments, animals are checked regularly for any signs of the development of abnormal behaviours or evidence of stereotypic behaviours. As most animals (~90%) are tested in behavioural tasks involving reward, we can closely monitor wellbeing and expect animals to only show subtle changes in their behaviour, which can only be detected at a group level. If any animal shows signs of below normal task performance they will be reviewed and the vet contacted. Some animals may be exposed to aversive training methods so that behavioural responses to negative emotions can also be investigated. This will affect only a small number of animals used in the overall programme and will use aversive noise or air puff where possible. If footshock is needed, the intensity will be kept below the level that induces fear and freezing behaviour and animals will normally be able to escape by pressing a lever or moving to another part of the cage. For some experiments, surgical interventions are required to alter the function of a specific part of the brain or to enable stimulation. This will involve surgery with recovery and will therefore be moderate severity. All animals will be given appropriate pre- and/or</p>

	<p>post-operative analgesia and careful intra-operative and post-operative care. At the end of the experiments all animals will be killed. This may be carried out as part of the protocol to permit the collection of tissue for post-mortem analysis or using a Schedule 1 method.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>These studies require an intact living brain in order to achieve the objectives. In order that the work is directly translatable to the human brain, a mammalian species is also necessary. The majority of the studies described will use rats, as they are the most appropriate species for the achievement of the objectives. Mice are the species of choice for genetic studies so some work in this project will use both mice and rats. A small number of neonatal animals are included in this project. These are animals that will undergo procedures during the pre-weaning period to induce long-term changes in the adult brain. This is necessary as many psychiatric disorders have been linked to insults experienced in early life including pre-birth.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>Each of the experiments proposed have been carefully designed to achieve the project objectives whilst ensuring that the appropriate numbers of animals are used to achieve statistical power and validity to the data generated. We have now generated more than 10 years of data using these methods so can accurately predict the variability of the data associated with any given method as well as a meaningful effect size. These factors are included in power calculations such that any subsequent experiment uses the lowest number of animals.</p> <p>The animal numbers over the course of this licence are based on power estimates, our current funding and projections for future funding over the next 5 years. We also work to an ~80% success rate as not all animals will successfully train in the tasks and reach criterion for inclusion in the experiment.</p>

3. Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The behavioural tests primarily use positive reinforcement and punishment is achieved through a 'time out' procedure where the animal is unable to obtain an outcome to its responses during this period. In a small number of behavioural tests, an aversive stimulus is used. Mild aversive/stressful stimuli may also be used to manipulate the emotional state of the animals, something which is also necessary to achieve the project objectives. We have refined the techniques to use the minimum level of aversive stimuli necessary to trigger the behavioural outcome we are investigating. This has also meant we have developed husbandry and procedural techniques which reduce stress in control animals helping also to refine the stress/aversion needed for the induction of a depression-like change in the animal.

The surgical interventions use methods which do not cause overt changes in behaviour and post-operative analgesia is provided. Within 48 hrs post-surgery, animals return to normal and are usually group housed.

When we induce a model of disease we will use the least invasive approaches and we focus on social or environmental manipulations or treatments with pharmacological agents. These procedures compare well with factors that have been shown to influence human psychiatric conditions and also induce changes in behaviour that reflect depressive-like and/or anxiety-like behaviour.

In all the protocols, animals may be exposed to mild stressors and/or receive drug treatments which may involve mild discomfort during administration. Animals will be habituated to handling and dosing procedures, and monitored to ensure they are not developing any sensitisation to the procedure. Control animals will also be housed and handled using methods which optimise welfare.

Endpoints: When animals exhibit signs of poor performance in tasks, this is used as an early indication that they are showing signs of ill health and will be discussed with the animal care staff and/or vet and animals removed from the

	<p>experiment and killed if they do not return to normal behaviour. To monitor the impact of cumulative suffering, animals will be routinely monitored for body condition, weight and the presence of abnormal or stereotypic behaviours including aversion to handling. Together, these measures provide endpoints which are used to limit the overall severity of the protocols.</p>
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Project	Neurochemical effects of prenatal ethanol exposure	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Foetal Alcohol Spectrum Disorder (FASD) is a condition that affects approximately 1% of all babies born in the UK. It is caused by the unborn baby being exposed to alcohol during pregnancy and its severity can range from behaviour and learning difficulties at school to more severe impaired growth and development with marked problems with behaviour. It is not known what are 'safe limits' for alcohol during pregnancy or at what stages of pregnancy alcohol presents the greatest risk, but animal studies suggest that 'binge drinking' during early pregnancy increases the risk of severe problems like growth impairment whilst drinking the equivalent of two large glasses of table wine per day during late pregnancy can cause problems with behaviour, learning and memory.</p> <p>The problem of FASD cannot be controlled by health education alone. At least half of all pregnancies are</p>	

	<p>unplanned and it is common for women to continue drinking alcohol before they are aware that they are pregnant and 3% of UK women have problems with alcohol dependence. 4-5% of women continue to drink 3 - 14 units of alcohol per week throughout pregnancy.</p> <p>There are currently no drug treatments to prevent or reverse the effects of alcohol on the unborn baby. There are some therapies that help overcome the changes caused by the alcohol, but their success depends on successful diagnosis. FASD shares many symptoms with other conditions such as attention deficit hyperactivity disorder (ADHD) and autistic spectrum disorders (ASD), but the treatments differ. For a child to be diagnosed with FASD the doctor needs evidence that there was exposure to alcohol before birth. This requires, for example, the mother of a 6-year old child to report having drunk alcohol during pregnancy over six years ago. In many cases the mother and child have become separated by the time FASD is suspected. Because the doctor is unable to get reliable evidence that the baby was exposed to alcohol before birth, a high proportion of FASD children are misdiagnosed as either ADHD or ASD, and therefore do not receive the best therapies.</p> <p>The aim of this project is to use a mouse model of FASD to look at the biochemical changes caused by alcohol.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>We believe that we might detect changes that can still be seen in young adults. Such a finding would allow the development of a test for use in young children to see if there is a likelihood that the child was affected by alcohol before birth. The results of such a test would allow more confident diagnosis of FASD and would mean that affected children would get the most appropriate treatment. Our research will also tell us whether the biochemical changes caused by the prenatal alcohol might be corrected by drugs. For example, it will tell us whether drugs currently used to improve memory in old people might also be useful to improve learning and memory in children with FASD. The final aim of this research is to explore whether the changes caused by alcohol exposure before birth can be passed on to future generations. This would tell us whether the results of our proposed test for prenatal alcohol exposure could be affected if the mother or father suffered from FASD.</p>
<p>What species and approximate numbers of animals do you expect to use over what</p>	<p>Approval is sought to use 60 mice (20 males and 40 females) over 5 years. These breeding colonies will be used to generate 500 'FASD' mice. A further 200 normal mice will be used as controls</p>

period of time?	
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The mouse model to be used involves pairs of male and female mice being given alcohol in their drinking water. The strength of alcohol given is equivalent to lager, and the maximum blood alcohol levels reached are equivalent to the drink-driving limit in the UK (80mg/dL). Levels fall to zero when the mice have been sleeping. The mice do not get 'drunk'. The mothers receive the alcohol until the young mice are weaned. After the young are weaned, they are switched to plain drinking water and allowed to develop normally. We know that this low dose of alcohol has no adverse effects on the mice, there are no symptoms of distress or impairment, and we know that the mothers are as successful at raising their litters as normal mice. We detect only very subtle changes in behaviour and learning when we test the 'FASD' mice. The tests that we use do not cause pain or distress. The first test simply records the movements of a single mouse in a quiet, empty cage that it has never encountered before. We record how far it travels while exploring the area. The second test involves placing mice on a raised runway, half of which has high walls and the other half is open. The mice are initially more comfortable on the walled runway but quickly gain sufficient confidence to explore the open arms. The amount of time spent in the closed portion is a measure of anxiety. The final test involves mice exploring two identical objects made from plastic building bricks. One day later they get to explore one of the familiar objects together with a novel object made of different sized, different coloured bricks. The mice typically spend longer exploring the new object, which is a measure that they remember the familiar object. These tests allow us to assess anxiety and learning and memory. As part of the experiments the mice might receive drugs via their food or drinking water or by injection. Only drugs known to be side-effect free at the doses used are selected and mice are not unduly disturbed by a single injection. All animals are killed humanely for the collection of tissues at the end of the experiments.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>In all of our experiments we only use animals when no suitable alternatives exist and we aim to progress our studies into humans as soon as possible.</p> <p>We have previously studied the effects of prolonged alcohol exposure on enzymes in cultured cell lines. The results demonstrated a tendency to cause movement of the enzyme</p>

	<p>away from the cell membrane into intracellular vacuoles. Such studies, however cannot predict the effects of prenatal alcohol exposure on neuronal cells in a developing foetus with maintained homeostasis. Furthermore, cell culture techniques cannot be used to fully explain the effects of ethanol exposure on future complex behaviours and cognition.</p> <p>In the case of the effects of alcohol on a developing organism and the effects months later in an adult, it is not possible to use cell culture models or computer modelling.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We are always careful to calculate the lowest possible number of animals required for the study so that the results are reliable. We are able to conduct experiments on this low sample size as we are very careful to breed and maintain animals under standardised, controlled, conditions so that variability of the animals' experiences is kept to a minimum and thus variability of behaviour is minimalised. These processes improve reproducibility, negate the need to repeat experiments and reduce animal numbers by decreasing the effects of confounding factors on behaviour.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The behavioural tests used cause no distress. The training of our researchers ensure that the animals have the highest possible standards of welfare.</p>

Project	Neurodegeneration: the role of macrophages and microglia	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	A great deal of knowledge has been acquired recently on the types of genetic alteration that promote the development of neurodegenerative conditions like Alzheimer's disease. However, an understanding of how genes and pathways lead to the development of disease is lacking, as is a fundamental understanding of how the diseases develop. The overall aim of this project is to further understand how genes associated with immune and related pathways (genes of interest) are associated with and contribute to neurodegenerative diseases. We will address this by: studying the cell types involved in the disease process; how the genes of interest contribute to the function of those cells both during disease and	

	<p>development; and whether these insights identify novel therapeutic opportunities</p> <p>The key elements of this project licence are to:</p> <ol style="list-style-type: none"> 1. To identify inflammatory and neurological cell types involved in pathological processes of neurodegeneration. 2. To determine how the genes of interest and their pathways influence specific cell functions 3. To determine how the genes of interest and their pathways influence the development of disease processes and contribute to the function of normal and disease tissue. 4. To establish if the genes of interest and pathways influence the development of the CNS (including cell types) and/or specific functional aspects of the brain. 5. To test the efficacy of novel therapeutic interventions in neurodegenerative or inflammatory contexts.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>A greater understanding of how alterations in patient genes lead to neurodegenerative conditions via altered cellular function and brain composition will lead to the development of novel and potentially effective therapeutic interventions.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mouse, 10,000 (largely through complex breeding programmes) over the course of the project.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Many of the experiments will be mild in severity, in that they will involve aging of mice with a natural predisposition to develop neurodegenerative conditions. The mice may show the beginnings of cognitive impairment (after about 6 months), but the majority will not be retained into these later stages of disease. In the main, mice with defined genetic alterations associated with neurodegenerative disease will be examined for changes in cellular function and development in the context of the whole</p>

	<p>tissue in health in otherwise normal mice and in disease prone mice. Additional stimuli will be used, for example, to evoke a self-resolving transient inflammatory response, which will be studied with various interventions to understand how such challenge influence disease. Some procedures will involve stimuli of moderate severity, where clinical signs of adverse effects will be more evident or longer lasting. In some cases we will study mouse models of spontaneous chronic disease development, to understand how they develop and how we may intervene. In all cases, the presence of adverse effects is specifically monitored for and in most cases mice will be killed if exhibiting evidence of such effects.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Wherever possible we have developed <i>in vitro</i> systems for the addressing our specific questions, for example producing our own cell lines as a direct replacement. Cell lines do not display all the characteristics of the cells found a living animal and whilst cell culture techniques have dramatically improved, the distribution of multiple cell types and the presence of many diverse cell:cell interactions cannot be replicated <i>in vitro</i>.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>As part of our standard procedures, every experiment involves, prior to commencement, an assessment of the design. This including statistical analysis or equivalent where possible of the number to be used. This ensures the correct numbers of animals are used to be able to have a realistic chance to address the scientific question.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>A mammalian species must be used because of the complexity of biological systems studied. Mice are also the most appropriate species because of the advantages of genetic manipulation in mice. We refine our procedures by titrating doses, administering substances by the least adverse methods (for example, using pipette feeding instead of oral gavage) and using low-dose challenges first. We will add to this by, for example, exploring the use of implanted minipumps as an alternative to repeated injections. We also use animal welfare scoring systems with 'humane experimental end-</p>

	<p>points' to limit any suffering and risk of adverse effects. In cases of surgery, we will use anaesthesia and analgesia as appropriate. The neurodegenerative disease models studied have the potential to cause serious implications, but we use defined experimental end-points to prevent unnecessary suffering. The majority of animals studied under this project will be used in breeding programmes, others will be aged for the development of pathology, but experiments will be mostly be terminated before development of symptoms that affect the quality of life. However, a smaller group of animals will be aged to later stages of disease, which may begin to affect quality of life and in these cases they will be monitored carefully for signs of such deterioration to minimise any suffering. We may also induce disease by administration of substance. In these cases we will monitor welfare and use end-points to limit adverse effects.</p>
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Project	Neurodegeneration: understanding the causes and investigating therapeutic mechanisms in zebrafish	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Many of the neurodegenerative diseases affecting humans (such as Alzheimer's disease and Parkinson's disease) are caused by proteins that form clumps inside nerve cells. These diseases are devastating to both the patients and their families and are currently incurable. The build-up of these proteins is like a build-up of rubbish inside cells. We work on a naturally occurring process which occurs inside cells where they are able to "eat up" rubbish. This process is called autophagy. We have shown that we can speed-up autophagy and this helps the cells clear up the build-up of these harmful clumps of	

	<p>proteins. However, speeding up this process might not work for all patients or for all types of neurodegenerative disease. There are likely to be other ways to treat neurodegenerative disease but we need to test lots of possible different compounds (medicines) or test different genes to find new treatments. We do this by testing large collections of compounds or performing genetic screens (removing one gene at a time to find the genes which make the disease better when they are removed).</p> <p>There are three main aims of this project:</p> <ol style="list-style-type: none"> 1. What is the best way to speed-up autophagy (and hence the clearance of clumps of protein) and is that safe if we do it in all the cells in the body? 2. We have already found ~8 genes and compounds that make the disease better in our models. We need to find out how they work and whether they will be safe in humans. 3. We will identify new ways of treating neurodegeneration by testing compound collections and performing genetic screens.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>At present, there are no known treatments that slow down or reduce the severity of neurodegenerative diseases. These commonly affect elderly people (such as Parkinson's and Alzheimer's disease) but in rare cases, can also affect children (for example, Multiple Sulfatase Disease, which is a rare neurodegenerative disease which occurs in children). This project will help to find compounds which slow the disease down and that do not have side-effects. This will help companies which make medicines to develop new ones to test in patients.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This project will use zebrafish. We estimate that we will use ~80,000 zebrafish over the 5 years of this project (approximately 16,000 per year).</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the</p>	<p>Most of the procedures carried out in this work are unlikely to cause any pain or suffering (these are termed "mild" procedures). There are some procedures which involve the use of genetically altered strains of fish which develop the early stages of disease (termed "moderate" procedures). In some procedures, the fish are anaesthetised and on rare</p>

end?	occasions the fish do not recover from anaesthesia and so will be culled by a humane method. The other expected adverse effect results from the immobilisation of juvenile fish to allow us to perform microscopic observations. On rare occasions, bruising or damage occurs while getting the fish into the correct position. If this happens, the fish will be killed immediately by a humane method. The second type of moderate procedure is used to find out the amount of drug that it is safe to give without causing harmful effects. At high concentrations of drugs, some fish will show signs of toxicity, such as increased heart rate or failure to swim in the correct position. Fish will be killed by a humane method as soon as any toxic effect is observed. There is one protocol on the licence where we will need to perform studies on animals with signs of disease to determine when the disease pathology occurs and how rapidly it gets worse. In some cases, this protocol will be used in combination with drug treatments to determine how long the drug is effective for. This is defined as a “moderate” procedure. All animals will be humanely killed at the end of procedures.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We use cells grown in the lab for much of our work. However, to understand biological processes in diseases that affect tissues like the brain, we need to study these processes in living animals. We use zebrafish as they have a high level of genetic, tissue and pharmacological similarity with mammals (humans and mice). We are one of the main groups pioneering the use of zebrafish models to reduce and replace the use of mammalian (mouse) models on our research. We need to be able to assess pathology in non-dividing nerve cells within a living brain, with the appropriate connections. When testing compounds which might be suitable as treatments for neurodegenerative disease, we need to examine possible side effects in all body tissues and to test whether these therapeutics can get to the tissue we are trying to treat (e.g. can compounds get into the brain).</p>
<p>2. Reduction</p> <p>Explain how you will assure the</p>	<p>We have considerable experience in developing zebrafish models of human disease. We do this by making animals which have an extra gene and this</p>

<p>use of minimum numbers of animals</p>	<p>gene causes the disease – these are called transgenic animals. We have developed assays in zebrafish which have short duration times (typically 5-8 days long). We have also tested what is the smallest group size we can use to obtain meaningful and statistically significant results (typically 5-10 animals per treatment). All our new transgenic fish are generated with a coloured marker to allow us to genotype the animals (e.g. green colour in the heart, red colour in the eyes). This can be seen when we use a microscope and we can identify the transgenic (coloured) fish at 1 or 2 days old when they are still in the chorion (the transparent egg shell) and therefore do not need to be anaesthetised. We can select and grow up only the transgenic offspring and therefore do not raise wildtype siblings that are not used. This means we do not need to cull adult animals because we only grow the transgenic ones and so has reduced the number of animals we need to cull by 50%.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Zebrafish are small tropical fish that have many advantages as an animal model for this work. For example, one pair of adults produces 100-200 fertilised eggs per breeding and young fish are transparent allowing internal organs such as the brain to be seen without surgery. Also, there is a high level of similarity between the genes and tissues in man and other vertebrates. We have carefully developed genetically modified zebrafish which have aspects of human disease but in which we have limited the severity of the disease, e.g. by expressing the disease-causing gene in only one cell type in the eye. When these cells degenerate, only this one cell type dies (and then the fish cannot see well in the dark but have normal vision in daylight). We also use a special technique (Gal4/UAS transgenic technology) so that parent lines do not express disease-causing proteins and do not have any signs of disease. The disease protein is only expressed the when two carrier fish are mated together. Following mating, only the offspring produce the disease protein and show disease pathology. Using such lines, we only generate offspring for experimental purposes and the adult animals that are kept to maintain a breeding colony are viable and healthy, with no sign of disease.</p> <p>Zebrafish are a social species - we have refined our protocols to reduce the number of fish which are</p>

	<p>kept in single tanks (for example, when identifying transgenic founders) by housing these with wildtype fish with a different pigmentation pattern (e.g. if transgenic fish is “stripy”, it can be housed with “spotty” wildtype fish). For adult fish in the aquarium, we will use plastic plants to enrich their environment.</p>
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Project	Neuroendocrine mechanisms regulating reproductive physiology and disease
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research <input type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Objective 1: A brain hormone called REDACTED (REDACTED) has been found to regulate puberty and fertility. This is demonstrated since mice and humans in which the gene is inactivated display arrested puberty and infertility. However, REDACTED is present in distinct populations of cells throughout the brain, which appear to have unique roles in controlling reproductive functions. Thus, the primary aim of this project is to further characterise how each population of REDACTED cells is connected throughout the brain and is regulated to control reproduction and fertility.

	<p>Objective 2: More recently it has been suggested that gene mutations in hypothalamic proteins (e.g. REDACTED) can cause pubertal disorders by misregulating the functions of REDACTED cells in children. Importantly, altered REDACTED expression has been associated with either triggering early puberty or delaying puberty, making it a crucial setpoint for puberty. We aim to determine how these proteins interact with REDACTED cells to control puberty onset.</p> <p>Objective 3: It has been identified that women are nearly twice as likely to be diagnosed with anxiety disorders than men. The sex-difference in anxiety disorders begins at puberty, where sex hormones are thought to play a role. A region of the brain called the medial amygdala contains REDACTED cells which are sensitive to sex hormones. We have recently shown that activating these cells can increase circulating sex hormones in male mice but not females. Previous studies have shown that steroid treatments are protective against anxiety-related behaviours. So, the third aim is to determine how this population of REDACTED cells influence sexual, social and affective behaviours differently between the sexes and if these neurons protect males from anxiety-related behaviours.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The prevalence of infertility in developed countries is becoming an increasing problem with 15-20% of couples in Western Europe experiencing infertility. Increasingly, infertility is linked to impaired hormone secretion from the brain. In addition to impacting fertility, reduced levels of reproductive hormones are linked to depression, loss of sexual drive and metabolic syndrome. These studies will be 'blue-sky' basic research and are designed to improve our fundamental understanding of how brain signalling networks control puberty, reproduction and behaviour at the cellular level. These studies are not designed to affect human care directly but will increase our understanding of the underlying biology and pharmacology which will underpin the development of therapeutic strategies targeted at pubertal, reproductive and mood disorders or to diagnose patients with hypothalamic disorders.</p>

<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The proposed studies will use rodents (mice) because they are considered to be the least 'neurophysiologically sensitive' mammals that still possess a high degree of functional similarity to humans. In addition, mice have a highly characterised reproductive axis and the ability to genetically alter the mouse germ line is superior to other mammals. In addition, there is a greater availability of probes and antibodies that enable us to accurately identify relevant cellular relationships. This project will use laboratory mice. It is expected that 3000 animals will be used over the 5 year period, however before any experiment is finalised, data from similar experiments (published literature or previous work done by our group) will allow us to determine exact numbers.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Our protocols will be of mild to moderate level of severity. Animals in the moderate severity band will undergo surgery to manipulate the brain and reproductive system. Brain surgeries (1-2 hours) typically are used to make very small holes in the skull and introduce non-toxic substances or to place small probes (no longer than 5 mm) to sample or active brain circuits. Following this everything is held in place with small screws/dental cement, and incisions are sealed with surgical glue. Gonadal surgeries (less than 1 hour) typically consist of removal of the ovaries or testes after which everything is held together using sutures or staples. In addition, we may insert capsules under the skin that release hormones slowly. Although, these surgeries are classified as moderate severity, most animals normally recover within a few days post-surgery. Animals will be given painkillers and post-operative care just like people recovering in hospital. We expect only a few adverse effects however the most prominent will be decreased in body weight due to poor recovery from surgery. Surgery will be performed following accepted guidelines for surgery/post-surgical care, anaesthesia and analgesia to minimize the risk of infection. Substances administered are not expected to cause any harm or discomfort on their own and to avoid causing anaemia in animals, blood volume will be drawn according to accepted guidelines. Bodyweight will always be monitored as well as general behaviour (changes in</p>

	<p>coat/activity and pain severity using the mouse grimace scale). Weight loss is limited to a maximum of 15% of an adult mouse's free-feeding weight in age, sex- and strain-matched controls. Humane endpoints will be used to ensure the adverse effects do not go beyond the minimum required to achieve the scientific objectives. END POINT: Weight loss more than that stipulated then the animal will be euthanized by an appropriate humane method. Animals will be killed by a humane method and tissues taken for analysis after death.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The brain signals governing the control of reproduction are highly complex, often involving interactions with multiple cell types in their microenvironment, and regulation by both cell-cell contact and secreted factors, which cannot be effectively evaluated by the use of cells/tissue in a culture dish. Our hypotheses must be tested and refined in models where the complex environment of the intact brain and reproductive axis are present. We currently do not have the ability to reproduce these conditions outside an organism. However, we have recently begun in vitro experiments using new immortalized REDACTED cell lines to test if we can use them to replace or augment some of our study objectives, but our pilot studies indicate that some of these cell lines do not maintain their characteristics over time.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Reduction of animal use is built into the design of this project at several levels.</p> <ul style="list-style-type: none"> ● The minimum of animals will be used; experimental group sizes will be determined based on experience, previous experiments and pilot studies using power calculations whenever possible with reference to statistical readouts so that the number of animals is sufficient to achieve statistically significant results. ● We will also reduce the number of animals required by employing multiplexed analysis combined with highly sensitive and information-rich detection techniques to

	<p>maximise the amount of information extracted from test samples.</p> <p>Experiments will be designed that where possible an animal can act as its own control.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse is the species of choice for these studies due to its highly characterized reproductive axis and the high degree of functional similarity to humans. The ability to genetically alter the mouse germ line is superior to other mammals and the availability of probes and antibodies enable us to accurately identify relevant cellular relationships. In particular, we will use animal models that accurately reflect characteristics observed in human patients, so that the observations obtained will be more precise and provide more relevant information about human disease.</p> <p>By using more refined models we will limit off-target effects that often disturb the wellbeing of the animal and confound data analysis. To minimize suffering we will have developed precise protocols to interrogate specific brain cell populations using vectors, which are not known to cause any adverse health effects.</p> <p>My considerable experience in conducting studies with rodents has led to effective and safe procedures, thus minimizing animal suffering, distress and long-lasting harm. Any staff will be trained to be competent in the appropriate surgical techniques and be able to identify adverse effects/behaviour.</p> <p>Non-schedule 1 methods of killing are required in order to obtain tissue of sufficient quality to obtain scientific outputs.</p>

Project	Neuroimmunity in obesity	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Obesity is an unmet medical need. Our project aims to understand the crosstalk between the immune system and the nervous system, and how it controls, physiology, fat mass and metabolism.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Nearly one third of adults in the UK are obese (https://files.digital.nhs.uk/publication/0/0/obes-phys-acti-diet-eng-2018-rep.pdf), yet no safe medications are available to patients. The health burden of obesity is overwhelming: diabetes, high blood pressure (hypertension), heart disease, stroke, nerve damage, kidney disease, blindness, limb amputation, among others — all contribute to the reduced quality of life and reduced life expectancy experienced by people living with this disease. In addition, the financial burden on healthcare systems is significant: £10billion each year (10% of the	

	<p>annual NHS budget) is spent only managing diabetes (www.diabetes.org.uk), let alone the financial burden of all other diseases associated with obesity. Finally, the loss of productivity in the workforce due to ill health or social stigma of obese patients is substantial. As the global population is facing an obesity and associated diabetes epidemic, research into this area is a medical, economic and ethical priority. For all of the aforementioned reasons, my work programme will pave the way to the development of a new generation of anti-obesity medications through the identification of druggable molecular and cellular mechanisms involving neuroimmune interactions that control fat mass.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>20,000 mice over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Mice will undergo procedures to test their ability to control body weight and metabolism. Most of the tests are regarded as of mild severity, as they involve little more than a single injection and several small blood samples being drawn from the tail. The tests listed have been refined over many years to cause the least disturbance to the mouse possible, whilst gaining suitably robust data to answer our research questions. It is important to remember that stress or pain will impact metabolism and subsequently confound our experimental data sets: therefore, there is a strong scientific as well as ethical rationale for us to avoid inducing stress or pain. Furthermore, our breeding strategy and mouse lines exhibit mild (if any) adverse effects. Where multiple tests are required upon the same mouse, sufficient recovery time between tests will be allowed. At the end of the protocol all mice will be culled by schedule 1 or terminal procedure (under appropriate general/terminal anaesthesia).</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>It is essential that our research project is carried out using mice, as there is simply no alternative. Adipose (fat) homeostasis, food intake, hormone secretion, action, stability and clearance are regulated by a myriad of circulating factors (each with their own pattern of systemic regulation) and the contributions of multiple organ</p>

	<p>systems, which cannot possibly be recreated <i>in vitro</i>: therefore, these processes must be investigated in a living mammal to ensure our results are physiologically relevant.</p> <p>It is neither practical nor ethical to conduct these interventional experiments in human volunteers and we have opted to study mice (see reasons under refinement below). Notwithstanding, we will always replace <i>in vivo</i> experiments with <i>in vitro</i> experiments where possible, such as testing expression or basic function of a gene/protein or optimising drug dosage. These data will then inform the subsequent <i>in vivo</i> testing which will establish physiological relevance.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Mouse numbers used in experimental protocols will be reduced to the minimum required to achieve our experimental goals. We have taken a number of steps to achieve this:</p> <ol style="list-style-type: none"> 1. Ensuring technical competency of researchers 2. Appropriate experimental design and power. 3. Using rigorous and robust controls: 4. Efficient and appropriate breeding strategies. 5. Systematic tissue collection and banking (for future lab projects). 6. Sharing of samples, resources, models and data.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We have opted to study mice due to 1) they are a good model of human metabolism and endocrine function; 2) there is a wealth of existing data on mouse physiology; 3) there are a large number of genetic tools available; 4) mice breed readily and quickly.</p> <p>Our mouse models and techniques have been selected as they: (i) are relevant to understanding human metabolic disease (e.g. obesity , diabetes, etc.); (ii) can be used to address basic biological questions about the normal regulation of whole-body metabolism, body weight , blood glucose, hormone/neurotransmitter secretion, and immune homeostasis; (lii) have the least impact on the animal's welfare, compatible with our scientific objectives.</p> <p>Our protocols have been refined over many years, ensuring that studies will be conducted with the least disturbance to the mouse whilst gaining suitably robust data to answer our research question. Furthermore, all personnel will undergo exhaustive training prior to</p>

	independent conduction of experiments.
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Project	Neuronal circuitry of the spinal dorsal horn	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	<input type="checkbox"/> Basic research <input type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Although the spinal cord plays an important role in transmitting and modulating sensory information that is perceived as pain and itch, we still know relatively little about how this information is processed at the spinal level. The objectives of the project are to identify and characterise functional populations of nerve cells in the spinal cord that are involved in pain and itch, to establish how they are organised into nerve circuits that either increase or decrease these sensations, and to determine how they contribute to pathological pain after nerve injury.</p>	
What are the potential benefits likely	There is a lack of effective treatments for both	

<p>to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>neuropathic pain and chronic itch, and these therefore represent major unmet clinical needs. Understanding the nerve circuits that convey pain and itch, and characterising the different cells that are involved, is likely to lead to the recognition of potential targets for new analgesic and anti-pruritic treatments. In addition, understanding changes that occur in the spinal cord following nerve injury is necessary if we are to develop improved treatments for neuropathic pain.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>These experiments will be performed on mice, because of the availability of genetically-modified animals that allow specific types of nerve cell to be identified and have their functions altered. Approximately 10000 mice will be used during the 5 year course of the project.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Most of these mice will be used to for breeding and maintenance of genetically modified lines, and since the vast majority of these animals will have no behavioural abnormality, this is classified as "mild". Many of the mice will undergo procedures that are carried out under general anaesthesia, from which they will not recover, and these are therefore classified as "non-recovery". A further group of mice will undergo procedures such as injection of harmless tracer substances into the brain or spinal cord, or spinal injections of agents that will activate or inactivate different nerve cell populations. These procedures are performed under general anaesthetic. These animals will receive post-operative pain-killers and should experience no more than transient discomfort resulting from the operation. Some mice will either have a nerve injury operation or injection of irritant chemical into the hindlimb, both of which may lead to a mild form of pain with increased sensitivity to touch or warm stimuli, or in some cases increased itch. The animals' ability to move around their cages is not reduced after these procedures, and they eat and drink normally. These last two groups of procedures are classified as moderate. At the end of the study, the animals will be killed, for example by perfusion fixation, while under</p>

	terminal general anaesthesia, or else by a humane killing method.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Since the aim of the project is to investigate the organisation and function of nerve circuits within the spinal cord, it can only be carried out on animals. It would not be possible to obtain appropriate samples from humans, and it is not possible to use cultured cells, since these do not have the complex organisation and interconnections of the intact spinal cord.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Numbers used for anatomical studies are usually around 4-5 per group, to minimise the risk of inter-animal variability. For physiological recording studies the experimental numbers will be determined by the need to obtain a sample that is sufficient to identify the various populations of nerve cell in the spinal cord or to determine whether the responses to a specific treatment are expressed to a significantly different extent between different groups of cells. Behavioural experiments are normally carried out on group sizes of 5-8 animals as this compensates for variability between animals. In all cases the number of animals used will be the minimum required to provide statistically significant data, and these will be determined by the use of power calculations where appropriate.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the lowest animals in the evolutionary tree on which these experiments could be conducted. Most similar studies have been performed on mice or rats, and mice are now increasingly being used because of the availability of genetically-altered lines, which allow identification and targeting of specific populations of nerve cells.</p> <p>All of the neuropathic, inflammatory and pruritus models that we will use are well-established and widely used in studies of pain research. They have all been refined during the course of previous studies by many laboratories to model clinical conditions. For</p>

	<p>those models that would cause prolonged discomfort, survival will be limited to a maximum of 3 days. None of the models should cause any significant alteration to the behaviour of the animal, and this will be monitored regularly.</p>
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Project	Neuronal circuits of cortical plasticity	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our perceptions, thoughts and actions depend on the coordinated activity of billions of neurons in our brain. These electrically excitable cells are wired up into networks. These networks are established during the development of the brain but some of their properties can be modified throughout our lives. Indeed, our ability to learn relies on the potential of these neuronal circuits to change through experience. These changes are mediated by the loss, the formation or the regulation of the communication pathways between neurons, the synapses. Revealing the nature of these modifications is essential to understand how our brain adapts to new circumstances and allows us to learn from our	

	<p>experiences. In addition, understanding how brain functions can be strengthened by experience will bring about new potential strategies to promote recovery of function after brain injury.</p> <p>The objective of this proposal is to understand how neuronal circuits involved in visual perception are modified by experience. The brain areas receiving visual information from our eyes have become a popular system for studying how neuronal circuits encode sensory information and how they can be modified by experience. Due to recent ground-breaking developments in imaging and genetic tools, it is now possible to use optical methods to image the activity of individual synapses in the living brain. In the context of this proposal, I will use an imaging approach to determine how experience modifies the activity specific classes of neurons.</p> <p>Finally, I will apply this knowledge to find which neuronal types and neuronal properties are disrupted in neuronal circuits of mouse models of autistic spectrum disorders and in particular, mouse models of Fragile X, the most widespread single-gene cause of autism. Individuals with autistic spectrum disorders experience hypersensitivity to sensory stimuli and perceptual deficits including well-studied visual deficits in face recognition and motion perception. Several studies suggest that abnormal sensory processing contribute to social and communicative deficits in autism.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The results of this proposal will increase our general knowledge about how the brain stores information and adapts to new environments. In addition to this fundamental knowledge, these results have direct impact into research about pathologies affecting the brain. Knowing how brain functions can be modified by experience will guide strategies to promote recovery of function after brain injuries such as strokes or brain trauma. It will also facilitate the development and the integration of efficient visual aids and hearing aids. With an ageing population, there is an increasing need for visual and hearing aids: an efficient</p>

	<p>integration of these devices requires functional sensory brain areas that can process this information. Increasing the capabilities of these brain areas to adapt to new stimuli should enhance sensory perception. In this project, we will also elucidate potential specific defects in the cortical circuits of mouse models of autistic spectrum disorders and intellectual disabilities. These results will give insights into whether and how targeted drugs to specific neuronal sub-populations would be of therapeutic value in these disorders. Finally, these results will be used as a reference for testing how proposed pharmacological treatments can rescue cortical activity deficits in these brain disorders.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mouse, I will use approximately 3200 mice over 5 years (including about 1500 for breeding).</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Some mice will be used for breeding only. The highest severity will be moderate. The experiments proposed in this study require surgery, for stereotaxic delivery of viruses to specific brain regions and for the placement of a recording chamber on the skull of the animal. For these experiments, pain will be controlled during surgery by general anaesthesia and post-surgery by analgesics. Deaths resulting from anaesthesia or surgical complications are uncommon (<1%) and will be minimised by correct dosing of anaesthetics, by accurate weighing and by maintenance of body temperature during and post-surgery e.g. use of heat pads. Risk of infection will be minimised by good surgical and aseptic techniques. At the end of each protocol, animals will be killed by using approved humane methods and tissues from these animals may be analysed. Training mice relies on motivating mice to perform a task. Recent studies have demonstrated that water restriction is an effective approach for motivating mice to perform a task. The health of all mice undergoing water restriction will be monitored and scored daily. Two-photon imaging should not cause adverse effects. Animals will be closely monitored while</p>

	<p>undergoing imaging. Behavioural training will be conducted in the animal facility where noise will be kept to a minimum to avoid unnecessary stress. All experiments will be conducted in a dedicated room.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The experiments in this proposal are designed to improve our understanding of synaptic plasticity and neuronal circuit function in the visual cortex of the mammalian brain. In order to record neuronal activity evoked by visual stimulation, these experiments require studying intact neuronal circuits in living animals. It is therefore impossible to avoid the use of animals for addressing these questions.</p> <p>Mice are the most appropriate animals for these experiments because:</p> <ul style="list-style-type: none"> - Basic mechanisms of synaptic plasticity and neuronal circuit functions are likely to be preserved in all mammals including humans. - Essential knowledge has been accumulated over years of research about the anatomy, the physiology and the plasticity mechanisms in the visual cortex of this species. - State-of-the-art imaging techniques allowing recordings of neuronal activity in the living brain have also been developed in mice and will be used in this study. - Transgenic mice offer the unique possibility to study specific neuronal populations, such as inhibitory neurons, in the mammalian living brain. Since this proposal aims at investigating the role of specific classes of interneurons these transgenic mice are highly valuable and necessary for this project. - Mice have emerged as valuable models of human genetic disorders, offering the opportunity to understand how brain circuits can be altered in genetic disorders and, hopefully, lead to ways in which these disorders could be treated. I will use mouse models of autism spectrum disorders in order to understand how neuronal circuit functions

	are altered by these diseases.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of animals will be minimised wherever possible, and animals and brain tissue will be shared across experiments as much as possible. Experimental work will be complemented with theoretical modelling to further minimise the number of experiments and animal use. I will use computational modelling to make predictions that can be used to guide the design of future experiments. I will also ensure that experiments are effective at testing hypotheses and therefore reduce the probability of unnecessary or unhelpful experiments being carried out.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Pain will be controlled during surgery by general anaesthesia and post-surgery by analgesics. Deaths resulting from anaesthesia or surgical complications will be minimised by correct dosing of anaesthetics, by accurate weighing and by maintenance of body temperature during and post-surgery e.g. use of heat pads. Risk of infection will be minimised by good surgical and aseptic techniques. Surgical sites will be monitored for signs of inflammation and infection. Appropriate effective treatment e.g. antibiotics will be administered under the advice of the Named Veterinary Surgeon if required. In addition, principles for good surgical practice will be followed throughout.</p> <p>For chronic recordings, prior to training (1-2 days after the surgery) animals will be handled extensively to become familiarised with the surroundings of the recording area and to the experimenter. Animals trained on the cylindrical treadmill will be free to run, walk, groom or remain motionless thus emulating natural mouse movements.</p> <p>Behavioural training will only start once the animals have become fully habituated to being head restrained. This will be achieved by placing head-restrained animals on a friction reduced treadmill where animals can choose to remain still or walk freely. Behavioural training will be conducted in the animal facility</p>

	<p>where noise will be kept to a minimum to avoid unnecessary stress. All experiments will be conducted in a dedicated room. After each training session animals will be returned to their home cages. They may be maintained in a reversed light/dark cycle to facilitate their well-being by synchronizing their activity with experimental schedules. The health of all mice undergoing behavioural training will be monitored and scored daily.</p>
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Project	Neuronal interactions underpinning perceptual decisions	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Every day, we have to make countless decisions based on the information we receive from our senses, for instance when to cross the road or what to eat. In order to make even simple perceptual choices about the visual appearance of an object, our brain has to integrated many different types of information. These can be different types of visual information that have to be combined, like motion, colour and depth. It can	

also include other types of information, e.g. what is the pay-off for a certain choice or what have others decided. While it is clear that all these factors affect decisions behaviourally, it remains unclear how they do this.

We investigate where in the brain and how different types of visual and contextual information are integrated and evaluated in specific brain circuits and how this affects perceptual decisions. To do so we record the responses of a number of neurons and study how they interact. We will generate models of these processes that can be applied and tested in different contexts. Altered decisions about sensory information, as studied here, are hallmarks of mental disorders like schizophrenia and autism. ay, we have to make countless decisions based on the information we receive from our senses, for instance when to cross the road or what to eat. In order to make even simple perceptual choices about the visual appearance of an object, our brain has to integrated many different types of information. These can be different types of visual information that have to be combined, like motion, colour and depth. It can also include other types of information, e.g. what is the pay-off for a certain choice or what have others decided. While it is clear that all these factors affect decisions behaviourally, it remains unclear how they do this. We investigate where in the brain and how different types of visual and contextual information are integrated and evaluated in specific brain circuits and how this affects perceptual

	<p>decisions. To do so we record the responses of a number of neurons and study how they interact. We will generate models of these processes that can be applied and tested in different contexts. Altered decisions about sensory information, as studied here, are hallmarks of mental disorders like schizophrenia and autism.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The results from this study combined with human behaviour and imaging using the same task will allow us to probe and build models of how primates make decisions that can be used by others. Our findings will generate knowledge and contribute to the larger body of work required to understand the brain mechanics of how we make cognitive decisions from input to our senses, through to the behavioural response. How this knowledge is used has relevance to areas as diverse eye witness accounts, economics and the law. The quantitative models we generate will better explain how the relevant brain processes operate in healthy subjects. They also provide insights into and means of investigating, how visual perception and decision making heuristics might be altered in such disorders as autism and schizophrenia.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>8 Rhesus macaques, of which up to 4 are continuously studied for a period of 6 years and up to 4 for less than 1 year.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Animals will have been introduced to the apparatus and tests of their visual judgements slowly and progressively. Rewards will be used to motivate animals to make and report judgements with a press of a touch screen or a movement of their eyes. Animals have been trained to earn a part of their daily fluid intake through responding to images on a computer screen. Animals will be carefully monitored for health and well-being throughout the study. The neurophysiological recording and testing require that the animals' heads are restrained to remain still, and the animals will be very gradually</p>

	<p>accustomed to achieve this with strict limits on timing. Surgery is required to implant devices on the skull that allow the neurophysiological recording from the brain. At the end of the experiment, animals are euthanized, so their brain connections will be studied as part of this project.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The study of brain mechanism of perception and decision-making with the relevant spatial and temporal resolution (brain cells and milliseconds) requires to-date invasive experiments, which cannot be carried out in humans. The same applies to linking the circuitry of such neurons to their connections. Human imaging methods, which we use in parallel, measure brain activity either indirectly or not on the right scale to allow the study of the underlying brain computations. Neuronal responses in brain slices cannot be linked directly to behaviour and therefore to perception and cognition. While we use our data to build computational models of perceptual decision-making, we are still at a point where we do not know enough to have a 'definite' model of perceptual decision-making, rather experiments are needed to test and develop such models.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The required number of animals is small, because the experimental unit is not the individual animal but the neuron or recording site.</p> <p>Neurophysiology and electrical stimulation leave the structure and function of the brain intact. By collecting data from a number of neurons simultaneously, we can reduce the number of sampling days while also studying how neurons interact in real-time.</p> <p>Experiments built around the same behavioural task. Therefore, a minimum of four animals is needed for the neurophysiological experiments. An additional two-four animals might be needed for the histological analysis of the circuitry.</p> <p>Different parts of the protocol are carefully staged in a sequence such that we can achieve</p>

	the main objectives with a minimum of four animals.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The macaque is the only suitable animal model for this project. Lower species are unsuitable because of a lack of cognitive ability and/or because the underlying brain circuitry is not directly relevant to man, and such invasive studies cannot be undertaken in man.</p> <p>There are currently no other, non-invasive methods available to elucidate neuronal mechanisms and functional circuitry at the level of single neurons in real-time. To study the neural basis of cognitive tasks like perception, brain activity and behaviour must be linked statistically robustly. The behavioural task is based on judgements monkeys (and humans) have to make implicitly on a daily basis when they move through their environment.</p> <p>Animals will be socially housed and carefully monitored for well being. They will gradually be trained to carry out a behavioural task for fluid rewards. Training schedules and rewards are tailored to the individual animal. Fluid protocols and monitoring schemes are designed to ensure each animal's wellbeing.</p> <p>The risks associated with general anaesthesia, magnetic resonance imaging and surgery for skull implants is similar to those for humans; we work to the same aseptic standards. All implants are formed from medical-implant grade materials to osteo-integrate. The movement of electrodes within the brain is painless. Eye movements are recorded non-invasively.</p> <p>Animals will be monitored daily by researchers and veterinary staff. Animal care and veterinary staff are closely involved in the care and monitoring of all animals under study and in the development of protocols.</p>

Project	Neuronal mechanisms of pain	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Chronic pain is arguably the biggest clinical challenge of the age and leads to substantial socioeconomic burden and ongoing suffering that is detrimental for the quality of life of patients. Nearly a third of the UK population have been in pain for more than three months, and this problem is set to worsen with an ageing population. Targeted therapies are lacking from different chronic pain conditions and the unmet clinical need for improved analgesia calls for a better understanding of the physiology of pain processing in the nervous system in order for us to identify new drug targets.</p> <p>The objective of our project is to increase the scientific understanding of what nerve cells and</p>	

	<p>products in pain pathways are involved in various types of chronic pain conditions, as well as the determining the factors that underlying the transition from acute to chronic pain. Our license covers various techniques applied to different animal models, which will allow us to explore the role of different types of sensory nerve cells with regards to processing painful inputs and driving the development and maintenance of chronic pain. Improved knowledge in cellular and genetic components of pain processing is necessary for providing new drug targets for analgesic therapies that more refined targeted for specific pain syndromes.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Our programme of work is dedicated to defining which nerve cells and molecules underlie different types of chronic pain in order to propose new therapeutic approaches and drug targets for relief from chronic pain. Targeted analgesic therapies are needed as many chronic pain conditions are addressed with similar classes of drugs that do not discriminate between different types of pain syndromes. By better understanding which populations of nerve cells in our nervous system and which genes are involved in pain processing, we will be able to target specific types of pain with better efficacy and provide better pain relief for patients suffering from different types of chronic pain.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use roughly 10000 mice over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Our protocols involve procedures of mild to moderate severity, and these are mostly based on models of human chronic pain conditions. Therefore, implementation of these pain conditions in animal models is necessary to advance our understanding of pain pathways and potential treatment for suffering from chronic pain. Expected adverse effects may include postoperative stress or discomfort, but these will be quickly identified and we have measures in place to minimise suffering. In all cases if any unexpected clinical signs appear,</p>

	<p>we will consult our NACWO and NVS. Our protocols fall under a mild or moderate severity limit, where animals with persistent mild pain would be classified under a moderate severity limit. At the end of each procedure animals will be euthanised according to a certified Schedule 1 method and tissues will be isolated for further studies.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Pain is defined as a whole body experience that is more than just a sensation; there's a huge emotional component and often the body's motor system is often implicated. In this project we propose to investigate the role of candidate genes in pain pathways by producing and studying transgenic mice where the gene is deleted. In some case, cell-based <i>in vitro</i> studies can be used to reproduce neuronal signalling processes, but only whole animal studies permit the study of an integrated and physiologically intact nervous system for the processing of pain where pain responses are normally coordinated. We will use cell culture systems where possible, but further investigations into the role of candidate genes in pain pathways of the nervous system and diseased states would only be feasibly replicated in live animal models.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We carefully design our animal studies using statistical power analyses to ensure that the minimum number is used to achieve our experimental aims and to measure differences between groups and treatments. Moreover, animals will be tested in multiple paradigms to maximise the data collected for the minimum number of animals. For example, the same animal will be tested through behavioural and electrophysiology assessments. Cell culture models and gene expression data will also be examined for initial experiments, where appropriate, in order to reduce the numbers of potential candidate genes we are studying so that those with the most potential benefit will be taken further into live animal models.</p>

3. Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

In our project we use mice because they represent the most appropriate species for our studies as their physiological systems and genetic makeup are largely similar to humans. Importantly, key structures and pathways for pain processing are similar between rodents and humans; many of these were originally identified in rodent and other animal models before being validated in more recent human imaging studies. The mouse is also highly amenable to genetic modification, allowing for investigation of genes and cellular mechanisms of pain that are relevant to our project.

Several decades of pain research using the mouse model has provided us with efficient techniques for neuroscience studies. We use established protocols that we have learned and adapted over many years to minimise stress in mice and to ensure the best welfare of all animals whilst maximising scientific output from our studies that requiring consistent animal handling. We have adapted electrophysiology techniques in the rat to use in the unconscious mouse (to record activity of nerve cells) to reduce suffering.

Project	Neuron-glia-vascular networks in the nervous system	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>In order for the brain to function it depends on the supporting "glial" cells and on an adequate blood supply provided by local capillaries. Our projects focus on the functions of glia and the vascular system in the brain and spinal cord, which are poorly understood, and play an important role in development, normal physiological function, and pathology. Specifically, we are interested in how changes in blood supply which occur in pathology, can affect the white matter, which houses the glial cells that allow fast conduction of signals in nervous tissue. White matter damage (e.g.</p>	

demyelination) is prevalent in stroke, dementia, multiple sclerosis and other diseases.

The interaction of the cells in the brain with each other is particularly important, and occurs through the release of signalling molecules such as neurotransmitters and cytokines. Here, using mice which express fluorescent proteins in glial cells or blood vessels, or mice in which a gene of interest has been genetically removed (**Protocol 1**), we can study these interactions.

Glial cells also affect local siphoning of potassium. Potassium concentrations regulate the voltage in the brain and regulate the cells propensity to seizure. We will also breed mice with a higher propensity to seizure using **Protocol 2**.

As general anaesthetics inhibit the proteins we are studying, we use cervical dislocation as our chosen method of schedule 1. However, we cannot use this method in neonates as it leads to destruction of the cerebellum. Therefore, in neonates (<P13 in rats and <P12 in mice), in order to preserve the tissue, we have **Protocol 3** to use decapitation instead of cervical dislocation.

As demyelination is a major feature in neurodegenerative diseases, we have **Protocol 4 and 5** to study this and the remyelination that occurs during recovery. In protocol 4, the rodents will ingest curpizone to induce demyelination. In **protocol 5** we will inject demyelinating substances (e.g. LPS or a TRPA1 modulator) into the spinal cord.

To determine whether the genetic changes in the mice bred with Protocol 2 have increased the mice's propensity to have a seizure, we will time how long it takes to induces a seizure with **Protocol 6**. Once the mice have a seizure, they will be culled.

During these protocols, we can apply potential therapeutic regimens to determine their affect on the pathogenesis of the disease. If we identify a possible therapeutic target, this could have major health and economic benefits.

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>We hope to advance our understanding of the following issues. (1) How loss of blood flow affects the cells in the white matter. By understanding how cells die in ischaemia and during demyelination we can prevent this from occurring (2) How myelin forms around axons and how axons signal to myelin to improve axon function. (3) How myelin is disrupted in diseases such as multiple sclerosis, stroke and cerebral palsy. Loss of myelin causes physical and mental impairment. By understanding how it forms and is damaged we may be able to treat disease of myelin loss better. (4) How the immune cells in the brain (microglia) function in stroke and demyelination. (5) How glial cells regulate global brain excitability, by studying what proteins regulate the induction of seizures in epilepsy. Glial cells are important in diseases like multiple sclerosis and stroke, and they also regulate the development of the brain, but we know little about how their activity is controlled. By improving this knowledge we would open up novel therapeutic targets for treating neurological diseases.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice: about 8000, Rats: about 2500</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Protocol 1 – Breeding and maintenance of GA mice (mild) Most experiments will involve acquiring tissue from genetically modified rodents bred with Protocol 1. We do not expect adverse effects caused by the genetic mutations as these animals have been bred elsewhere previously and none were found. Most of these animals will be killed humanely via a schedule 1 method, however, some will move to protocols 3-6. Protocol 2 – Breeding and maintenance of GA mice with a propensity to have mild seizures (mild - moderate) We have some mice (TRPA1 knockouts) which may have a propensity to seizure. The seizures are mild, transient and rare, and have not been found to affect the wellbeing of the mice. We may acquire another GA mouse which has a similar or slightly greater propensity to seizure during the project. Under this protocol the mice</p>

will be killed humanely via a schedule 1 method, or moved to protocols 3-6. Protocol 3-6 Administration of substances (moderate) to evoke transgene expression or to modify the activity of targets within the cells. All the following protocols start with an initial optional step to apply compounds that are needed to evoke expression of the right genes in the transgenic mice or to modify the activity of target molecules within cells. The compounds may affect the animals and therefore these protocols are considered moderate. It is essential to give the animals these substances (e.g. a drug called tamoxifen which is used to treat breast cancer in humans, or a higher fat diet) in order to carry out research which will provide information that may in the long term help the development of therapeutic drugs.

Protocol 3 – Decapitation of neonates (moderate) In this protocol we aim to obtain the tissue in the most humane method that can be used to obtain viable tissue. The neonates will undergo decapitation without anaesthetic (if under P13 for rats, and P12 for mice) or perfusion fixed.

Protocol 4 - Inducing demyelination with cuprizone (moderate) This protocol involves causing demyelination in the brain. This is considered to be a moderate protocol as the mice have some motor deficit, but this only detected by performing behavioral tests. They can move around and feed themselves. The demyelination decreases the speed of the signals and potentially conduction block. This is not expected to be painful. At the end of this protocol the animals will be killed via a schedule 1 method, perfusion fixed, or culled by exsanguination.

Protocol 5 – Inducing demyelination (moderate) This protocol involves causing demyelination in the spinal cord. This is considered to be a moderate protocol as the mice may have some small motor deficit and surgery related adverse events. However, this is rare as we make small lesions. The lesions will cause demyelination which will decrease the speed of the signals and potentially conduction block. This is not expected to be painful. At the end of this protocol the animals will be killed via a schedule 1 method, perfusion fixed, or culled by exsanguination.

Protocol 6 – Inducing

	<p>epilepsy (moderate) These rodents will be given a bolus injection of an agent which induces epilepsy. They will be monitored for up to an hour after the injection for the development of seizures, after which they will be culled regardless of whether one occurred. When the rodents reach status epilepticus, they will be killed via a schedule 1 method within 10 minutes or perfusion fixed under general anaesthetic.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Where possible we will use cell lines to study the action of molecules within the tissues, however because the work studies interactions between different cell types, it can only be done on tissue from real animals –, partly because cells change the proteins they make once they are put in culture. Wherever possible we also employ computer modelling if it can replace animal experiments, and we also hope to use live human tissue to check that our animal work is relevant to humans.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We ensure that the minimum number of animals is used by sharing tissue from each animal between different researchers and, when possible, by designing experiments to use the minimum number of animals sufficient to achieve a desired level of statistical significance in the results. Using transgenic technology to express coloured dyes in particular cell types for identification also reduces the number of animals that we need to use for experiments.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rodents have been chosen for this work as the lowest species which mimic the human nervous system well enough for our work to be relevant to human disease. We minimise suffering by either killing animals humanely and then taking tissue from the dead animal, or by anaesthetising them and killing them after the experiment is complete but while they are still under anaesthesia. Changing the proteins made in the animals' cells using transgenic technology is not harmful to the animals for the</p>

	experiments we propose.
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Project	Neurophysiological mechanisms of sleep regulation	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Sleep is a vital process, necessary for well-being, but its neurophysiological substrates and specific functions are poorly understood. This is especially relevant since sleep disturbances are common in most neurological, psychiatric and metabolic disorders. The overarching aim of this project is to attain better understanding of the effects of sleep deprivation on the brain and the body, and of the benefits of sleep for metabolic regulation and cognitive functions. The metabolic state torpor will also be studied to investigate this link further and understand the metabolic and central regulation of torpor.	

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This project will have high impact for society, the economy and well-being because it will lead to a greater understanding of the mechanisms which regulate circadian entrainment, sleep/wake timing and sleep quality. Circadian rhythms and sleep/wake timing are commonly disturbed by social and lifestyle factors, such as jet lag and shift work. Poor sleep is among the most prevalent complaints observed in many epidemiological studies, and the second most common overall complaint reported in primary care settings after pain. Disturbances in circadian rhythms and sleep/wake timing have a major impact on quality of life, resulting in impaired cognitive performance, increased risk of accidents as well as effects on immune function, hormone levels and cardiovascular performance. The amount of sleep and its quality also deteriorate with increasing age. As a result, elderly people are the main consumers of hypnotics, which have various side effects. The work detailed in this program has therefore direct relevance to clinical conditions. A greater understanding of how circadian and sleep responses are disturbed by disease is necessary to enable therapeutic intervention. For example, data from this project are expected to provide a greater understanding of how sleep is affected by dysfunctional neurotransmission, typical for a range of neuropsychiatric disorders. In addition, there is a well-known trend for increased incidence of obesity, diabetes and metabolic dysregulation, which is often associated with reduced sleep quantity and quality, or disrupted wake/sleep patterns. Finally, this project will determine neurophysiological links between brain mechanisms underlying sleep and torpor. Inducing a reversible hypometabolism that mimics natural torpor in humans could have important influences on critical medical situations, including myocardial or cerebral ischemia, haemorrhagic shock, septicaemia, and organ transplantation. Controlled hypothermia and metabolism are already widely used in clinical practice, such as during cardiac surgery and to protect tissues from damage when blood flow is reduced, such as after a stroke. The main difficulty with replicating spontaneous torpor is that we do not know how animals start and maintain the process, and our research should provide important insights.</p>
<p>What species and approximate</p>	<p>In this project I will use mice and hamsters. It is</p>

<p>numbers of animals do you expect to use over what period of time?</p>	<p>expected that over 5-year period, up to 10,000 animals will be used for breeding/maintenance purposes. Approximately 2800 animals will undergo procedures, such as behavioural training/testing, surgery for implantation of electrodes in the brain, sleep deprivation or stimulation.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>All the procedures used in this project are well established, and are not expected to result in any adverse effects exceeding Moderate level of severity. Recording of brain signals involves surgically implanting devices in the brain, which will be the highest severity procedure in this project (severity: moderate). Other surgical procedures used in this project include the implantation of telemeters/transmitters, vessel cannulation and continuous glucose monitoring. Special care will be taken to minimise suffering related to the surgical procedure. Surgical procedures may last approximately 3-4 hours and may be performed in aged or transgenic mice that may be more susceptible to potential complications. Surgery can result in a transient post-operative pain or discomfort, which will be treated with analgesics, and single housing of the animals. Behavioural tests require the use of food as a reward, which requires limiting the animal's normal food intake. Animals will be weighed daily, and the target body weight will be 85% of baseline body weight. We will also examine the effects of drug treatments, which involves administration of substances to modify circadian or sleep signalling pathways throughout the body. In addition, we will study the effects of genetic mutations on sleep, brain activity and behaviour. Some of the mutant/transgenic mice used in this project are established or emerging models of neuropsychiatric disorders, such as schizophrenia, neurological and neurodegenerative disorders, such as Parkinson's disease, ocular diseases or sleep/circadian disturbances. We do not expect, however, that the full-fledged disease will develop in any of these models, but only specific, relatively subtle symptoms will be apparent. In some animals, torpor will be induced by food restriction in mice, or by shortening the photoperiod in hamsters. Weight loss by up to 40% associated with short photoperiod in hamsters is physiological, and occurs even when food is provided ad libitum. Sleep deprivation will be performed by the most ethologically relevant way, i.e. by providing naturalistic stimulation. Humane</p>

	endpoints as described in the Project Licence will be closely observed, and animals will be humanely killed at the end of the experiments.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Sleep, torpor and waking are complex behavioural states, which require use of live animals. Numerous brain areas are implicated in various aspects of memory, cognition and sleep-wake control and cannot be fully emulated in an vitro preparation or using computer modelling. The processes associated with cognition involve multiple distributed brain systems and therefore can also only be studied in alive freely-behaving conscious animals. Laboratory mice and hamsters are the best species for this project, as the techniques for chronic neuronal recordings and sleep studies have been well established in these species. These are also the phylogenetically lowest species of mammals commonly used in the laboratory with a brain sufficiently large enough to accommodate the recording electrodes as required by this project.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use two different species, to be able to address specific question using animals, most suited for a specific goal. Biostatisticians will be consulted regularly while specific experiments are being designed. Breeding of genetically altered animals will be closely monitored to prevent over-production. Animal numbers will be reduced by careful experimental design (e.g. power calculation). Finally, we will employ randomisation and blinding to ensure our results are reproducible, as well as employ within-subjects experimental designs where each animal can act as its own control.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>A variety of measures will be used to reduce the amount of suffering, pain and distress to the absolute minimum. This is critical for the current project, as sleep and behaviour are greatly affected by pain and distress and so this could also confound the results. The animals kept in isolation will have appropriate environmental enrichment provided. All experimental procedures used in this project are well established and routinely used in the field and in my laboratory, and we will routinely evaluate technical improvements to improve experimental</p>

	<p>conditions, specific procedures and animal welfare. All surgical procedures will be conducted with aseptic techniques with appropriate analgesia and post-operative monitoring. Transgenic mice will be monitored closely to identify signs of genotype-related adverse effects. Animals subjected to aging will be also monitored carefully, including the identification of any signs age-related health impairments.</p>
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Project	NEUROPLASTICITY AND COGNITION IN HEALTH AND DISEASE	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Plasticity describes the changes in the nervous system that result from experience. For example, this could involve changes in the strength of the synaptic connections (chemical junctions) between nerve cells in the brain. Plasticity is thought to play an important role in learning and memory, and deficits in plasticity (too much or too little) are thought to lie at the heart of various neuropsychiatric and neurodegenerative disorders (e.g. schizophrenia, depression, Alzheimer's Disease). The aim of this project is to understand how different kinds of plasticity, in different neural circuits within the brain, support different kinds of learning and</p>	

	<p>memory, and choice behaviour. In particular, we want to understand how different neurochemicals (including primary neurotransmitters such as glutamate, and neuromodulators such as dopamine and serotonin) and their receptors support different aspects of cognition and why this goes wrong in disease.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Given the fundamental role that plasticity is likely to play in learning and memory, and adaptive behaviour more generally, then not surprisingly it has been a major target for trying to develop new treatments for numerous brain disorders. However, there has been limited success so far. This likely reflects our failure to understand fully the role of different forms of plasticity in cognition. The outcome of this project will hopefully be a better understanding of the role of different kinds of plasticity in different kinds of learning and adaptive behaviour. It is essential to better understand the functional significance of plasticity in different parts of the brain if we are to understand and treat these different diseases. Ultimately this could lead to the development of new drugs or treatment strategies, or to a better utilisation of existing treatment options.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Rat (<5900 in experiments; 1,000 breeding) Mouse (<8700 in experiments; 14,000 breeding) during the 5 year project</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The experiments will involve testing the ability of rats and mice to perform behavioural tasks in which they have to learn and remember crucial information such as where a food reward is or how to escape from a pool of water. We also assess anxiety, for example, by asking the animal whether it wants to explore a new place or remain in a safe location, or using mild footshock. We will examine the effects of brain lesions, drug treatments, genetic mutations, optogenetic/pharmacogenetic manipulations and sleep deprivation on these behaviours, and record signals of brain activity while the animals perform. The animals will readily learn what to do to get a tasty food reward or how to climb out of the water. Recording of brain signals involves cranial implantation of microelectrodes and could involve single housing of the animals. The expected adverse effects would include brief periods of mild</p>

	<p>distress during some of the behavioural tests (e.g. after a mild footshock). There may also be transient pain and discomfort after brain surgeries. Our extensive experience of these kinds of experiments is that they are all of moderate severity. Animals will be humanely killed at the end of the experiments.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>In order to show that a particular bit of the brain, or a particular chemical neurotransmitter or receptor, is important for the brain to work properly, it is necessary to remove or silence that bit of brain, or remove or block the neurotransmitter from working. This is not ethical (or practical) in humans. Computer simulations of the brain actually rely on the information that we will provide and so cannot replace the work that we do.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will minimize the numbers of animals used by making both the behavioural tests and the experimental manipulations (e.g. lesions, genetic modifications) as accurate and sensitive as possible</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We work on rats and mice because they are the lowest vertebrate group which reasonably resembles humans.</p> <p>Operations on the brain are done very carefully and in state-of-the-art surgical theatres, and the animals are given pain killers after the operations until they have fully recovered. Soon after the operations you would not be able to tell the difference between treated animals and controls in terms of the way they behave in their home cages. It is only with the sophisticated tests of learning and memory that you can begin to tell them apart.</p>

Project	Neuroprotection and treatment of dyskinesia in rodent models of Parkinson's Disease.	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Parkinson's disease is the second most prevalent neurodegenerative disease after Alzheimer's. There are no cures but the effects of drugs such levodopa (sinemet or careldopa and madopar or co-beneldopa) that improve movement in people with Parkinson's disease are limited to a brief time-frame; after a few years these drugs lose their effectiveness. Moreover, the non-motor symptoms, symptoms that are not related to movement such as the excessive urination, constipation, cognitive deficit (performing mental tasks such as remembering the months of the year) and psychiatric disorders such hallucinations are not addressed by current drugs that improve walking and general movements . Importantly, although the gold standard treatment of the disease after a brief period of around 5 years	

	<p>levodopa results in secondary movement disorders such as on-off symptoms and abnormal involuntary movements (dyskinesias) that limit the effectiveness of treatment of the movement disorder. The aim of the project is therefore to test compounds which have been shown to be effective in preventing cell death in culture and to apply them to animal models of Parkinson's disease that exhibit motor and non-motor problems. If these drugs are able to prevent the death of dopamine producing cells and prevent the onset of movement abnormalities, then these drugs have a great potential as agents used in the treatment of Parkinson's disease and all the problems associated with this disorder. Moreover, the other important aim is the treatment of dyskinesia. Once the disease has been established, neuroprotection will be of little value but the associated side effect of dopamine replacement therapy, namely levodopa-induced dyskinesia would be an important motor disorder that requires treatment.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>By increasing our knowledge and understanding of the mechanisms involved in neurodegeneration, and better understanding of the pharmacology of non-motor symptoms and levodopa induced dyskinesia it might be possible to identify better molecular targets for intervention. Treatment of motor and non-motor symptoms as well as abnormal involuntary movements might render Parkinson's disease a more benign malady.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>It is expected to use less than 500 mice and 500 rats for the duration of 5 years. All animals used will be wild-type (genetically normal).</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The animals will undergo various treatments that include administration of toxins by injection peripherally or directly into the brain following brain surgery. Animals will be subject to implantation of small pumps which will administer drugs continuously without the need for repeated injections or administration of drugs by oral gavage, intraperitoneal (injection into the gut cavity) or subcutaneous (injections under the skin) routes. In the case of the former, anaesthesia followed by analgesia will be used whereas administration of drugs by the latter routes will result only in moderate discomfort. Consequently, the animals may experience some symptoms of the disease such as in-coordinated movement, circling movements, excessive urination, constipation, weight loss or excessive involuntary movements. Anaesthesia, analgesia will be used to mitigate the pain associated with</p>

	<p>surgery and analgesia will be given to reduce the possible post-surgical pain and discomfort. There are also limits to the number and frequency of any injections, blood sampling and behavioural assessment that any one animal can experience. Overall, the severity of this license is expected to be moderate. At the end of the experiments the animals will be humanely killed and tissues may be used for biochemical investigations.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The understanding of mechanisms involved in the CNS is still at very early stages to allow us to model neurodegenerative diseases effectively in non-mammalian or non-animal models. Consequently, we are bound to undertake some of our work in animals. However, prior to <i>in vivo</i> testing compounds will be screened using a range of <i>in silico</i> (computer based) and <i>in vitro</i> testing to ensure efficacy.</p> <p><i>In vitro</i> and <i>in silico</i> techniques are also not sufficiently advanced to model the integrated actions of the nervous system. It is imperative that models that display cardinal motor signs e.g. slowness of movement and rigidity, loss of smell and constipation or levodopa-induced dyskinesia that is common PD so that a potential treatment could cure these symptoms. These features could not be replicated in flies, worms, cells or in computer modelling in a meaningful manner (for example worms, flies or cells do not allow detection of motor abnormalities seen in PD).</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>In order to use the lowest number of animals we will carry out an <i>a priori</i> estimate of the sample size needed to achieve the smallest number that would yield statistically robust meaningful data. Therefore, before the onset of any study a power analysis will be carried out to inform the experimenter of the lowest numbers needed to measure 25-30% change from mean from historical data and the published literature. By using good experimental design, consideration of statistical advice, taking steps to make sure experiments do not have to be repeated, minimal number of animals will be used to provide statistically meaningful data with the lowest number of animals used.</p> <p>I have considerable experience in this type of work, and have published extensively in peer-reviewed journals where reduction of animal use has been one of the main objectives of the experimental design. Thus, I already have a very good working knowledge of the optimal way to design and execute</p>

	these types of experiment.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Whilst, MPTP-treated primates are the gold standard models of Parkinson’s disease, recapitulating nearly all motoric aspects of the disease, use of this model at early preclinical stages of therapy is unwarranted. Therefore, rodent preclinical models will be used instead. Mice and rats have been used extensively in studies of this kind with very good level of translational potential to the human condition. Rodents have very similar neuroanatomy and neurophysiology and many agents display a pharmacological profile in rodents that is similar to man. Moreover, the genetics of rodents are also increasingly well documented which allow a more direct comparison with man. The behavioural repertoire of mice and rats are well documented and are easily assessed after discrete lesions of the CNS. In primates, MPTP treatment requires careful special controls especially at the early stage of MPTP treatment (i.e. hand feeding, careful control of body temperature and close monitoring of body weight) in rodents, these special measures are less critical but are nevertheless very important for reproducibility of lesions. In rodents, adoption of refinements such as controlling gender, age, and body weight are also factors that modulate MPTP sensitivity as well as reproducibility of the lesions.</p>

Project	Neurorestoration following nervous system injury	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Overall Aim: the aim of this Project is to develop new therapies for disorders such as spinal cord injuries, which lead to severe disability and for which there is currently no cure</p> <p>Clinical need: Injuries to the central nervous system (the brain and spinal cord) cause devastating and permanent loss of function such as paralysis, loss of the use of the arms and hands, bladder and bowel incontinence and loss of sexual function. Spinal cord injury is a particularly strong example of a life-changing event that can lead to a lifetime of disability and high-dependence care, devastating the lives of individuals and their families. Over 2.5 million people worldwide are</p>	

currently living with a spinal cord injury and many new injuries are suffered each year. Healthcare costs to treat and care for individuals with spinal cord injuries are among the highest of any neurological disorder. Spinal cord injury therefore represents a major medical challenge and a huge burden to society. There is no cure and no regenerative therapies available to spinal injured individuals. The only available treatments offered to patients are rehabilitation and medication for managing complications such as pain and depression. Therefore, new regenerative therapies that enable tissue repair and new nerve connections to be made and which would lead to some functions being restored are drastically needed. This could improve quality of life of the many thousands of individuals living with severe disabilities.

Key objectives:

1. To advance the clinical development of a promising new gene therapy which degrades spinal injury scar tissue. This scar tissue normally blocks new nerve growth, but the gene therapy enables new growth and new nerve connections and can lead to recovery of important functions such as the use of the hands. A main objective of this project is to work out the best timing and doses of this therapy and to improve its safety so that it can be developed into a treatment for human spinal cord injury.
2. To develop new approaches to improve the ability of spinal nerve fibres to regenerate and to overcome the spinal injury scar tissue. We will use newly developed gene therapies that can be switched on and off and that can be targeted to specific cells, as well as new drug therapies that destabilise the growth-blocking elements of the scar.
3. To determine whether the therapies developed in objectives 1 and 2 will work when the treatment is given some time after the injury (when the scar tissue and pathology is well established). This will determine whether our therapy could be useful to the many individuals living with long-established spinal cord injuries.
4. To study the cellular changes that happen in the

	<p>days and weeks after a spinal injury occurs (called the “secondary injury”). By understanding the molecules that cause pathological inflammation and scarring we can develop new therapies to target these molecules.</p> <p>5. To use genetically modified mice to identify genes that are important for regeneration and recovery and to develop new therapies which would increase the expression of these genes and improve recovery.</p> <p>6. Develop rehabilitation methods for improving the function of hand and arm muscles. We will focus on abilities that are top priorities for individuals with tetraplegia (where all 4 limbs are affected), such as the ability to pick up and grip an object. Recovering the use of the hands would give individuals greater independence (e.g. the ability to wash, feed and dress independently) and an improved quality of life.</p> <p>7. Determining the best treatment combinations which have the potential for maximising recovery of function after injury (e.g. a regenerative gene therapy combined with intensive rehabilitation).</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The work under this Project has multiple potential benefits, both for the advancement of science and, in the longer term, for improving human health. Novel tools and therapies developed under this project will be made available to other researchers to use, which may benefit both their research programme and the wider field of regenerative medicine, since they may be used for improving our understanding of the basic biology of tissue injury and repair. This could be important for developing therapies for traumatic brain and spinal cord injuries, and for other nervous system disorders. Clinicians and neurologists may also benefit from this work, since novel rehabilitation-based strategies developed here may be implemented in the clinic, and candidate biomarkers of injury that may be discovered here could be evaluated in patient samples. The pharmaceutical industry may also benefit, should they invest in the clinical development of our new gene therapy strategy, enabling safety and toxicity studies to be performed and clinical grade vectors produced. The long-term aim of this project is to</p>

	<p>have practical application in the development of a clinical therapy that could benefit patients living with spinal cord injuries (as well as other stakeholders such as families, caregivers, charitable foundations). The regenerative therapies that will be developed in this project have a realistic potential for improving the functional outcome for thousands of individuals living with paralysis and other severe disabilities. A small gain in function, such as recovering use of the hands, would dramatically change the quality of life for individuals living with tetraplegia (affecting all four limbs), enabling them to carry out basic everyday tasks such as feeding, washing and, dressing themselves, giving them independence and autonomy and enabling greater participation in society.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use approximately 200 mice and 500 rats per year. For any procedures we will undertake power calculations to estimate the minimum number of animals we need in order to obtain statistically meaningful results.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>1. Cell culture work involves removal of tissues after humane killing; no additional adverse effects are expected. Severity level is mild. 2. Nervous system injury work, including spinal cord injuries, involves surgeries performed with anaesthesia and analgesia. We aim to use injury models that cause partial damage to precise areas of the brain and spinal cord as well as more “clinically-relevant” models that replicate the typical pathology of human spinal cord injuries. In both cases we aim use the most minimal injury that will enable us to assess muscle and limb weakness and dysfunction, but that will keep the animals in as best health as possible. For example, none of our protocols induce complete paralysis in the experimental animals. Following injury, animals will show varying levels of impairment depending on the severity of the injury they have received. Typically, for the first two or three days after partial brain or spinal cord injury (or up to two weeks after clinically relevant injuries), rodents require special care because they may be transiently weak or partially paralysed, may feed and drink less, and appear unkempt. We provide them special intensive care including soft bedding, additional</p>

	<p>fluids (by injection), pain relief medication, additional food (feeding by hand if necessary). One of our procedures is classified as “severe” because the loss of mobility is greater than other injury models. However, this is the most clinically relevant model and therefore important to use in some cases for assessing and developing new therapies. General well-being of the animals will recover quickly following injury (within the first week) and typically this will be followed by dramatic functional improvements such that animals will be able to support body-weight on their affected limbs by 2 weeks post-injury. We continuously strive to improve the welfare of our animals and to reduce their suffering. Animals that do not recover with this additional special care will be humanely killed. However, we have considerable experience caring for animals of this kind and the majority recover as expected. At the end of the studies, animals will undergo euthanasia and their tissues will be used for further analysis of treatment effects; making maximal use of tissue will reduce the number of animals.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Our work aims to develop novel therapies for promoting recovery following spinal cord injury. This work requires the demonstration of robust effects in clinically relevant animal models. Additionally, many mechanistic questions and experimental strategies require invasive techniques that are not possible or feasible at present in humans. Cell culture and computer simulation techniques are not significantly advanced to model the integrated actions of the nervous system, in particular they cannot mimic the complex inflammation and scarring processes and cellular reactions that lead to destructive tissue pathology within a central nervous system injury environment. For this, there is no alternative to using in vivo systems, therefore animal models are required.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of</p>	<p>Animal numbers will be kept to a minimum by carefully designing and planning all studies to ensure that the group sizes are kept to the smallest possible size at which a meaningful effect can be detected. Additionally, we aim to measure</p>

<p>animals</p>	<p>many different variables in each animal, thereby reducing the numbers used. Typically, after a nervous system injury (performed under anaesthesia) a therapy will be administered, and simple tasks (e.g. gripping a bar and reaching for sugar cubes) will be used to assess any improvements in function. At the end of the study, tissue will be used to obtain molecular and anatomical data from the same animal.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rats will be used for the majority of studies as they closely mimic the pathology of human nervous system injury. Mice will be used in some instances however, specifically when the use of a particular gene can reveal valuable information. these species represent the lowest sentient mammalian species, and a great deal is known about their anatomy, neurophysiology, genetics and behaviour.</p> <p>The models we will use will either be discrete injuries of nerve fibre pathways to study how they respond to injury and regenerative therapies. Or, we will use clinically-relevant models which closely mimic the pathology, disease progression and functional readouts observed in human patients. In these cases, we can test promising therapies in these valuable pre-clinical models as a first step towards translating a therapy to the clinic.</p> <p>We have experience of all the techniques detailed in this project. All animals are subject to regular inspections by the scientists, NACWO and veterinary surgeon and mild health problems are dealt with accordingly. In the event of any unexpected adverse reaction during experiments the animal will be humanely killed.</p>

Project	Neurovascular inflammatory mechanisms	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall purpose of this programme is to define mechanisms involved in the stimulation and functions of sensory nerves and related systems. The sensory nerves transmit pain sensations and are involved in the disease processes. This includes the study of substances (e.g. 'TRP activators') released from sensory nerves and the cell and mediator systems that they interact with in the body. Current funded projects are: to investigate the role that sensory nerves play when exposed to adverse temperature changes that the skin and body undergo. This is relevant to, not only, cold-induced injuries; but also the high number of	

	<p>deaths that occur during the coldest months of the year that are not flu-related, in the elderly in winter. Another project is to investigate effects of sensory nerves and inflammation sometimes involving temperature changes on the joints and the relationship with pain. We especially study arthritis and joint inflammation. Over the last five years we have shown that a new TRP substance, is beneficial in arthritis and we now have further funding to investigate this. Similarly we have recently shown it is 'protective' in heart disease and want to build on this and recent findings that a sensory neuropeptide is protective in heart failure in mice. Studies for the next five years will progress the findings involving TRP activators and neuropeptides to increase our understanding of their role in disease and whether the new findings can be used to develop new medicines</p> <p>Our main aims are to:</p> <p>(1). Identify sensory nerve stimulants, the effect they have in disease and to learn more about the mechanisms by which they act, once activated, and how this links with inflammation.</p> <p>(2). Use the information to investigate the effect of blocking agents or gene modification on the sensory nerves, neuropeptides and inflammatory disease processes.</p> <p>(3). Establish the role that these have in cardiovascular and inflammatory models</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The project is designed to allow new treatment pathways and new therapeutic agents (medicines) discovered. For example in REDACTED we showed that REDACTED, a newly discovered substance at the time, had effects on blood vessels. It increased blood flow (was a vasodilator). In the following years this REDACTED was characterised by us and other groups. It was realised that blocking its actions had a beneficial role in some pain syndromes, such as REDACTED. In REDACTED REDACTED have been cleared for the treatment of REDACTED pain in the USA and Europe including the UK. Thus our research, which is fundamental in nature has been proven to lead to clear human benefit.</p>

<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice, approximately up to 17,000, depending on funding, over five years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In this project mice may become develop high blood pressure (hypertension), suffer heart failure, become arthritic or suffer inflammation. We will use mice with genetic alterations in specific pathways of interest relevant to the project. These include mice with disease conditions eg diabetes, arthritis and treat with medicines that either accentuate or ease these conditions. All the animal models are well defined and characterised within our laboratory. Animals will be monitored closely for adverse effects and for the extent of their disease. This may be by direct observation, or may involve taking small blood samples for analysis. Some mice will undergo surgical procedures to implant devices for the monitoring of disease conditions (by measuring blood pressure, heart rate, activity or temperature) or to allow drug delivery. During and after surgery mice will receive painkillers and will be monitored closely during their recovery. The level of care and monitoring is increased for mice after surgery and when a disease has been induced in them. Many of our techniques involve non-contact monitoring, so the mice are not disturbed or handled. Thus any stress and discomfort is minimised. Occasionally we will change their diet and include test medicines or added nutrients in their water or food. However, we will ensure that they eat and drink normally through weighing the food and water. Inflammation and arthritis will usually be induced by a single injection of a pro-inflammatory substance into the paw or joint. This is carried out under anaesthetic and animals recover without signs of pain. Normally studies involve measurement of the time taken for a rodent to move away from a heat source that is shone onto skin, or a pressure probe. This involves transient discomfort. These models are associated with minimal discomfort. Some mice will undergo long term models of arthritis or limb ischaemia/skin wound healing (up to several weeks), involving several injections. Whilst this is associated with discomfort, our experience is that they do not exhibit lack of well being and eat and</p>

	groom normally. At the end of the study animals will usually be subject to a non-recovery terminal procedure and the mice humanely killed. We usually collect tissues and body fluids for analysis.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The factors involved in sensory nerve and blood vessel biology includes the activation of nerves and release of the biologically active substances which act on a range of cells and body systems, including blood vessels. It is not possible to simulate these biological systems completely in a test tube. For some parts of our research single cells or tissues are studied in isolation and this is important in order to establish basic mechanisms. The findings need to be evaluated within animal systems as we cannot accurately recreate in the isolated cells in the laboratory the complex structures that work together in the body and in disease conditions such as hypertension or arthritis that include blood vessels, tissues, bones and organs. Therefore, there is an essential need to examine how sensory nerve activation and neuropeptide activity influences and interact with whole body systems.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Our breeding programmes are monitored on a regular basis in order to keep numbers to a minimum. We use mathematical analysis to calculate minimal breeding and group sizes needed to reach scientific objective. We design experiments based on mathematical calculations to determine the smallest numbers needed in each group to reach statistical significance, considering the changes expected.</p> <p>i) Using imaging techniques. This allows study of animals by imaging of body systems by either ultrasound or laser systems. This results in the use of fewer animals per group, and better information on disease progression as the disease state is followed in each animal over a number of imaging sessions.</p> <p>ii) Implanted sensor probes that measure blood pressure, temperature, activity and ECG enable continuous monitoring of the conscious mouse in</p>

	<p>the home cage. This enables reductions in numbers used, especially with respect to long-term studies, as we can take readings before the start of the disease model and do a 'before (control) and 'after' (test) evaluation of those readings in the same animal.</p> <p>iii) We use a control site within the animal, rather than a separate animal, for multiple site experiments, such as in skin. Additionally, experimental design involving multiple skin sites allows reduction of animal numbers without compromising animal welfare.</p> <p>iv) Cells, fluid and tissues from experiments are used for analysis after the mouse is humanely killed. This enhances the accumulation of knowledge obtained from any one animal undergoing a procedure. This allows us to reduce the number of animals used for any one study. The study is also supported by analysis of human cells where possible.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>All our studies will be carried out in mice, where we now have an advanced expertise on mouse models of human and animal disease and also access to genetically modified mice. The use of genetically modified mice means that we can remove genes of interest to us from mice. We can then investigate if that alters, for example benefiting the disease. This can lead to large steps forward in our understanding. The animals will be housed in our modern facility with environmental enrichment, designed to allow the animals a wide range of natural behaviours and some privacy, provided by environmental enrichments, such as tunnels. The husbandry of these species is according to best practice, involving highly trained staff and is under regular review by our institution.</p> <p>Many regulated procedures are typically completed over the course of one day and thereafter animals are monitored accordingly until end of experiment with anaesthesia used where possible to reduce stress of restraint and injections. This allows us to gain information on mechanisms and modulating systems, with minimal discomfort.</p> <p>Longer term studies involve pre-treatments with</p>

	<p>procedures such as injections and implantation of sensors, involving anaesthesia followed by recovery. We routinely use medical pain relief at surgery and sterile techniques to reduce the chance of infection. We now sometimes, when suitable, administer agents via palatable food (e.g. peanut butter), which the mice see as a treat. At all times and more frequently after anaesthesia/surgery we monitor animals for adverse effects and treat accordingly. The implanted sensors allow measurements of blood pressure activity and body temperature to be carried out remotely on conscious rodents, without them knowing. This both avoids any potential discomfort and stress for the animals from handling and provides very reliable data as the animal is carrying out its normal behaviour whilst the data is being collected by the sensors].</p>
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Project	New approaches for prevention of tuberculosis and other infectious diseases	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Tuberculosis (TB) is a major human disease, killing millions of people worldwide. Current control measures are seriously compromised by poor efficacy of the current TB vaccine (BCG), the increasing incidence of HIV/TB co-infection and the emergence and spreading of multi-drug resistant disease. This project endeavours to explore novel treatment and prevention approaches against TB. This will include generation and testing of novel TB vaccine approaches and novel immunotherapies that could be applied in HIV/TB patients or those	

	<p>suffering from multi-drug resistant disease.</p> <p>We have developed several new and promising vaccine approaches which now need further testing to determine if they could be translated toward human clinical trials. For this, it is important to first establish that they perform satisfactorily in animal experimental model of infection and reduce the risk of failure at later stages. Furthermore, the novel vaccination approaches we developed for TB, will also be tested for other infectious diseases including dengue, Buruli ulcer and possibly others. Some of the technologies we developed for vaccine delivery can be easily modified to make them applicable for multiple diseases and this will further enhance the benefits from animal experimentation and maximise our research outputs.</p> <p>Immunotherapy of multi-drug resistant tuberculosis (MDR-TB) would be highly beneficial to patients, since the current treatments are very protracted, toxic and poorly effective. We propose to test the potential of immunotherapy with antibodies and cytokines, both natural components of our immune system, to shorten MDR-TB treatment and improve treatment efficacy in mice before this approach could be considered for human application.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Primary benefit (i.e. control of disease) concern the potential application of new vaccine candidates for prevention of infectious diseases (i.e. TB, dengue, Buruli ulcer and possible others) in man. This could have a substantial health impact on millions of people globally. In addition, our objective is to determine if immunotherapy for TB has the potential for human application for treatment of multi-drug resistant TB (MDR-TB). Secondary benefit of this study concerns scientific community. The scientific impacts may be multiple and will include: 1) advancement of the vaccine immunology field; 2) better understanding of the immune responses to infection, and 3) novel research tools and technologies (i.e. nanoparticle and spore vaccine platforms, molecular engineering, novel animal models of</p>

	infection). As the results generated from this project will have multiple applications, we anticipate that the findings will be of interest to both academia and Small/Medium Enterprises.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will require up to 6000 mice, over the 5 year period. These will include wild type mice and also genetically modified mice. A typical experiment will have 6-8 experimental groups of 6 mice/group, including controls and test groups. Experiments will last 1-6 months, typically 3-5 months. To ensure reproducibility, each experiment will be repeated at least once, either on its own, or as a component of a subsequent experiment, where possible.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The level of animal suffering in this project will be mild or moderate for the vast majority of animals. Immunisation procedures (which account for > 80 % of animal experimentation in this project) will generally cause only minor and often transient adverse effects. For example, local haematoma may occur during blood sampling, brief respiratory discomfort may occur during intranasal inoculation, localised dermatitis (skin lesion) may occur in some animals immunised with the BCG vaccine, oral gavage may cause a temporary discomfort etc. None of these adverse effects is expected to cause any lasting harm to the animals. Very occasionally, vaccination via respiratory route may cause an excessive inflammatory response in the lungs and if that happens and animals show any signs of distress or suffering (hunched posture, poor eating and grooming habits) they will be humanely killed. Infection of mice with Mycobacterium tuberculosis bacteria (that cause TB) is a well described experimental model. Though animals may harbour significant numbers of bacteria in their lungs, they show very little sign of clinical disease, as they are naturally more resistant to this infection than other species (eg. guinea pigs or humans). It takes a protracted course of infection (typically more than 3 months) for mice to begin to exert clinical symptoms of TB such as weight loss, irregular breeding, reduced mobility etc.). This project will not include such prolonged infection studies as all experimental

	<p>readouts can be obtained before the onset of clinical symptoms. The footpad model of <i>Mycobacterium ulcerans</i> infection (causing a neglected tropical disease called Buruli ulcer) is also well described and does not cause any major suffering to animals. The infection causes painless (due to attacking nerve endings) swelling of the footpad which can be objectively categorised and experiments concluded before it becomes excessive as to significantly impair animal mobility or cause visible ulcerations of the tissue. In one of our experimental protocols (aerosolised infection of mice with <i>Mycobacterium tuberculosis</i>), a very small proportion of animals (in our experience, typically <2 % animals from this particular protocol, and < 0.5 % for the overall project) may unfortunately be lost to sudden death due to equipment shortcoming or failure and this is being continually addressed with the manufacturers and improvements have been made and will continue to be made. Animal welfare will be always a primary consideration and all measures and precautions will be taken to minimise animal suffering and distress. Where necessary, anaesthesia will be used prior to initiation of a procedure and appropriate care given to the animals after the completion of the procedure. At the end of the experimental protocol, animal will be humanely killed.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Use of animals is justified, as this is the most appropriate experimental model for testing of immunotherapy and vaccine immunogenicity and protective potential, proposed in this study. Mouse models of tuberculosis is well described and it serves as a good initial screen of the vaccine immunogenicity and efficacy. Extensive <i>in vitro</i> evaluation of the proposed vaccine candidates using dendritic cells and tonsil tissue culture will be performed prior to deciding whether to proceed with immunisation of mice. Similarly, effects of immunotherapy will be tested <i>in vitro</i>, using human cell lines or blood samples, to select the most active/therapeutic antibodies and determine the best</p>

	combinations.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The experimental design in this project took into account previous experience with similar work and the published information from other research groups. Importantly, the statistical aspects (including power calculations) and data analysis have been given full consideration. Furthermore, the replacement protocols described above (i.e. tonsil tissue culture, human blood, dendritic cells) will identify non-performing candidates, thus reducing the need to use animals for their testing. Wherever possible, multiple tests will be performed using the same control groups, thus avoiding repetitive use of control animals.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Experimental tuberculosis in mice shares many immunological features with the infection in man, and therefore, the mouse model is the most appropriate for the proposed work. None of the objectives listed above could be achieved satisfactorily without the use of animals. However, extensive <i>in vitro</i> evaluation will be conducted on all new vaccine candidates and only those that 'pass' the <i>in vitro</i> test will be considered for evaluation in mice. Over the years, I have gained significant experience (as evidenced in my publications) of work with experimental vaccines and treatments in animals and this means that the proposed protocols and experimental designs have been continually refined to improve animal welfare and reduce stress and suffering during experimentation.</p>

Project	New biomaterials for tissue regeneration	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project will develop new materials that improve the healing of tissues that have been lost or damaged. Proteins and other molecules important for healing processes often diffuse and escape from the site where they are delivered, leads to a reduction of the effectiveness of some of the current treatments. We expect that these natural and synthetic materials will enhance the effects of these molecules by delivering them locally in the injury site and increasing the residence time in the site where they are required. We will test prototypes in experiments with animals, an important step for the posterior clinical translation these new technologies.	

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The loss of tissue due to trauma or infection is one of the most frequently and devastating causes affecting human and animals' welfare. Our technology provides a novel platform where proteins and other important biological molecules with critical functions for tissue healing can be delivered in a local and controlled way, which is expected to enhance the cell response during processes of tissue repair and regeneration. This will improve the current procedures by reducing the required doses and frequency which the patient need to be treated with.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use male C57BL/6 mice, a very common line of mouse used in lab research, during up to 12 weeks long experiments. In those cases, where grafts with human MSCs will be implanted, we expect to use immunocompromised male mice (NSG). Special sterile conditions will be applied before and after surgery. We expect to use a minimum number of implant compositions, depending on the final results from the previous in vitro cellular experiments, therefore the overall total number of animals will not exceed 1000 animals.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Based in our previous experience with this animal model, we expect the animals to recover quickly after surgery, displaying normal behaviour and mild pain level, since the model will consist basically on a skin incision to create a pocket under the skin where the material will be implanted. The surgery will be performed under anaesthesia. We do not expect that the animals experience wound infection, but the wound will be treated if necessary. Because of the need to collect all the implant samples for the planned testing, at the end of the experiment the animals will be humanely killed.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-</p>	<p>The responses of cells and proteins properties to the implanted materials used are not possible to fully reproduce in the lab, and often different in an animal or in a cell culture. Therefore, animal</p>

<p>animal alternatives</p>	<p>experiment are a critical step on this project, to validate the efficacy of the platform in an environment similar to a human body, and are an essential validation in these technologies before a clinical application.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>At least two samples will be implanted per mouse. This will significantly reduce the number of animals used without causing an additional pain or discomfort to the animal, and will allow us to combine the different compositions to be tested in such a way that we will be able to compare the response of the same animal to different materials implants. Before starting the animal experiments we have planned extensive research on cell cultures of the most promising systems. A power calculation will be used to determine the minimum number of animals will be used to achieve statistical relevance and meaningful results.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We have chosen the subcutaneous mouse model to evaluate our technology. The subcutaneous implantation site is a commonly used and appropriate model to evaluate host responses to implanted biomaterials as well as the capacity of cells to perform specialized functions during tissue repair. This model is the least invasive, which exclusively cause a skin wound on the back of the animal. This model does not involve any alteration of internal organs, which significantly reduces animal pain and risk of infection compare to other more invasive animal models.</p>

Project	New methods for diagnosis and treatment of cataract
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Basic research
	X Translational and applied research
	X Regulatory use and routine production
	Protection of the natural environment in the interests of the health or welfare of humans or animals
	Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Cataract is a condition in which the lens of the eye becomes cloudy. This causes vision problems. Cataract is the main cause of blindness in the world. The current treatment is surgery to remove the affected lens. The surgery is extremely delicate, requiring special facilities, equipment and a highly-trained eye surgeon. A better way to detect and treat cataract is needed.</p> <p>A new device has been invented that aims to detect and treat cataracts. It will do this by shining light into the eye and onto the lens. Cataract will be detected by the signal coming back from the light hitting the lens. A different beam of light will be used to clear the clouding</p>

	<p>of the lens. No surgery will be required.</p> <p>This project will test whether the device is safe and works as expected. This will be done by testing it in pigs with cataracts. The pigs have cataracts because they have been genetically-modified to develop diabetes. Diabetes is a common cause of cataracts in people and animals. If the device is shown to be safe and work in this project then it can move forward to be tested in human volunteers.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The immediate benefit of this project will be to show that the device is safe and can detect and treat cataracts. The results of this project will be used to support applications for clinical trials in humans and animals. In the future, this device could be used to detect cataracts in people at an earlier stage. It could offer a new, non-invasive way to treat cataract. This would be cheaper and more widely-available.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This project will use pigs because their eyes are similar to human eyes. To test the ability of the device to detect and treat cataracts, we will use pigs that have been genetically altered to be diabetic. Diabetes causes cataracts and these pigs start to develop cataracts at a young age. A small number of healthy pigs without eye disease (up to 12) will also be used to test the safety of the device. Overall we expect to use up to 40 pigs in this project. Most of the testing will take place over a 12-month period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The device will always be used on anaesthetised pigs. General anaesthesia is required so that the light from the device can be shone directly into the eye. The device itself is not invasive. Previous tests have shown no harmful effects. Therefore, healthy pigs will only have negative experiences related to the process of anaesthesia. These are expected to be mild. Pigs with diabetes will have negative experiences from the disease itself. These will mainly be increased hunger and thirst. Like diabetic people, their blood sugar level will be checked regularly. They may also receive insulin injections to</p>

	<p>help control their diabetes. The pigs will be trained to accept these procedures, but they may still cause some discomfort. Cataracts may affect how well the pigs see. However, they are not expected to go blind. Overall, diabetic pigs are likely to have negative experiences of moderate severity. In studies where the testing period is long (6-12 hours), the pigs will be killed at the end. They will not recover from anaesthesia. When the testing period is shorter (under 6 hours), the pigs will be recovered from anaesthesia and monitored. This is to see how long the treatment effect lasts. It will also allow any later side effects to be detected. Some pigs will have more than one anaesthetic. This is to allow their eyes to be re-examined using the device. Different protocols for treatment can also be tested in this way. At the end of the study period, the pigs will be killed. This allows the eyes to be removed for further testing.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The first tests of the device did not use experimental animals. Instead pig eyes from abattoirs were used. Tests were also carried out on human lenses removed during cataract surgery. Further safety tests are required before the device is tested in humans. Tests in live animals can show if unwanted effects occur in the living eye after treatment.</p> <p>Studies in animals with cataracts are necessary to see if the device can detect and treat cataracts. The pig is a good model because pig eyes are very similar to human eyes.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>The project is designed to use the minimum number of animals. The most appropriate animal and cataract model have been chosen. Testing will be step-wise. For example, the first studies are non-recovery. These will confirm that the device can detect and treat cataract. Only after this will the recovery studies begin. If testing identifies a problem, later studies will not go ahead unless this is</p>

	<p>resolved.</p> <p>The study results will be reviewed as they proceed. This means that studies can stop as soon as their objectives are achieved.</p> <p>Variation between animals will occur. However, the other eye will be used as a control. This means that fewer animals will be required.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The pig was chosen because pig and human eyes are very similar. Also, a model of naturally-occurring cataract exists in diabetic pigs. We believe that this model offers greater benefit and less harm than artificially creating cataracts with chemicals or radiation. Testing in pigs with cataracts is the best way to determine if the device is safe, effective and suitable for testing in humans.</p> <p>Pigs will always be anaesthetised while the device is used on their eyes. In the first studies, the pigs will not recover from the anaesthesia. Only when the device has been shown to be effective will animals be recovered. Anaesthesia will be carried out by veterinarians in a facility with advanced monitoring equipment.</p> <p>These pigs have cataracts due to their diabetes. To maximise their welfare, their health will be monitored closely using a range of criteria. This will include regular testing of their blood sugar. Insulin may be given to control blood sugar levels. The pigs will be trained to accept these procedures without restraint. We shall also investigate further ways to refine the care of these animals. This could include the use of glucose sensors applied to the skin, similar to those used in people.</p>

Project	New methods for percutaneous needle access to the chest
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Basic research
	X Translational and applied research
	X Regulatory use and routine production
	Protection of the natural environment in the interests of the health or welfare of humans or animals
	Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Lung cancer is the most common cancer in the world. Almost 2 million people die of lung cancer each year. Accurate diagnosis requires a sample of lung tissue (needle biopsy). Taking this sample can cause a collapsed lung in around one-third of patients. A collapsed lung can be harmful and painful. It can need further hospital treatment.</p> <p>This project will test a new device for needle biopsy of the lung. The device has been designed to reduce the chance of lung collapse. It combines a new needle design with a sealant.</p>

	<p>The main aims of this project are to:</p> <p>1) understand how the sealant behaves. How long does it take to degrade? Does it cause any response from the body? Is it safe?</p> <p>2) show that needle biopsy with the new device causes less lung collapse than the standard technique.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The immediate benefits of this project will be to show that the device is safe and effective. The results of this project will support applications for clinical trials in humans. In the future, the device could be used for needle biopsy in people with suspected lung cancer. A safer device, causing less lung collapse, would have multiple benefits. Patients will have fewer complications and this will save money. Doctors will be more willing to carry out the procedure. This could lead to earlier diagnosis in more patients.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This project will use pigs because their lungs are similar to human lungs. The project is divided into two phases. Phase 1 will study the sealant behaviour. Approximately 20 pigs are required to obtain samples at different time points after the biopsy procedure. If the sealant is modified based on the results of testing, Phase 1 may be repeated on up to two further occasions. Phase 2 will compare the new device with a standard needle biopsy. This will use around 40 pigs. Overall, up to 100 pigs may be used in this project. Most of the testing will take place over an 18-month period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Needle biopsy will always be carried out on anaesthetised pigs. The negative experiences of the pigs will be related to the process of anaesthesia and any after-effects of needle insertion into the chest. These procedures are likely to be of moderate severity. The most likely complication of needle biopsy of the lung is a collapsed lung. Imaging (a CT scan) will be carried out during and after needle biopsy to check for this. Pigs will not be recovered from anaesthesia if the lung is collapsed enough to cause more than moderate discomfort. Pigs will be monitored closely as they recover from the</p>

	<p>procedure. If the pig shows signs of breathing difficulty it will be killed immediately. Pigs may have further sedation or anaesthetics. These are needed to image the chest (a CT scan). This is not invasive. At the end of the study, the pigs will be killed. This allows lung tissue to be removed for further testing. This may occur at the end of a final anaesthetic from which the pig does not recover. During this final anaesthetic more needle biopsy procedures may be carried out. This will provide more data about the safety and effectiveness of the new device without increasing the number of animals used.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The first tests of the device did not use experimental animals. Instead pig lungs from abattoirs were used. Further safety tests are required before the device is tested in humans. Tests in live animals are needed to understand how the sealant behaves for several weeks after the procedure. Tests in live, breathing animals are also needed to confirm that the device works as expected to decrease the risk of lung collapse after needle biopsy.</p> <p>The size of the device prevents its use in non-protected animals. The pig is a good model because pig lungs are very similar to human lungs.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>The project is designed to use the minimum number of animals. Testing will be step-wise. Phase 2 will only proceed after successful completion of Phase 1. The study results will be reviewed as they proceed. This means that studies can stop as soon as their objectives are achieved.</p> <p>Each phase has a clear primary objective. However, additional data about the device and the animal will be collected at the same time. This will maximise the amount of data obtained from each animal. It will reduce the number of experimental animals needed to test this device in the future.</p>

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The pig was chosen because pig and human lungs are very similar. Testing in pigs is the best way to determine if the device is safe, effective and suitable for testing in humans.</p> <p>Pigs will always be anaesthetised for needle biopsy of the lung. Long-acting painkillers will be given. Anaesthesia will be carried out by veterinarians in a facility with advanced monitoring equipment. The needle biopsy procedure carries a risk of lung collapse. Needles will only be inserted into one side of the chest, so only one lung is at risk of collapse. Advanced imaging (CT) will quickly detect lung collapse during or straight after the procedure.</p> <p>To maximise their welfare, we will monitor the pigs closely as they recover. We shall also investigate other ways to refine their care. This may include monitoring oxygen levels in conscious pigs. If a conscious pig develops breathing problems it will be killed immediately.</p>
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Project	New opportunities for improved control of sheep scab disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	3 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Sheep scab disease caused by parasitic mites, is an increasing problem in the UK, with limited control options available consisting of injections or dipping sheep in chemicals. There are growing concerns over the welfare of animals being treated, residues left in meat and pesticide resistance in UK mite populations. This project aims to improve the health and welfare of sheep by researching three aspects of sheep scab mite control:</p> <p>1. Assess pesticide resistance in UK mite populations and develop a test to detect</p>	

	<p>pesticide resistance in mites which will determine the effectiveness of current treatments for sheep scab mites.</p> <p>2. Identify alternative control measures for sheep scab mites by finding new biological areas within the mites' genetic code where pesticidal activity works, by using computational and molecular biology techniques.</p> <p>3. Develop an artificial breeding method for sheep scab mite so mites can be produced without using sheep, for research purposes in the future. Mites cannot be currently bred without sheep.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The overall benefits of this work are expected to improve the health and welfare of sheep. 1. If a test can be produced to understand if mite infestations are resistant to a particular pesticide, alternative pesticides can be used and the sheep would not receive an ineffective treatment, saving time and money. 2. The development of new control measures would provide alternative treatment options, thereby reducing disease incidences. There are limited options currently available for the treatment of sheep scab mites. 3. Developing an artificial breeding system for sheep scab mites would reduce the need for using sheep in the future (on which the mites need to feed and develop) and provide large numbers of mites for research purposes.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Sheep, approximately 36 over 3 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Mites cannot be currently obtained without the use of live sheep. Therefore, to obtain mites for use in experiments, live sheep are required on which the mites develop and feed. Sheep used in this work will be kept in outside pens before being infested with mites. They will then be brought into internal pens within a secure building. Low numbers of mites (about 30) will be added to sheep by parting the fleece and adding the mites to the surface of the skin. The mite population will be allowed to develop for</p>

	<p>approximately 6-8 weeks. Sheep will be monitored daily as part of the sheep maintenance regime and at least weekly to determine the spread of infestation. Due to the frequency of examination it is not expected that the mite population will increase considerably to pose a serious welfare issue. However, susceptibility to mites in individual sheep can vary and this is considered during the examinations. The waste products produced by the mites cause an allergic reaction in the sheep which makes their skin very irritated and itchy. Reactions to the infestation can include head twitching and nibbling when handled, spontaneous biting or scratching and areas of pulled out fleece. The animals will be humanely killed when the infestation covers >25% of the total body surface area or if the reactions persist and palliative treatment is unsuccessful. The mites will be removed from the sheep skin post-mortem.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Sheep scab mites live on the external surface of sheep skin and are entirely dependent on the sheep for feeding, reproduction and survival. Previous studies have shown that it is possible to keep mites off of a sheep for limited periods, with some developmental progression, but a complete life cycle has yet to be achieved. It is therefore not currently possible to do research on sheep scab mites without using sheep. This project aims to investigate whether this can be achieved.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>The sheep will be used to provide mites for use in laboratory experiments. Previous experience has shown that mites removed from individual sheep should provide sufficient numbers for multiple experiments thereby maximising data yield from each animal and minimising the overall number of animals used. The mites will be used in different experiments, thereby reducing the need for additional sheep to be infested. Whilst the development of the infestation in individual sheep can be unpredictable, sheep will be sourced from previously used organisations which were found</p>

	<p>to provide good quality animals which responded well to the mite infestation, thereby reducing the need for further animals.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Sheep scab mites need sheep for survival and development. The rabbit ear canker mite is closely related, but there is no realistic alternative to using sheep, as variations in responses may occur depending on the mite species. Also sheep scab extracts from natural infestations are required to determine the nutritional and developmental requirements of the mite in order to investigate the development of an artificial breeding system.</p> <p>Due to the frequency of examination and removal of scab mites post mortem, it is not expected that the population of scab mites would increase sufficiently to pose a serious welfare threat, however the susceptibility of individual sheep to scab mite can vary. It has been found that Poll Dorset breeds of sheep tolerate scab mite infestations better than some other breeds, and these will be used by preference. To monitor the well-being of the sheep, they will be inspected and monitored daily by the animal care staff as part of the maintenance regime (feeding, watering, cleaning out etc.), and by the project researchers at least weekly to determine the spread of individual infestations. Sheep are maintained as a minimum of two individuals per pen, such that there is always a companion animal.</p> <p>The measures used to assess welfare/well-being include degree of head twitch and nibble when handled, spontaneous biting or scratching, fleece displaced (pulled/tagged), scab infestation covering <25% of the total body surface area and demeanour and feeding. Symptoms exceeding these, such as superficial open wounds caused by scratching, more extensive lesions (>25% of the total body surface area), or secondary infection in wounds are notified immediately to the vet for advice / palliative treatment. If symptoms persist the sheep will be humanely killed to prevent undue suffering.</p>

Project	New targeted nanomedicines for cancer therapy
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research <input checked="" type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The aim of this work is to develop new delivery systems able to carry anti-cancer therapeutic DNA and drugs specifically to the tumours, without secondary effects to normal tissues.</p> <p>The objectives of this study are:</p> <ul style="list-style-type: none"> - to characterise novel drug and gene delivery systems <i>in vivo</i> - to determine the efficacy of anti-cancer therapies delivered by these systems - to determine the ability of these systems to reach the brain following intravenous

	administration
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	At least 1 in 3 people in the UK will be diagnosed with cancer during their lifetime. Therefore, improved treatment of cancer would greatly reduce suffering and save many lives. The efficacy of conventional therapy is often limited by its difficulty to selectively reach tumours after intravenous administration, without secondary effects to normal tissues. Developing novel targeted treatments for cancer will not only kill the tumour cells but minimise the death of normal cells in the body. They will therefore reduce the painful side effects associated with conventional therapies and improve the likelihood of patient survival.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice are the species of choice for these studies because they demonstrate many features of the human diseases and the genes involved are common to both species. We expect to use a maximum of 630 mice per year. The number of animals to be used is the minimum that will give any statistically significant results. If fewer mice are needed to get the results required, then lower numbers of mice will be used.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	New delivery systems will be extensively tested in cell culture and only those of proven efficacy are advanced to in vivo studies, first to establish suitable dosing, then to evaluate their biodistribution. The delivery systems showing suitable biodistribution will then be tested for efficacy in a tumour-bearing animal. Efficacy will be measured as tumour growth delay, by calliper measurement of subcutaneous tumours. A pilot study with just a few animals will indicate if further work would be appropriate. In all experiments the mice will be monitored closely to ensure that no unforeseen adverse reactions cause distress to the animals. Tumours will be established in mice by a single injection of cancer cells and allowed to grow until they reach a suitable size for distribution and therapy studies. Tumour growth will be measured before and after treatment to determine the response to the novel therapy. The mice will be continually assessed for any (rare) signs of distress. We will take every measure to avoid any animal suffering. Following humane

	<p>killing of the animals, tissues such as liver, lung and tumours will be removed for analysis.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>In order to avoid as much as possible the use of animals, the new nanomedicines will first be thoroughly tested <i>in vitro</i>. However, a total replacement of <i>in vivo</i> experiments cannot be achieved in our research project, as the overall aim is the development and evaluation of improved drug formulations for the delivery to distant tumours and metastasis after intravenous and other ways of administration. We have fully considered alternative approaches such as computer modelling and using non-protected species such as nematodes, however a whole mammalian organism is necessary to verify the delivery of these therapeutics to their target, the absence of any unspecific distribution, the general toxicity which could eventually occur, as well as any changes in the behaviour of the animal as a result of the treatment.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>This research project pays due regard to using the minimum numbers of animals required to meet the objectives of the programme of work. The sample size will be adjusted based on the experimental outcome. The aim is always to be able to detect differences between treatment groups with the minimum number of animals necessary.</p> <p>A small pilot study with just a few animals can indicate if further work would be appropriate.</p> <p>For imaging experiments, in order to minimise the number of animals involved, it may be more appropriate in some cases for the control to be based on the animal itself, e.g. pre-treatment vs post-treatment or pre-contrast agent –contrast agent-wash out.</p> <p>Increasing the number of imaging sessions per day with the same animals will reduce the number of mice needed to obtain significant data.</p>
<p>3. Refinement</p>	<p>The mouse has been chosen for these experiments, as it is a well-characterised model</p>

<p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>for biodistribution, gene expression and therapeutic efficacy studies. We have extensive experience of this animal model acquired during our previous experiments.</p> <p>Athymic mice will be used for the majority of the experiments as most of the tumours will be derived from human cancer cells such as the epidermoid carcinoma A431. The athymic mouse is the model of choice for these studies as the animal has a depressed immune system, and therefore can grow tumours of human origin. The mice will be kept in suitable barrier housing to protect them from the environment.</p> <p>Animals will be housed in groups in cages with soft bedding and environmental enrichment (i.e. plastic houses). Good husbandry, daily monitoring and care by a team of well-trained animal technicians will ensure that animal welfare is paramount.</p> <p>Methods which cause the least harm to the animals and which are the most likely to produce satisfactory scientific results will be chosen in priority. The use of imaging techniques such as bioluminescence / fluorescence to monitor tumour development and to evaluate the targeting of new therapeutic systems to tumours is a significant refinement of experimental technique.</p> <p>Anaesthesia and analgesia will be used whenever appropriate and possible to minimise the pain, suffering, distress or harm caused to the animal. The <i>in vitro</i> study of the cytotoxicity efficacy of the new therapeutic systems on cancer cell lines, prior to any <i>in vivo</i> experiment, will allow obtaining essential data for choosing earlier endpoints, reducing the administered doses and the injection frequency, in order to cause the least suffering, distress and lasting harm to the animal. The animals will be checked at regular and frequent intervals. At the end of the experiment, the most humane method of euthanasia will be chosen.</p>
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Project	New treatment strategies for myocardial infarction and aortic aneurysm	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Blood vessel obstruction is commonly caused by Atherosclerosis - a 'furring up' of the inside of a vessel. This leads to heart attacks and loss of heart muscle, when blood flow is interrupted in the arteries supplying blood to the heart, and to strokes when blood flow to the brain is interrupted.</p> <p>Alternatively, weakness of the artery wall leads to a 'ballooning' of the vessel, also known as an aortic aneurysm: devastating if this tears or bursts. REDACTED is an example of this kind of disease.</p>	

	<p>Under this licence we will:</p> <ol style="list-style-type: none"> 1. Determine the origins of the cells that repair the heart following heart attack and how these cells are activated and switched on when needed. 2. Establish whether stem cell-derived cells can be used to regenerate the damaged heart muscle following a heart attack. 3. Identify the signals that trigger aneurysms in large blood vessels using human stem cells, test whether the same signals cause aneurysms in animal models of these conditions and develop new treatments for aortic aneurysm.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<ol style="list-style-type: none"> 1. Understanding how heart and blood vessels develop and their responses to injury and disease – new scientific knowledge. 2. Developing a way to regenerate injured heart muscle after a heart attack, using stem cells. 3. Identifying new treatments for aortic aneurysms.
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Approximately 8600 mice and 650 rats over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>For objective 1, genetically modified mice in which we can track heart cells will be used to identify which cells contribute to repair after a surgically induced heart attack and what genes may control this repair process. Some animals will be treated with medication to see if we can alter the repair response. For objective 2, we will try and regenerate the damage done to the heart after a surgically induced heart attack by treating rats or mice with stem cells aiming to restore heart function. For objective 3, we will use mice that develop aortic aneurysms either due to a mutation in their genes or caused surgically or by a drug treatment. The work will identify exactly how aneurysms develop and new treatments to prevent this. The majority of animals on this licence will be under mild or moderate protocols, and suffer minimal adverse effects. However, to test and develop</p>

	<p>new treatments for heart attacks and aortic aneurysms, that could one day be used in patients, we need to use some animals that also suffer a heart attack or aortic aneurysm. These are serious conditions and frequently lead to death in patients, so these animal protocols are severe in category. However, death usually occurs suddenly with only transient suffering. If the animals are suffering, they will be given suitable treatment and if this does not alleviate the suffering promptly, they will be killed humanely. All animals will be killed humanely at the end of the studies. The enormous burden and severity of heart attacks and aneurysms in patients warrants the use of severe category animal protocols in order to find new treatments.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>My group is a world leader in using human stem cells generated from patients to develop cell culture based models of human disease that can replace the use of animal models in some circumstances. Together with collaborators, we have replaced the use of a mouse with a genetic abnormality that predisposed to heart disease REDACTED with a human stem cell model instead.</p> <p>However, it is not possible to model many of the complexities of cardiovascular disease such as interactions of different cell types, immune response and blood flow in culture. Some aspects of disease including assessment of new treatments still require animal studies. Indeed it is usually not possible to take new treatments forward to patients without comprehensive animal studies.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Using the human stem cell disease models that we are pioneering, many aspects of understanding disease mechanisms and testing new treatments can be carried out in cell culture so greatly reducing the number of animals required for final validation. As an example, we have developed a human stem cell model of REDACTED- a genetic abnormality which is passed down in families</p>

	<p>and results in REDACTED. Using just patient derived stem cells, we identified a new disease causing signal, and published the results in a prestigious scientific journal REDACTED with no animal usage at all. Testing of new treatments and final validation of new mechanisms does however require animal models, although these are minimised by the extensive cell culture work already carried out.</p> <p>Similarly, we are generating heart cells from human stem cells and making engineered heart tissues from these in culture. We can test many combinations of cells and materials in culture this way in order to optimise how we regenerate damaged hearts. This strategy of testing engineered heart tissues in culture will again reduce the number of animals finally used in the definitive tests of this approach.</p> <p>In addition we also aim to use noninvasive imaging such as ultrasound (recently purchased for £300,000) that can be used repeatedly in the same animal with minimum discomfort, so needing fewer animals to obtain information from multiple time points.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Animals are housed according to the best recommendations in an appropriate and enriched environment. We collaborate extensively with experts including those in the USA to obtain the benefit of their experience in refining the protocols; so we minimise the effects on the mice and rats and subsequently pain, distress and suffering.</p>

Project	New treatments for inflammatory and chronic skin diseases	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We wish to test potential new treatments for inflammatory skin conditions including psoriasis, atopic eczema, and pruritus (itchy skin) in a mouse model system. Some current experimental treatments use antibodies, but these have to be injected on a regular basis to remain useful. It would be much better for a person to make their own antibodies in response to a carefully designed vaccine.	
What are the potential benefits likely to derive from this project (how science could be advanced or	In general, it is not possible to conduct clinical trials of new therapies in human patients without first obtaining good proof-of-concept data in	

<p>humans or animals could benefit from the project)?</p>	<p>rodents. We plan to move therapeutic vaccines targetting important skin diseases that we can validate as safe and effective in mice into human clinical trials.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Some of the mouse models of skin disease involve specific genetic alterations and we shall have to maintain breeding colonies of these animals (up to 2,000 over five years) We expect to use about 1000 mice to test whether candidate vaccines actually induce good immune responses. We expect to use another 8000 mice in which skin conditions have been induced in a very small area (normally on their ears) and up to 200 mice where more widespread skin disease has been induced in order to test whether promising vaccine candidates actually work, and how they compare with current treatments for the human equivalent diseases.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We do not expect either the mouse breeding programmes or the testing of the immune responses to candidate vaccines to be of more than mild severity. Some of the skin conditions may cause moderate itchiness, but most of the animals should only have mild itchiness or inflammation in a very small area of their skin. We shall use scoring systems to monitor any reddening or itchiness of the skin in the animals so that we can intervene if the animals appear in any way distressed and to ensure that it is never more than scientifically justified. The vaccines and other treatments are expected to cause significant relief of any itchiness and won't cause any harm (other than brief discomfort during at the time of injection). Animals will be killed humanely at the end of the procedures, for tissues to be collected for further laboratory analysis in detail.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Vaccines only work in a whole organism. They require many parts of the immune system to work. This cannot be modelled in test tube experiments. Regulatory bodies will not permit any clinical trials to proceed in the absence of</p>

	proof-of-concept data from studies in rodents that show principle efficacy and safety of new medicines.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We will limit our studies to models of skin diseases which have already been shown to be of no more than mild discomfort to affected animals.</p> <p>Vaccines will have been formulated in such a way so that they should not be toxic to the mice in any way. When starting our investigations of a new vaccine type, initial animal numbers will be kept very small in pilot experiments to ensure the absence of unexpected and significant toxicities.</p>

Project	New treatments for metabolic disease and its complications	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	4 Years 6 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The aim of this project licence is to discover new drugs for the treatment of diseases caused by the so-called western diet, rich in fat and sugar. These diseases include diabetes, obesity, liver and kidney diseases. In 2016, it was estimated that 1.9 billion people are were over-weight, of which 650 million were obese. There are 425 million people worldwide living with diabetes. Chronic kidney disease affects 10% of the population and remain silent until advance stages; as a consequence, over 70% of patients with diabetic kidney disease are dead within 5 years of diagnosis. There are no drugs available for chronic kidney disease, and drugs to treat diabetes and obesity are insufficient to</p>	

	adequately control the disease, as their prevalence in continually increasing. Therefore, new drugs are needed combat these debilitating diseases.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This program of work is expected to identify and select approximately 6 potential new therapeutic agents, with demonstrable efficacy in animal models of metabolic and kidney diseases, for clinical development. This will result in developing new treatment for metabolic and kidney diseases, an enormous potential benefit for patients. This project will advance our understanding of the cause of these diseases, and our intention to publish the research findings in publicly available journals will benefit the scientific community.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use rats and mice during this program of work. Some animals will be genetically altered to modulate a specific gene, or to produce a specific disease state such as diabetes, obesity or high blood pressure. We expect to use approximately 7000 mice and 1000 rats for diabetes, obesity and metabolism studies and 5400 mice and 1900 rats for chronic kidney disease studies over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	1. Healthy or diseased animals (including genetically-altered animals) will be used to harvest cells from different organs and will experience mild or moderate severity, respectively. Healthy animals will not experience any adverse effects. Diseased animals will experience weight gain, high blood sugar, high blood pressure, excessive urination which will last for the duration of the experiment but will not reach a level that is likely to cause pain or death of the animals. 2. In vivo healthy animal studies: young adult animals will undergo the following interventions: a) Administration of substances either orally, under the skin, into the abdominal cavity, directly into the blood stream or into the brain. Some of these will require a surgery under general anaesthesia. i. Under the skin administration might require placement of a minipump. This is done by making a superficial cut into the skin (approx. 1 cm in length), inserting the minipump under the skin and

	<p>closing the cut with sutures. ii. Direct administration into the blood stream may require placement of a permanent cannula into a blood vessel. This is done by making a cut into the skin (approx. 1 cm in length), inserting a thin flexible tube into a blood vessel and securing it with ligatures and a special glue and closing the cut with sutures. iii. Direct administration into the brain will require placement of permanent cannula into the brain. This is done by making a small incision in the skin on the head of the animal at an exact location, drilling a tiny hole through the skull to insert a thin flexible tube directly into the brain. The tube is maintained in place by a surgical polymer and the incision is closed with sutures. We will ensure full recovery of the animal before performing an experiment.</p> <p>b) No animals will undergo more than 2 surgeries. c) Measure of body function: blood pressure, kidney function and metabolism. d) Imaging under general anaesthesia. e) Animals could be singly housed. f) Blood and urine collection. g) Animals will be killed by a humane method and tissues taken for analysis after death by highly trained and competent individuals. h) Impact on animal experience: i. The overall impact is moderate. ii. Transient pain will be associated with blood withdrawal or substance administration. iii. Pain will be associated with surgery. iv. Stress will be associated with isolation from single housing. v. Transient stress will be associated with restraint for blood pressure measurement. vi. We do not expect any animal to die because of these procedures. I) Mitigation for impact on animal experience: i. Animals are expected to recover quickly from the surgeries. Post-surgical pain will be monitored at least daily and alleviated using painkillers. ii. Use of environmental enrichment in housing to relieve stress of isolation. iii. Acclimation to restraint before blood pressure measurement.</p> <p>3. In vivo disease model studies: young adult animals will undergo the following interventions: a) Induction of disease using either genetic alterations, modified diets or surgical procedures under general anaesthesia (i. removal of one kidney, ii. removal of 5/6th of the total kidney mass or iii. obstruction of urine flow). i. For the removal of one kidney, a 1-cm incision will be</p>
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	<p>made in the flank of the animal, the kidney will be exposed and surgically removed, and the incision will be closed with sutures. ii. For the removal of 5/6th of total kidney mass, one kidney will be removed as described above and a second surgery will be performed one week later to remove 2/3 of the remaining kidney. For this, an incision will be made in either the abdomen or flank of the animal, the remaining kidney will be exposed and part of it will be removed. The incision will be closed with sutures. iii. For the obstruction of urine flow, an incision will be made in the flank of the animal and ligatures will be placed around the ureter, the canal that carries urine from the kidney to the bladder. The incision will be closed with sutures. For all the above, no experiment will be performed until full recovery of the animals. b) Control groups will contain healthy animals or sham-operated animals where appropriate. c) Administration of substances either orally, under the skin, into the abdominal cavity, or directly into the blood stream or into the brain. Some of these will require a surgery under general anaesthesia (surgeries detailed in paragraph 2.) d) No animals will undergo more than 2 surgeries. e) Measure of body function: blood pressure, kidney function and metabolism. f) Imaging under general anaesthesia. g) Animals could be singly housed. h) Collection of blood and urine. i) Animals will be killed by a humane method and tissues taken for analysis after death by highly trained and competent individuals. j) Impact on animal experience: i. The overall impact is moderate. ii. Weight gain, high blood sugar, high blood pressure, excessive urination will last for the duration of the experiment and will not reach a level that is likely to cause pain or death of the animals. iii. Transient pain will be associated with blood withdrawal or substance administration. iv. Pain will be associated with surgery. v. Stress associated with isolation from single housing. vi. Stress associated with exposure to cold temperature. vii. Transient stress associated with restraint for blood pressure measurement. viii. We do not expect any animal to die because of these procedures. k) Mitigation for impact on animal experience: i. We will not mitigate the clinical signs of disease because we will test the</p>
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	<p>ability of the drug substance to reverse them. However, diseased animals will be monitored closely for the inability to feed, groom, nest, walk or breathe normally. Presentation of these signs will result in animals being humanely killed. ii. Animals are expected to recover quickly from the surgeries. Post-surgical pain will be monitored at least daily and alleviated using painkillers. iii. Use of environmental enrichment in housing to relieve stress of isolation. iv. Exposure to cold temperature will not be mitigated because this is a required component the experimental design. v. Acclimation to restraint before blood pressure measurement.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>There is currently no in vitro or in silico system capable of simulating complex whole animal physiology and metabolism. Metabolic and kidney diseases have a complex pathophysiology with multiple components interacting to manifest the disease. Our therapeutic agents target specific biochemical responses or physiological mechanisms that in vitro systems cannot replicate.</p> <p>Individual mechanisms can be probed in vitro, and we conduct extensive studies to characterise these as far as possible before conducting in vivo experiments. In this case we expect to access human tissues and cell lines and use these to understand at a basic level what mediators and mechanisms are involved.</p> <p>Regulatory authorities such as the FDA and EMEA require compelling data packages to support the development of a new medicine in humans. In vitro potency data are seldom sufficient to provide confidence of efficacy in man, and demonstration of activity (and mechanism) in animal models is becoming increasingly important.</p> <p>We are currently implementing the use of human kidney organoids which reflects the complexity of the human organ in a dish. We anticipate that characterising these and investing in this technology will enable less animals to be used in</p>

	the future.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use relevant statistical tools (e.g. power analysis) to guide the design of our studies. Reference will be made to key texts (e.g. Festing, The Design of Animal Experiments, RSM Press 2002).</p> <p>Study designs will be consistent with accepted scientific methods and will include relevant positive and negative controls as applicable. For example, we will minimise unwanted sources of variability by ensuring that wherever possible experimental and control animals are studied side-by-side on the same day by the same person.</p> <p>We have access to in house statisticians with whom we consult as necessary when planning in vivo studies.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice and rats are small and easily handled species with a highly characterised immune system and well-defined biology.</p> <p>Mouse and rat models of metabolic and kidney disease have been established by other groups and reported in the literature.</p> <p>The inclusion of mice enables us to use mutant or genetically modified animals for early hypothesis testing, target validation and humanization of target as necessary.</p> <p>Our models will be the minimal severity possible to answer the scientific question being studied. Pilot studies will be conducted for new protocols to ensure the methods used provide for the maximum animal welfare in relation to the experimental objective. We will also aim to implement new ways, as technology evolves, to further improve the welfare of the animal over the course of these experiments (e.g. by embracing non-invasive measurements).</p> <p>Best practice, for example the use of analgesics after surgical implantation of continuous delivery devices, will be employed to minimise suffering.</p>

Project	Non-coding genes in osteoarthritis and musculoskeletal ageing	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Arthritis is a common disease that causes joint pain and results in failure of the joint cartilage. Age is an important factor in the development of arthritis. We think non-coding RNAs (such as snoRNAs and microRNAs), which are novel and important molecules that control other RNAs and processes in cells, have a role in how cartilage alters in arthritis and why there is an increased risk of arthritis as we age. We also think that the genes that contain snoRNAs may also have a role in arthritis. We will identify which non-coding RNAs change in cartilage ageing and arthritis. For some specific non coding RNAs we will find out	

	<p>what their role is in arthritis. This is because we believe in the long term development of ways to alter non-coding RNAs will allow techniques for early diagnose and new treatments for arthritis.</p> <p>The musculoskeletal system is severely affected by the ageing process. Articular cartilage is susceptible to age-related diseases, such as OA. OA is the most common degenerative joint disorder worldwide, affecting 8.75 million people in the UK, and presents with degradation of articular cartilage, leading to loss of joint mobility and function, accompanied by chronic pain. There are few treatments available beside total joint replacement and pain relief at present.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>We will develop an understanding of the role of non-coding RNAs in cartilage ageing and OA. This is important as OA is a major burden on millions of individuals in the UK as well as society and the NHS. Therefore as an ever increasing ageing population, in which OA is predominant, patients (for which there is no treatment for OA apart from pain relief or a new joint), society (through less sickness leave) and the NHS will benefit. Animals will also benefit. In particular dogs and horse, also with an ageing population suffer substantially from OA. Potential new targets for diagnostic markers and treatment options are a likely outcome.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The project will use mice that have been genetically altered so that they are not able to regulate their genes properly. Over 3 years we will be using 1670 mice.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In humans the effects of osteoarthritis include a significant reduction in mobility, altered gait and pain. In the mice, the procedures do not exceed moderate severity because we set a humane end point that does not impede their mobility. The animals will undergo joint surgery under general anaesthetic. Potential adverse effects would be wound breakdown at the surgery site. At the end of the experiment mice will be sacrificed and tissue collected for analysis. At the end of the experiment mice will be sacrificed and tissue collected for analysis.</p>

Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The DMM model is an in vivo OA model. It is used to assess changes in the joint in a surgically induced arthritis. Mice in which targeted snoRNAs have been removed from cartilage will be used in order to determine the effect on normal cartilage ageing and OA. The only other models available are in larger animals such as sheep.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The project licence applicant and surgeon for this project is a trained veterinary surgeon. Surgical techniques will be practiced on cadaveric mice and specific training given by experts in the field of DMM surgery.</p> <p>Experiments have been designed so that multiple outcome measures can be obtained from one experiment.</p> <p>We have undertaken power analysis based on previous similar studies and following expert advice in order to define the minimum number of mice required in order to determine an affect.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Transgenic mice will be used in the study. Some of these mice will be aged prior to surgery in order to assess the effect of age. The DMM mouse model is the most well-known and characterised for evaluating OA allowing us to interpret the results of our studies. We anticipate that the DMM model will only be required for approximately six months within the three year project.</p>

Project	Non-invasive stress assessment in rodents using thermography	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Reliable measurements of stress and mental state in animals are extremely important tools for research and welfare assessment within a laboratory setting. While there are some well-established approaches using hormones and behaviour, these are either time-consuming, invasive, or are difficult to interpret. Stressful events stimulate rapid changes in the pattern of blood flow from the periphery to the core. This 'stress-induced hyperthermia' is characterised by increased core temperature and cooler surface temperatures. Surface cooling can be measured remotely by thermal imaging (infrared	

	<p>thermography, IRT), potentially providing a novel non-invasive method to assess stress. REDACTED the effect of acute stress on surface temperature in rodents has not been systematically investigated. We will validate IRT as a non-invasive approach to assess acute stress in rats. Using measurements of surface temperature, we will determine whether temperature changes and their patterning provide information on the strength of aversive stimuli and whether we can distinguish between positive and negative emotional states. Importantly, there is a wealth of existing information on the behavioural & physiological responses of rodents to stress and they have several potential ‘thermal windows’; including eyes, ears, tail, and paws, allowing reliable IRT measurements at multiple body parts. IRT will allow us to produce a temperature-time series for each animal, identifying and potentially quantifying stress and positive states.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Identifying and quantifying stress states are crucial to protect laboratory rodent welfare. This project will provide proof of principle and also practical measurement protocols for the use of infrared thermography to provide a validated non-invasive welfare assessment approach. As well as providing a route to refinement in numerous rodent models where traditional stress measures currently take place, the work addresses the increasing concern of researchers and the public for methods to distinguish different welfare states particularly with regard to housing systems and routine procedures such as blood sampling and handling. It is also likely that the results will be highly applicable to other laboratory rodents or possibly even pest control contexts. Currently, the typical stress assessment tool is the measurement of stress hormones in the blood. Although some non-invasive routes to these measures exist (e.g. salivary and faecal), they have important limitations. Thermal imaging has the potential to provide an immediate, non-invasive and non-contact method of stress detection and quantification. It is also continuous, so that ongoing monitoring of an animal’s state is possible, opening up new possibilities for detailed data collection with regard to stress</p>

	<p>responses. Our previous work in birds shows that this approach has excellent promise for welfare assessment and it is ideally suited to the laboratory environment. A rapid, non-invasive, non-contact system of welfare assessment could also contribute to the assessment of cumulative lifetime experience.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use 500 rats over the five year course of the project.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The project has mild severity because we will expose rodents to short term stressors (e.g. noise, light or handling) once only, and we have carefully chosen the stressors to reflect previous stimuli used in stress research or that reflect the routine experience of laboratory rodents. Approximately 50% of the rodents will be blood sampled so that we can identify physiological responses to the same stimuli, and this is also a mild procedure. At the end of the experiments the animals will be humanely killed.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>There are no alternatives to the use of rats for this work because intact, conscious animals are required for the study of novel methods of welfare assessment. We have fully considered alternatives but since many body systems contribute to the animal's experience of stress and positive states, this cannot be adequately reproduced by other methods.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We have carefully calculated the minimum meaningful numbers of animals for each experiment (some of which will test a single stressor at different magnitudes, others will compare different stressors), based on previous studies of responses to stress in rats and surface temperature changes in birds. Where appropriate, we will employ a factorial statistical designs to maximise statistical power and allow identification of interactions between our measures and causal factors, minimising animal numbers. We will randomly assign animals to experimental groups and we will use animals'</p>

	own baselines in our calculations of surface temperature changes to maximise accuracy.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We will work on rats because large numbers of these animals are routinely exposed to stressful events in the course of research and assessing their welfare is an important goal. Rats are also the animal species most commonly used in research on stress. The durations and intensities of stimuli we apply will be carefully controlled to evoke an appropriate acute stress or positive response. The animals will be habituated to handling (and where appropriate to the filming arena), where handling is not part of the stressor paradigm. We will house the rats at low stocking density with additional environmental enrichment to minimise underlying stress from housing.</p>

Project	Non-rodent/Large Animal Models of Surgery, Toxicity and Safety
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input type="checkbox"/> Basic research <input checked="" type="checkbox"/> Translational and applied research <input checked="" type="checkbox"/> Regulatory use and routine production <input checked="" type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project enables a range of studies to evaluate efficacy and safety/toxicity in domestic animals for the following purposes:</p> <ul style="list-style-type: none"> • To support the development of safe and effective veterinary medicines and other animal health products by generating data to determine their efficacy, safety, tolerance and toxicity in the target species. • To support the development of safe and effective human pharmaceutical products by generating data to determine their safety, tolerance and toxicity in relevant animal

	<p>models.</p> <ul style="list-style-type: none"> ● To assess the efficacy, safety and tolerance of medical devices (or other treatments) used in connection with human or veterinary surgery or disease treatment, including surgically induced models of human or animal disorders. ● To use animal models of skin and mucosal wounding and wound healing to assess the safety, tolerance and efficacy of medical devices, medicines and surgical treatments on wound healing. ● To determine the metabolism and residue characteristics of veterinary medicinal products in the target species ● To determine the metabolism and residue characteristics of agrochemicals or other chemicals to which food producing animals may be incidentally or accidentally exposed ● To assess the potential of relevant chemicals to induce delayed neurotoxicity in humans using the chicken model. ● To obtain biological samples from live animals for use in ex vivo work and quality control, where this is directly related to the other purposes in the licence. ● To develop and/or validate new, alternative or refined procedures/techniques in order to determine new scientific endpoints, or to improve/refine data quality, or to improve/refine animal welfare.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Governments require, and the public expects, that substances/articles to which humans or domestic animals may be exposed are effective and safe and/or well-characterised. Therefore, new substances or treatments must be evaluated before they are made widely available for use; this is a mandatory legal requirement which requires the use of animals in studies to evaluate systemic exposure, efficacy and toxicity. The principal benefit of the project is the provision of data to facilitate sound decisions on safe/effective product development and appropriate regulatory decisions on clinical trial approval or marketing authorisation for new</p>

	<p>medicines or other substances or articles to which humans or domestic animals will be exposed, thus contributing to their protection and safety.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This project uses domestic animal species, which for the purpose of this project are defined as those species generally regarded as farm livestock, i.e. cattle, pigs, sheep, goats and poultry (chickens, turkeys); or as companion animals, i.e. cats, dogs; or species which may fall into either category, i.e. horses, rabbits. The evaluation of safety/efficacy of veterinary medicines and other animal health products, and safety of other substances to which they may be exposed, is self-evidently best achieved through testing in the target species. For studies in which non-rodent species are used as models for the assessment of human safety, species selection is made on a case-by-case basis according to various criteria including physiological, morphological and anatomical similarities with humans. In cases where conventional non-rodent models (pigs or, where justified, dogs or non-human primates) are unsuitable an alternative is needed, and large ruminants, particularly sheep, may often fulfil the necessary validity criteria for use as a toxicological model. Pigs, sheep and goats are all well-established models for surgical studies of various types, again based on suitability/validity criteria (for example, sheep and goats are used extensively in orthopaedic research because of similarities in their bone architecture and bone regeneration processes to those of humans). There is one case in this licence where chickens are used as an animal model for humans, in the evaluation of delayed neurotoxicity; this is because they share a specific biochemical characteristic with humans that makes both susceptible to this type of toxicity. Dogs, cats and equidae (horses) will be used only where the purpose of the study/programme of work cannot be achieved using any other species. In nearly every case, the justification for their use is that they are target species for veterinary/animal health products, where evaluation in the target species is mandatory. Cats and horses will not be used for any other reason under this licence. In rare instances, it may be necessary to use dogs in supporting studies such as pharmacokinetic and metabolism studies, where they have been selected – with appropriate justification – as the non-rodent species for safety/toxicity or pharmacology assessment studies that will be carried out under other project licences.</p>

	<p>Over the five-year duration of the project licence, it is estimated that approximate maximum numbers of animals used will be as follows: cattle 330, pigs 1080, sheep 610, goats 210, horses 160, cats 150, dogs 380, rabbits 230, chickens 3520, turkeys 1600.</p> <p>These estimates are based on historical usage under previous projects with the same overall aims, and on anticipated trends in regulatory and scientific requirements for safety and efficacy data in the subject species. The estimates actually represent total numbers of experimental uses rather than the total numbers of individual animals used, which may be lower due to re-use of animals in circumstances where such re-use does not add significantly to the overall harms experienced by the animals or confound the scientific objectives.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In general, animals are dosed/treated by the intended/likely route of human (or target animal) exposure, and observed regularly to monitor appearance, behaviour and clinical health. The main study types that are performed under this licence for each class of test item are: Veterinary medicines/animal health products: Efficacy – animals are dosed at clinically relevant doses and observations on expected parameters of efficacy are made. In some cases it may be necessary to administer a challenge treatment to elicit a condition against which efficacy can be assessed – for example an experimental infection with intestinal nematodes in sheep to test a veterinary worming medicine. Target animal safety - Animals are dosed at clinical doses and low multiples thereof, and observed regularly. Typical investigative procedures are similar to diagnostic procedures that might be used medically to monitor progress of a human patient (e.g. collection of blood samples for laboratory investigations, or ECG monitoring to assess heart rate/function). Terminal investigations will involve sampling and processing tissues for pathological assessment.</p> <p>Pharmacokinetic, metabolism and residue studies – Animals are dosed at clinically relevant doses and observed regularly. The metabolism, fate and distribution of the test item is investigated by analysing samples of blood, excreta, expired air, milk, eggs and tissues taken post mortem, as appropriate.</p> <p>Agrochemicals/chemicals: Pharmacokinetic, metabolism and residue studies – Animals are dosed at clinically relevant doses and observed regularly. The metabolism, fate and distribution of the test item</p>

is investigated by analysing samples of blood, excreta, expired air, milk, eggs and tissues taken post mortem, as appropriate. Human pharmaceuticals: Safety/Toxicity studies – Dose levels for definitive toxicity studies in animal models are determined in preliminary studies and are selected to investigate mechanisms of toxicity and a safe exposure level (no-effect level) that can be related to expected clinical exposure. Typical investigative procedures are similar to diagnostic procedures that might be used medically to monitor progress of a human patient (e.g. collection of blood samples for laboratory investigations, or ECG monitoring to assess heart rate/function). Terminal investigations will involve sampling and processing tissues for pathological assessment. Pharmacokinetic, metabolism and biodistribution studies – Animals are dosed at clinically relevant doses and observed regularly. The metabolism, fate and distribution of the test item is investigated by analysing samples of blood, excreta, expired air and tissues taken post mortem, as appropriate. Medical devices/surgical models: Surgical models included in this licence are designed to evaluate devices used in the treatment/correction of cardiovascular diseases (grafts, stents, cardiac pacemakers); orthopaedic treatments for bone and cartilage disease; techniques to improve kidney transplant technology; treatments for the ablation/destruction of cancerous tumours; devices for treatment of diabetes; neural devices for treatment of chronic pain and seizures; stem cell/device combinations for surgical repair of lung and bile system defects; treatments for lymph node regeneration; treatments intended to reduce operative or traumatic blood loss; and robotic surgery devices designed to improve surgical outcomes (reduced trauma/pain/blood loss, reduced surgical complication, improved outcomes and faster recovery times). The surgical procedures are performed under general anaesthesia with full monitoring of vital signs and pre-/post-operative preventive analgesia and antibiotic treatment. Animals are monitored closely during surgical recovery and appropriate investigations are carried out similar to those used in safety/toxicity studies. Terminal investigations will involve assessment of healing at the surgical sites and sampling/processing of tissues for pathological assessment. The protocols for all of the above studies have a moderate severity classification. However, most animals are expected to experience

	<p>no adverse effects, or only mild effects such as slight weight loss or transient discomfort due to dose injection or blood sampling. A small percentage of animals may show more significant adverse effects indicating moderate severity, e.g. more marked weight loss/reduced activity. A very small number of animals may experience severe adverse effects without intervention, but humane end-points are applied to avoid this and to prevent unnecessary suffering. Animals in surgical studies are normally regarded as experiencing moderate adverse effects (though they are given appropriate pain relief medication) and may, as a result of the surgical procedure, experience some adverse effects similar to those that might be experienced by human patients; for example, in the case of renal studies, animals may experience some degree of impaired renal function which could potentially lead to kidney failure without appropriate interventions. However, supportive treatments are given to eliminate or minimise these adverse effects, and humane endpoints are again applied. All surgical procedures are performed under anaesthesia, with full peri- and post-operative analgesic cover to reduce/eliminate as far as possible any pain or discomfort during surgical recovery, as would be the case for a human patient. In addition, there is one protocol in this licence with a severe severity classification: the assessment of delayed neurotoxicity in the chicken. Most birds used under this protocol will nevertheless experience no adverse effects or only mild to moderate effects; however, it is necessary to determine (and test for delayed neurotoxicity at) the highest non-lethal dose possible in order to provide clinically relevant data (because humans most at risk of delayed neurotoxicity include those who have been exposed to very high doses of organophosphorus compounds but have survived the acute effects due to medical intervention); this means that the occurrence of severe effects and potentially death in a few birds is likely. However, these potential effects are minimised as far as possible by sequential dosing and the use of antidotes where they are effective to protect birds from acute toxic effects.</p>
Application of the 3Rs	
1. Replacement	Although non-animal (<i>in vitro</i> , <i>in silico</i>) studies can provide useful supporting data to refine and reduce

<p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>animal studies, definitive assessments of systemic exposure, efficacy and toxicity can only be achieved in studies using intact animals, and this remains a mandatory legal requirement; currently, there are no scientifically, ethically or legally acceptable non-animal alternatives available.</p> <p>However, no studies in animals are conducted under this licence until an assessment has been made to determine that the specific study is necessary and justified, i.e. the study aims and objectives are consistent with the scope and purpose of the licence and cannot be achieved by any other means not involving the use of animals. This assessment will involve consideration of any potential non-animal alternatives, review of existing data on the test item and reference to any other relevant information (including literature review, in-house data, information on similar items).</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The numbers of animals used within each study are kept to the minimum commensurate with meeting study objectives, through careful assessment of results at each stage of testing, reference to all available sources of information on the test article under evaluation, compliance with guideline recommendations on minimum group sizes where applicable, and the appropriate use of statistical principles in study design.</p> <p>In some cases, numbers of animals used may be minimised by appropriate re-use of animals in more than one unrelated procedure; however, a rigorous harm-benefit assessment is made to ensure that the overall harms experienced by the animals are not significantly increased.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The animal species to be used under this project licence, and the reasons for their use, are as indicated in the first section of this summary.</p> <p>Sequential testing, with review of findings at each stage and modification of subsequent stages as necessary, maximises opportunities for refinement to achieve the desired scientific endpoints with the least risk of pain, suffering, distress or lasting harm to the animals.</p> <p>Where appropriate, positive reinforcement training (treat rewards) is used to encourage co-operation in</p>

	<p>(and minimise any stress of) handling/procedures. Environmental enrichments appropriate to the species are used within the animal facilities.</p> <p>Animals are monitored for clinical signs of toxicity or other effects on their health and wellbeing, and in order to prevent unnecessary suffering, humane end-points are applied under appropriate veterinary guidance (e.g. modification/withdrawal of treatment with the test substance, provision of palliative or therapeutic treatments, or humane killing of affected animals).</p> <p>In cases where study objectives do not require that animals are killed for terminal investigation, they may be kept alive after completion of the procedures and considered for re-use in further procedures, or release from the control of ASPA for rehoming as companion animals (dogs, cats and horses only) or for return to commercial livestock use (farm livestock species and horses only). The criteria for keeping alive, re-use and rehoming/return to livestock use are applied in accordance with the legislative requirements of ASPA.</p> <p>Where re-use of animals is considered as a strategy to reduce the numbers of animals used, this is assessed against the potential overall welfare harms to the animals, taking into account their overall lifetime experience. After each use, animals are assessed for suitability for keeping for re-use, and any animals showing significant adverse effects will not be re-used.</p> <p>The rehoming of animals as companion animals is subject to careful assessment and confirmation of health, suitability for rehoming including appropriate socialization procedures, and confirmation that the animals do not pose any risk to human health, animal health or the environment. In addition for animals released back to commercial livestock use, checks are made to ensure that any other applicable legislative requirements (e.g. DEFRA requirements on use of animal health products and withdrawal periods for food producing animals) are met.</p>
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Project	Novel immunotherapeutic strategies to treat cancer	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cancer affects 1 in 3 people in the UK during their lifetime and is the cause of over 40% of premature deaths. Treatment frequently involves surgery, usually accompanied by chemotherapy or radiotherapy. However, patients often relapse due to the survival of small numbers of tumour cells and despite decades of work on treatment regimens, the survival for many cancers remained unchanged until very recently. Clearly there is a need to develop new therapies for use either as an alternative to or in combination with conventional treatments. Utilising the patient's own immune system to seek and destroy remaining cancer cells (termed immunotherapy) is an attractive adjunct to current treatments as this can potentially be performed with	

	<p>maximal specificity and minimal toxicity. After many years of disappointing results recent success in cancer immunotherapy has reinvigorated the hypothesis that the immune system can control many if not most cancers, in some cases producing durable responses in a way not seen with many small molecule drugs</p> <p>The overall aim of this project is to explore the utility of new anti-cancer reagents for use in the clinic. The specific objectives are:</p> <ol style="list-style-type: none"> 1. To produce and characterize new anti-cancer reagents. 2. To determine the therapeutic effects of anti-cancer reagents, the mechanism of these effects, and how they may be improved. 3. To develop strategies to promote/modulate a patient's own immune responses to cancer and to understand the underlying immune mechanisms. 4. To understand the way in which tumours develop (tumorigenesis) with the aim of developing reagents that can inhibit the process.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Immunotherapy has the potential to provide long-lasting protection from tumour relapse in patients. The principles obtained from our work will also inform the fields of clinical infection, autoimmunity, transplantation and allergy as well as veterinary science. Animals will be used in experiments between</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Animals will be used in experiments between May 2017 and April 2022. On the basis of our current research, it is estimated that we will use approximately 33,000 animals during this 5 year period. We will apply for amendment to the licence if monitoring shows that this is likely to change significantly.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will</p>	<p>The vast majority of the experiments will result in no adverse effects. When using tumour models, mice will be culled at the humane endpoint. In some cases the administration of immunomodulatory substances may cause transient adverse effects, but</p>

happen to the animals at the end?	these will remain within the moderate severity limit, otherwise the mice will be culled.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We are committed to replacing mice where possible and we evaluate immunotherapeutic agents on cell lines in vitro when we can. However, immune modulating agents act upon multiple cell types across the body concurrently and this cannot be adequately modelled in vitro at the current time. Similarly, to study the interactions between an ongoing immune response and a growing tumour, or to evaluate immune-mediated pathology there is unfortunately no viable alternative to in vivo modelling using animals.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Mice used across experiments are inbred thereby minimising intra-group variability and allowing reduced mouse numbers for experiments. Experiments are always designed with the fewest animals consistent with obtaining statistically valid results. We have performed Power analysis to determine the numbers of mice required to deliver statistically significant results, although through experience we find we can often use smaller numbers of animals without sacrificing statistical significance. Where appropriate, small pilot experiments are carried out where simple factors such as dose or route of administration are not clear. Where multiple inter-relating parameters are to be evaluated, larger factorial experiments are performed to prevent use of excess mice as controls. In recent years significant technological advances have enabled more information to be obtained from one individual mouse than was previously possible (e.g. using multi-parameter flow cytometry and micro-array technology), enabling multiple parameters to be assessed simultaneously from small samples. These technologies thereby facilitate longitudinal studies and reduce the need to cull multiple mice at different time points to sample from the spleen for instance; we aim to fully exploit these new techniques fully where possible. Tumour cells will be stored frozen when possible to prevent mice being used to passage tumour in vivo.</p>

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the least sentient mammal species with an immune system similar to humans. Mice represent a relevant animal model for these studies and the clinical successes now being reported using immunomodulatory drugs against cancer were dependent on data arising from such murine studies. Numerous mouse cancers have been studied and the availability of genetically altered strains, and commercially available reagents aids this research. Environmental enrichment, good husbandry and frequent monitoring ensure high welfare standards. Few adverse effects are anticipated but, should any occur, rapid steps will be taken to ameliorate them or humanely cull affected animals.</p> <p>Death is not an acceptable end-point for cancer models: we have established end-points for humane culling before pain/distress occurs, based on accepted guidelines. Many tumour lines develop as subcutaneous nodules, allowing easy monitoring of tumour size. However, the visible or palpable size of the tumour is only one of the criteria used for determination of humane endpoint. Experiments will therefore be terminated before tumour size limits behaviours (feeding, drinking, movement) or before or at the first signs of, tumour associated symptoms or poor condition of the animal according to well defined guidelines (e.g. facial expression scales; www.nc3rs.org.uk/assessment-pain-using-facial-expressions-laboratory-mice-rats-rabbits-and-macaques). Occasionally, following therapy a subcutaneous tumour resolves from the inside out giving the appearance of ulceration; we have adopted a scoring system from Lloyd and Wolfensohn in the Handbook of Laboratory Animal Welfare and Management to ensure that these are managed with minimum adverse effects to the mice. While the maximum severity limit for much of the work to be conducted under this PPL is set as 'moderate', through experience and good management of the mice, we have found under our existing PPL that the actual severity of most experiments is 'mild'.</p>
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Project	Novel targets for anti-epileptic drug design	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The primary goal of this project is to develop new and improved drugs for the treatment of epilepsy. Epilepsy is a common disorder of the brain that affects approximately 1% of the worldwide population. Importantly, almost one-third of epilepsy patients do not respond to currently available antiepileptic drugs. Furthermore, current antiepileptic drugs can cause severe side effects, significantly affecting the quality of life of patients even when seizures are controlled. Thus there is a clear clinical need for better antiepileptic drugs. A greater understanding of basic central nervous system biology will greatly advance the development of better antiepileptic drugs, by finding new</p>	

	<p>mechanisms ('targets') that could cause seizure. This project is based on identifying such novel targets, for example a protein termed Pumilio.</p> <p>Increasing the amount and/or activity of Pumilio is able to reduce the occurrence and severity of seizures. We have identified a chemical compound that is able to increase expression of Pumilio. This compound is able to reduce seizures. However, the compound is not ideal in terms of its drug-like properties. This project will make a range of compounds related to the original, but that are better at being used by the body and hence more effective in controlling seizures. Following testing in mouse seizure models we will hopefully identify which of the new drugs are the best to take forward for clinical trials in human epilepsy patients.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>At present there are a large number of epilepsy patients that cannot be well treated by existing drugs. For many of these individuals surgery is their only option. However, brain surgery is not without significant risks. Thus, being able to find new ways to control seizures may provide a favourable alternative treatment for these patients. The compounds we develop will likely have good potential to meet this clinical shortfall. These compounds will also find significant use in basic research because they will allow researchers to modify the activity of brain cells to further study how seizures develop and how they impact on the brain.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use 312 mice over the course of this project.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We will induce seizures in 'normal' wildtype mice by injecting specific chemicals. This will allow us to test how effective our new drugs are in preventing seizures. Inducing seizures will change behaviour in the mice resulting in head nodding, pawing, rearing, falling and rolling. These behaviours are rated on a scale called Racine, with stage 5 being the most</p>

	severe. Animals that exhibit the most severe 'stage 5' behaviour will be killed immediately by terminal anaesthesia. All other animals will be killed at the end of the observation period by identical means.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Epilepsy is a complex disease that cannot, at present, be modelled by cell culture or <i>in silico</i>. Moreover, whilst lower organisms (e.g. <i>Drosophila</i>, zebrafish) can be used for epilepsy-related research, significant differences in the structure of the brain and how brain cells function mean that these alternatives are complimentary. Thus, it is essential to use mammalian models to better represent the complexity of the human brain.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>For all experiments we will use appropriate power calculations ($P = 0.05$ at 80%) to minimize the number of animals required to provide appropriate statistical power. We will, additionally, when testing new compounds for anticonvulsive efficacy, run small pilot studies to i) determine appropriate dose to use and ii) ensure no adverse toxicity.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse is a good model for the human brain. Moreover, this has been the animal of choice by most research groups working to understand seizure and identify new antiepileptic drugs over the last 20 years. Thus, our use of mice will allow better comparison of our data to that of others.</p> <p>Seizures can result in significant behavioural effects in the animals, as in epilepsy patients. However, as far as we are aware mice are probably not aware of the seizure or suffering, similar to humans. In most cases the seizures are short-lived and the animals do not show obvious signs of pain or discomfort post-seizure. Animal welfare will be continually monitored throughout the project. Seizure exposure will be controlled in all cases to prevent large and persistent convulsions and in those rare cases where these are seen, the</p>

	<p>experiments will be stopped or drugs given to reduce the seizures. In any surgical procedure animals will be given analgesics (pain killers) after surgery. We will always use the lowest number of animals possible to meet our aims. Wherever possible we will use alternatives, and are already doing work in <i>Drosophila</i>, which will reduce the number of mice used.</p>
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Project	Novel therapeutics for central nervous system disorders	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The objectives of this project are to validate drug targets for treating disorders of the central nervous system (the brain and spinal cord; CNS), to identify new drugs for treating CNS disorders, and to test for the side-effects of new drugs that may occur in the CNS.</p> <p>Compounds in early drug discovery research will be tested in rat and mouse in vivo models of anxiety disorders, schizophrenia, Parkinson's disease and epilepsy.</p> <p>Anxiety disorders are responsible for a large proportion of mental illness. Although a several medications are used clinically they have</p>	

	<p>limitations such as a delay before being effective and side effects when treatment is stopped.</p> <p>Schizophrenia is a mental disorder characterized by thought disorder, hallucinations, delusions and paranoia. Many patients do not respond to current treatments, which unfortunately produce a number of side-effects such as involuntary movements and weight gain.</p> <p>Parkinson's disease is a brain disorder characterised by reduced movement, tremor and rigid muscles. The disease has affects about 1 in 1000 in the general population. Existing treatments are somewhat effective against the symptoms of Parkinson's disease, but do not prevent disease progression, and have a limited effect and many side effects.</p> <p>Epilepsy is a brain disorder that causes with seizures. Epilepsy is one of the most common neurological disorders. Approximately 20-30% of patients have seizures that do not respond current medications. No medications prevent the progression of the epilepsy.</p> <p>Studies are carried out in rodent models of CNS disorders to decide whether a new compound is considered worthy for progression into Development, with the ultimate aim of discovering valuable new medicines that meet an unmet medical need.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The major potential benefit of this work is the discovery of novel drugs for the treatment of CNS disorders, an area of unmet medical need. This project licence will enable drug discovery projects that are aiming to produce treatments which are superior, in terms of effectiveness and side-effect liability, to current treatments. Specifically in the therapy of Anxiety disorders, schizophrenia, Parkinson's disease and epilepsy the aim it to produce new treatments that overcome the limitations of current treatments such as delay to onset, side effects that include involuntary movements, prevention of disease progression and treatment resistance, respectively.</p>

<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Approximately 3000 rodents will be used over the 5 years' of the project.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Adverse effects (eg mild sedation and salivation) are those associated with testing novel compound from early stage drug discovery projects, but the likelihood of occurrence is minimized. Close monitoring and use of pilot studies, will help to keep the incidence of adverse effects to a minimum. There will be behavioural studies, mild food and water retention and some animals will have surgery, in which case sterile techniques will be used, pain relief will be administered, and animals will be monitored. The maximum severity that animals will undergo is moderate. Animals will be humanely killed at the end of protocols.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The in vivo models described in this licence application are employed to generate information about how the whole body responds once it has been given a compound. It is neither possible, nor ethical, to use human volunteers in early drug discovery. It is therefore necessary to use other whole body systems, animals, to find out how a living organism responds.</p> <p>The studies covered by this licence typically follow on from in vitro models/assays performed by our clients. The in vitro models provide useful information about which are the best chemical leads from a particular chemistry program to be select for further study.</p> <p>However, at present, in vitro methods cannot entirely predict and replace the in vivo models described by this licence, as the technology does not exist to simulate the complexity and diversity of the whole body system.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of animals required per group and experimental design are determined on the basis of power analysis, advice from statisticians, published data and previous results that have consistently identified target effects in</p>

	<p>a clear and unambiguous manner.</p> <ul style="list-style-type: none"> • Whenever possible repeated measure analyses will be employed to increase precision, maintain smaller group sizes, and reduce animal usage. For example behavioural measures from one subject might be recorded, and compared, both before and after drug administration, or on multiple occasions over time. • Within each experiment a positive control is included to provide an internal control to compare the relative efficacy of the test compound and to assess the sensitivity/validity of the test procedure on a given test occasion. This good experimental design principle will avoid unnecessary replication of experiments.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Purpose bred, adult free living animals of assured health and genetic status will be obtained from commercial suppliers, or from breeding colonies.</p> <p>Animal suffering will be minimised by the following;</p> <ul style="list-style-type: none"> • Conditions in the animal house follow current best practice, and items such as bone chews are placed in rodent cages for their stimulation. • Competent personnel will perform all studies on this project licence and adverse effects will be minimised by careful handling and the application of good technique. • Guidelines on the limit of volumes of administration of substances and blood sampling will be strictly adhered to. <p>Clear-cut end points are described in the possible adverse event description for the protocol covered by this licence.</p>

Project	Novel therapies for age-related fibrosis	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Tissue scarring (fibrosis) is a natural process in which the proliferation and form of certain cells (fibroblasts) play a key role. While this can be a good thing (e.g. repairing injury), excess or unsolicited fibrosis, can be highly deleterious to many organ systems. It is also directly linked to the gradual process of ageing which results ultimately in organ failure. We have recently found that REDACTED a molecule that transfers a signal from one cell to another, plays a key role in fibrosis in many tissues by carrying a 'fibrotic message' to cell types that are responsible for the condition. Moreover, that blocking REDACTED signalling is highly effective at</p>	

	<p>limiting and indeed reversing the adverse effects of fibrosis in the context of diseases that manifest in a short period of time from a stimuli (e.g. injury) rather than chronic conditions that develop over a long period of time. In this project we propose to investigate the tissues of the ageing mouse and explore how REDACTED affects fibrosis, inflammation and organ failure in ageing. This is the first study to address this question and current information suggest that REDACTED will have significant therapeutic value in treating diseases associated with ageing.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Recently, we identified an increased amount of REDACTED molecules in tissue analysis of several diseases for which fibrosis is known to be an underlying cause. In this study, we are aiming to identify the role of REDACTED in age-related conditions that fibrosis has been shown to already play a role, which may lead to new therapeutic targets that can cure or even prevent age-related diseases.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>12,000 mice over five years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We will study how the cardiovascular system as well and metabolic organ systems (liver, kidney) work in ageing mice and see if inhibiting the REDACTED cytokine has beneficial effects on age-related diseases. Side effects from these studies, which mostly monitor organ function, are expected to be mild. We are in close communication with groups that have experience with ageing studies and we have put in place an assessment system of the overall health of each individual animal in order to avoid harming any of the animals involved in this study.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals</p>	<p>To understand ageing in humans it is necessary to study a mammal and we will perform these studies in mice. We have</p>

<p>and why you cannot use non-animal alternatives</p>	<p>already performed extensive analysis on the cellular and organ level, using cells and tissues that have been isolated previously from animals or humans, whenever it was possible in order to gather necessary information for our research and avoid the use of animals.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>The numbers of animals used will be minimized by designing experiments according to good statistical and scientific principles. For example, we will employ randomisation, cross-over study design, blinding and acclimatization strategies where appropriate. These approaches minimize experimental variation and therefore minimize the numbers of mice that need to be used and the numbers of times an experiment needs to be repeated to reach the physiological end-point increasing the validity and quality of our experimental outcomes. Breeding strategies and designs of alleles will permit the experimental cohorts to be bred in the most efficient manner. Longitudinal studies on the same mice (including non-invasive imaging and serial phenotypic studies) will reduce the overall numbers of mice required to reach the scientific end-points. Numbers will be determined using power calculations based on our published work on ageing which has largely been performed on an inbred C57Bl/6 background.</p> <p>To reduce animal usage we will also endeavour where possible to cryopreserve lines of our rodents as embryos or sperm to reduce the numbers of animals we maintain on the shelf. Prior to generating new lines we will ensure that these lines do not already exist by sending notices via mouse locator services and search mouse databases and the literature. We plan to actively form collaborations in order to optimise the quality of samples collected at the end of each study. We will also bank tissues from our longitudinal studies and share these with other investigators when possible so that they will not have to age mice themselves thus reducing mouse usage.</p> <p>We will follow the NC3Rs ARRIVE guidelines</p>

	in all of our research.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Ageing is a multifactorial condition that can only be studied using animals as we need to study a high number of physiological and cellular parameters in order to understand it. Also, we are interested in investigating the potential therapeutic value of drugs (e.g. antibodies) designed to neutralise the effect of specific harmful molecules, a study that needs a combination of physiological systems and can only be done in animals.</p> <p>All animals included in this study will be provided in advance with anaesthesia in order to be incapacitated during the procedures and analgesia to counteract any possible source of pain that might occur from any of the procedures, whenever it is needed according to official rules and regulations such as those designed by the Laboratory Animal Science Association (LASA). Furthermore, we are planning to use a variety of non-invasive techniques in order to study ageing in animals thereby minimising harm to animals. For studies of kidney function, we will use a skin-based analysis which is quantitative, time resolved and a marked improvement on one-off blood draws that measure toxins in the blood.</p>

Project	Novel therapies for pancreatic cancer	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	To evaluate the pharmacology and antitumour activity of experimental therapies (alone or in combination) in mouse models of cancer, focussing on pancreatic cancer.	
Why is it important to undertake this work?	Less than 1 in 20 people with pancreatic cancer survive more than 5 years, and the majority live less than 12 months from diagnosis, because currently available treatments are largely ineffective. Thus there is a need to (a) understand the underlying reasons why pancreatic cancer does not respond to therapies that are effective in some other cancer types and (b) to develop and test new therapies for treatment of pancreatic cancer. Before novel therapy can be tested in patients we need to identify the best way to administer that therapy (e.g. how often to treat, for how long, and at what dose level, and in what schedule	

	when combined with other therapy), and identify the best ways to measure the effect of the therapy. Ultimately, we need to generate evidence that it works in the most relevant cancer model systems (i.e. mouse models of pancreatic cancer) without being too toxic, before planning clinical trials.
What outputs do you think you will see at the end of this project?	The outputs will be data demonstrating the effectiveness of novel anticancer therapies, identification of which particular tumours (with specific genetic features) are most likely to respond, and identification of the best biomarkers that can subsequently be used in clinical trials to measure response. It will also generate new information on the underlying biology of pancreatic cancer. These data will lead to scientific publications and may generate Intellectual Property. These data will enable Go/No Go decisions on whether to progress the therapy into clinical trials in patients. In addition, the data will aid design of the dosing regimens for clinical trial protocols.
Who or what will benefit from these outputs, and how?	In the short term we anticipate scientific publications to arise from these studies, building a package of data to justify translation of the best treatments into the clinic. There will also be benefit from identifying those treatments that are not effective or have a poor therapeutic index, avoiding future patients from being exposed to non-beneficial, harmful, treatment. In the medium term (2 – 5 years) we anticipate some of our therapies to be made available to patients on phase 1 clinical trials. Longer term, these animal studies may contribute to patient benefit by identifying more effective cancer treatments.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	The proposed studies require collaboration with pharmaceutical/biotech companies who are developing the drugs we wish to test, and we have long-standing and productive collaborations that will continue to give us access to the best therapeutic agents that are commercially viable (and therefore developable). Most of the companies will not have pancreatic cancer as their primary tumour of interest for their drug(s) and so it is mutually beneficial for us to test their drugs in our pancreatic cancer models. We will have access to their scientific expertise and unpublished

	<p>data regarding the agent and the best ways to measure its effects.</p> <p>We will continue our existing multi-centre collaborations: REDACTED which is coordinating large scale efforts to make precision medicine a reality for patients with pancreatic cancer across the UK, by molecular phenotyping of each tumour to enable matching of patients to appropriate clinical trials – our preclinical mouse study data will feed into the portfolio of therapies REDACTED.</p> <p>We are also leveraging novel technology for maximising drug delivery via our multi-disciplinary, multi-Institution, collaboration REDACTED.</p> <p>We will disseminate new knowledge via publication. We would like to publish unsuccessful approaches if there were an appropriate route, as long as the data were not restricted as part of a commercial agreement.</p>
<p>Explain why you are using these types of animals and your choice of life stages.</p>	<p>The majority of animal models of cancer have been developed in mice, and there is a longstanding literature on cancer biology and pharmacology based on mice, which provides the background to our studies. Studies of the effects of drugs on the body and on the whole tumour in its complex microenvironment have to be performed in live animals. Studies on pancreatic cancer require adult mice in order to match the biology of the human disease. We will use some mice that are genetically modified to develop spontaneous pancreatic cancer. We will also use some mice as recipients for implanted tumours which are genetically modified, to either alter a natural gene of interest or to express a “marker” gene that enables detection of certain cell types.</p>
<p>Typically, what will be done to an animal used in your project?</p>	<p>Approximately 70% of the mice will be used in breeding, producing mice with the correct genes for use in experiments.</p> <p>The majority of the experimental mice (the other 30%) will either develop a pancreatic tumour or be implanted with a tumour (or occasionally 2 tumours). The most common site of implantation of tumours will be under the skin, with a very small number of mice having a tumour implanted surgically into the pancreas.</p> <p>Most of the mice on experiments will be treated for a period of up to several weeks with one or more anticancer drugs and/or radiotherapy, and a small number of mice may have</p>

	<p>the tumour under the skin removed surgically. Drugs may be administered either by mouth or by injection. Internal pancreas tumours will typically be monitored by ultrasound imaging under anaesthesia weekly. Typical duration of study for an individual mouse, from the time of tumour implantation, would be 3 to 6 weeks. The mice will be killed humanely at the end of the experiments, with blood and tissues taken for subsequent analysis to provide data on the pharmacological effects of the drugs.</p>
<p>What are the expected impacts and/or adverse effects for the animals during your project?</p>	<p>The majority of experimental mice will experience transient discomfort from drug dosing. Many will undergo short (<30 minute) periods of general anaesthesia (e.g. for imaging or surgical tumour implantation or radiotherapy). They may experience illness due to the presence of cancer (particularly those with pancreatic tumours), which may include weight loss, jaundice, build-up of fluid in the abdomen. They may also experience some adverse side effects from their anticancer drugs or radiotherapy (usually seen as weight loss, diarrhoea, and/or reduction in their normal activity levels).</p>
<p>What are the expected severities and the proportion of animals in each category (per animal type)?</p>	<p>Overall, 56% of the mice are expected to be subthreshold, with 17% experiencing mild severity. 27% are expected to experience moderate severity, with no more than 2% severe.</p>
<p>What will happen to animals at the end of this project?</p>	<p>killed</p>
<p>Why do you need to use animals to achieve the aim of your project?</p>	<p>We are developing drug therapy for cancer. By definition, pharmacokinetics (what the body does to the drug) and pharmacodynamics (what the drug does to the body) require the use of live organisms (animals or man). The Regulatory Authorities (FDA, MHRA, EMEA) will not allow agents to be tested in man until they have been shown to be safe and effective in animals. In addition, a large part of our research program involves exploiting the immune system to aid in anticancer therapy, and this requires the whole organism.</p>
<p>Which non-animal alternatives did you consider for use in this project?</p>	<p>We do perform many experiments in cancer cells grown in culture dishes, to screen different drugs and combinations to identify those most likely to be effective in mouse/man, and to investigate the drug mechanisms. We also use more complex mixed cultures– mixing cancer cells with other cell</p>

	types that in the body may support the growth and drug resistance of tumours.
Why were they not suitable?	We do use cancer cell cultures and complex mixed cultures, but once we have identified the best therapy options we have to test them in the context of the whole animal. It is very easy to kill cancer cells in a dish, but we have to identify those therapies that will not also be toxic to the normal body.
Enter the estimated number of animals of each type used in this project.	mice: 23,355
How have you estimated the numbers of animals you will use?	<p>For each study that we will perform, the number of mice per group will be determined by power calculations using knowledge of the effect size expected or desired, using data on variability in drug response, tumour growth, etc.</p> <p>In previous studies n=10 per group has typically sufficed for monitoring antitumour effect of agents in most xenograft models, but it depends on the variability in each model.</p> <p>Pilot studies are usually performed to characterise growth rate and variability of new models, to inform subsequent studies.</p> <p>For Pharmacokinetic (PK) studies (where we measure drug uptake and what the body does to the drug), 3 mice per timepoint usually suffices, but more may be required for Pharmacodynamic (PD) studies (where we measure what the drug does to the body), when biomarker expression is variable.</p> <p>In studies identifying the maximum tolerated dose (MTD) only 2 animals are used per group for initial dose-finding, to expose the minimum number of mice to potential substantial toxicity, expanding to a larger cohort (typically n=5) to confirm the tolerated dose.</p> <p>We have used these estimates of a typical number of mice per study, multiplied by the maximum number of each type of study we expect to perform over the 5 year period, to estimate the total numbers of animals to be used for the experimental studies in this programme of work (Protocols 3 to 8: 5,855 mice). We have then calculated the expected number of mice on breeding protocols 1 and 2 likely to be used to provide the required numbers of experimental mice, based on our previous 2 years' data on breeding these</p>

	same mouse lines for the same purposes (total 17,500).
<p>What steps did you take during the experimental design phase to reduce the number of animals being used in this project?</p>	<p>Because the speed of pancreatic tumour development in the genetically engineered mouse model can be variable, imaging (e.g. ultrasound scanning or MRI) allows recruitment of animals with pancreas tumours of a particular size, reducing variability and the number of animals per group.</p> <p>Imaging also permits measurement of certain features (such as pancreatic tumour size) at multiple different times in a single mouse, reducing the numbers required.</p> <p>Microsampling (taking tiny volumes) of blood permits multiple samples to be taken from each mouse for pharmacokinetic (PK, drug uptake), so that measurements of drug can be made at multiple times, reducing the number of mice required.</p> <p>For complex transgenic mouse lines with multiple genetic alterations (alleles), as many alleles as possible will be carried homozygously - with both gene copies the same, so that each offspring definitely inherits the genetic alteration. This reduces the breeding required to generate sufficient offspring carrying all the required alleles for completion of the goals.</p> <p>The tumour microenvironment is the environment around the tumour cells, including blood vessels, immune cells, fibroblasts, signalling molecules and biological matrix, with which the tumour cells interact constantly, and which plays a key role in therapeutic response of tumours. Characterisation of the microenvironment of tumours implanted under the skin (using either cell lines or tumour fragments), in genetically matched mice with complete immune system, has identified that these are indistinguishable from KPC tumours, and are suitable alternatives to KPC mice for many studies, so reduced numbers of KPC mice will be needed, with reduced breeding.</p>
<p>What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?</p>	<p>Pilot studies will be performed to characterise growth rate and variability of new tumour models, to inform subsequent studies. A Bioinformatics/statistics core facility will provide advice on the power calculations, study design and statistical analysis.</p> <p>If new breeding colonies are established, we will calculate the numbers of mice required to produce the number of mice with the correct genotype for the studies, and check</p>

	<p>this against the estimate for that protocol. At annual review the total numbers used on each protocol will be reviewed in relation to the number permitted on the licence.</p>
<p>Which animal models and methods will you use during this project?</p>	<p>We will use only mice. Rodents are the species with the lowest neurological sensitivity likely to produce data predictive of the effect in man. Also, the majority of animal models of cancer have been developed in mice, and there is a longstanding literature on cancer biology and pharmacology based on mice, which provides the background to our studies.</p> <p>We will grow tumours in mice by a number of different routes – injecting cancer cells under the skin, into the pancreas, into a vein to simulate metastasis, or via genetic alteration to cause mice to develop spontaneous pancreatic cancer (the “KPC” mouse). We select the tumour models most similar (biologically, genetically and in drug response) to the human cancer. Where the science permits we will use tumours under the skin, as those mice will have fewer health issues than KPC mice, reducing the suffering of individual mice used for therapeutic studies. Some of the mice that are tumour recipients will be genetically altered to express certain markers for detection of specific cell types. Others may be genetically altered to modify a gene suspected of being involved in the response (or lack of response) of the tumour to certain therapy. Mice that are to receive human tumour cells will need to be strains with defective immune systems, to avoid rejection of the tumours.</p> <p>Tumour-bearing mice are required to investigate the activity of anti-cancer agents. We need to test individual agents and also combinations of multiple drugs, possibly combined with radiotherapy and/or surgical removal of the initial tumour, to mirror the treatment that patients could receive. Where appropriate, non-invasive imaging methods are used to monitor tumour growth, and tissue sampling is normally post-mortem, but occasionally biopsies are required from tumours under the skin, to obtain samples from more than one time-point in the same animal (e.g. pre- and post-treatment). Animal suffering will be minimised by the use of anaesthesia and analgesia where appropriate, and environmental enrichment will be provided to promote the expression of species-appropriate behaviour.</p> <p>Most mice on studies will be killed before they show any signs of illness because the scientific endpoint of the study is reached before the cancer is too advanced, but mice will</p>

	be killed if they do develop signs of cancer-related illness or drug-related toxicity, to reduce pain, suffering, distress and lasting harm.
Why can't you use animals that are less sentient?	Rodents are the species with the lowest neurological sensitivity likely to produce data predictive of the effect in man. Also, the majority of animal models of cancer have been developed in mice, and there is a longstanding literature on cancer biology and pharmacology based on mice, which provides the background to our studies. These cancer models require adult animals.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	Via Mailing lists for initiatives from the NC3Rs, information circulated locally by Home Office Liaison Officer and Named Animal Care and Welfare Officers, and information from local annual 3Rs symposia. Changes to practice would be considered and pilot studies performed to ensure any changes do not compromise the ongoing science.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	We have been using these tumour models and most of the procedures for years already and so the methods are already refined. However, we will scrutinise new guidance as it comes available and will look to adopt new best practice when advances are published. For procedures we have not used before, such as surgical removal of the primary tumour, we will use pilot studies to develop the protocol and evaluate the best surgical method, post-op care and pain management protocols. As our understanding increases of the timescale for metastasis growth in our models we will refine our endpoints for those studies to avoid mice suffering from the presence of metastases.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	We minimise suffering by adhering to best practice guidance, currently the "NCRI Guidelines for the welfare and use of animals in cancer research" by P.Workman, et al., Br. J. Cancer (2010) 102, 1555-1577 . For surgical procedures: LASA 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery. A report by the LASA Education, Training and Ethics section. (E Lilley and M. Berdoy eds.). http://www.lasa.co.uk/publications/ For recording and reporting on experiments: Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG

	(2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLOS Biol 8(6): e1000412. doi:10.1371/journal.pbio.1000412
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Project	Novel Therapies for Severe Bacterial Infections	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Severe bacterial infections remain a threat to human health. In recent years, there has been an inexorable rise in marked antibiotic resistance within bacteria. Such drug-resistant infections can be very difficult to treat and in some cases no current effective therapies remain. There is thus an urgent need to develop novel therapies for bacterial infections. This project will explore a number of avenues that we believe will offer new therapies for bacterial infections. We will exploit natural antibiotics produced by bacteria, called bacteriocins, to establish if they can be used to treat bacterial infections. We will define key elements of the body's natural defences to these infections, to	

	develop new therapies that augment these responses that may then prevent or ameliorate infection.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The outcome of these studies will potentially lead to novel therapeutic interventions for severe human bacterial infections.
What species and approximate numbers of animals do you expect to use over what period of time?	We are using mice in this project and estimate to use about 4500 over a 5-year period
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The very nature of bacterial infection does mean that some of the models we use are severe in nature. The effects are on general characteristics, such as coat condition, movement and posture. Additionally, in animals infected by the respiratory route, they may show signs of laboured breathing. However, we have a very clear and well-defined monitoring process that ensures animals are humanely killed once their clinical condition has reached a pre-defined level of severity. At this point, animals will be removed from the study and humanely killed. Otherwise, at the end of the defined time points in the protocols, the animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The studies described in this licence cannot be done without the use of live animals. Extensive laboratory experiments outside of living organisms (“ <i>in vitro</i> ”) analysis is used to identify those experiments that can only be done using whole animals. The animal experiments are designed to study the host/parasite relationship using bacterial pathogens in animal models of human disease. These studies can only be done in systems with an intact immune system, vascular supply etc. To do this an infectious dose of bacteria is administered intended to produce clinical disease without overwhelming the animal. The pathogenesis of the disease

	<p>can then be studied by serial killing during its course. Analysis of the infectious process by bacteriology, immunology, histology, imaging and the use of bacterial and animal mutants allows new therapeutic and vaccine strategies to be investigated. Good reagents for studying these processes and genetically modified mice are available to study response to infection in animal models. Use will be made of <i>in vitro</i> models where simple cellular interactions between cells will be studied. However, the complex interplay between bacterial pathogens and the immune system can only be effectively studied with the use of live animals.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The dose range of novel reagents will be estimated initially using <i>in vitro</i> experiments, thus reducing the numbers of animals required to establish effective doses. Our experimental design has been carefully planned to use the smallest numbers of animals required to give statistically meaningful results. For example, where possible, a control group will be used as a comparator between several experimental arms to minimize animal use. Where exact effective doses of novel therapies are required, we have designed a protocol that will evaluate the results from all doses at the same time, which can then be manipulated mathematically, reducing the numbers of animals required.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mice models we employ are very good models of human infection with the pathogens we study and are very amenable to further experimental study because of the wealth of different reagents available for this species as well as the ability to use well-defined genetically modified animals. We have developed robust criteria for evaluation of animal well-being during the course of the experiments which allow the experiments to be terminated when these reach pre-determined levels. Staff employed on the project will monitor animals intensively following infection, as will the dedicated staff within the animal care facility. We have extensive prior experience of the models to be</p>

	<p>employed and have been able to intervene effectively to remove animals from studies where their clinical condition has exceeded a set limit, thus reducing the potential animal suffering. We will also employ where possible remote monitoring techniques, such as imaging of live bacteria within the animals that can provide a guide to the progress of infection without requiring animals to be humanely killed.</p>
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Project	Novel treatments for kidney disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Kidney disease is a common disease that has significant impact on quality of life and lacks effective treatments. This project aims to identify novel treatments for patients with chronic kidney disease by understanding at the cellular level what happens when people get damaged kidneys. In particular we have focussed on important molecules called non-coding RNA that are involved in the initiation and/or progression of kidney disease. These molecules work by changing expression of defined proteins that affect kidney function in cells. These molecules are essential for kidney function but they become abnormally active in kidney disease. Recently we have found they may also be	

	<p>important in repairing the kidney too.</p> <p>We are also optimising in parallel the production of substances that will carry genes into the kidney which can alter the expression of specific proteins involved in kidney damage which may slow or halt progression of kidney disease.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Chronic kidney disease (CKD) is common and the number of people suffering from this disease is increasing. People with CKD have kidneys that are not functioning properly where the normal kidney tissue has been replaced by scar tissue. Having CKD puts you at a greater risk of a cardiovascular related premature death. Patients with chronic kidney disease can progress to have kidney failure which ultimately results in the patient requiring dialysis and eventually a kidney transplant. The cost of treating these patients is a burden on the NHS. Treatments that can target this scarring of the kidney could reduce the numbers of patients with kidney failure, reduce NHS costs and potentially reduce premature cardiovascular deaths.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Rats and mice (wild-type and genetically altered) have been proposed to be used in these studies as they most published species when examining renal fibrosis and chronic kidney disease. Furthermore the results from these models in mice and rats have been translated to humans. We will use less than 650 rats and less than 1100 mice over a 5 year period. To allow us to perform studies in genetically altered animals we propose to breed less than 4000 mice and 1000 rats. In order to examine kidney disease an animal model has to be used as the disease process involves several cell types and an inflammatory response which we are unable to replicate in cell culture. However, were possible we will use in vitro models of kidney damage to investigate the mechanism of disease and for testing our gene transfer vehicles prior to use in animals.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the</p>	<p>Animals under this licence will develop renal disease under some of the protocols as a result of having to create models of kidney disease in</p>

<p>likely/expected level of severity? What will happen to the animals at the end?</p>	<p>order to identify novel treatments and to investigate and test potential treatments. In order to induce kidney disease animals may undergo surgery or be injected with a compound that damages the kidney. As a result of the kidney damage the animals may lose their appetite, have protein in their urine, develop high blood pressure and may lose weight as the kidney damage develops. Unfortunately, some animals may reach a severe level as a result of some of the models within this project licence but every effort will be taken to minimise this happening by having monitoring systems in place to minimise any animal suffering. Painkillers are used where required and animals are very closely monitored throughout these studies which are not long term.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Due to the complexity of renal disease and the involvement of multiple cell types it is not possible to mimic renal disease in cell culture. However, we have several cell culture models that we can mimic parts of the process for example tubular cells and fibroblast cell lines and we will use these to carry out some of our mechanistic studies prior to carrying out studies in animals.</p> <p>We now have the ability to examine other researchers published data (all published datasets are required to be placed in a freely available public website) and we are able to compare this to our own historical data sets we have. Therefore we are able to use bioinformatics to examine any potential targets by in silico means rather than having to perform additional animal experiments.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>Any experiments using our established protocols have power calculations from initial pilot studies performed by ourselves or collaborators identifying the lowest appropriate group sizes for each procedure.</p> <p>We use fully blinded, randomized studies (using computer generated randomization) and littermate controls were possible to ensure no</p>

	<p>bias in our experimental results and to control for variability. We use the NC3Rs experimental design assistant to help us improve the design of our experimental studies. Animal genetic backgrounds are also considered when designing experiments to ensure we are controlling variability.</p> <p>We actively use historical tissue we carefully bank when we cull animals on procedure. This ensures we have good quality tissue and histological blocks available which allows pilot studies for new avenues of research to be conducted without the use of new animals thus reducing the number of animals we use.</p> <p>All our gene therapy vehicles are extensive quality controlled (QC) before being used in in vitro models to assess the functionality and efficacy of the viral vector and to ensure that the gene expressed is produced. Only those vectors that pass the QC are allowed to continue to be used in animals.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We propose to use rats, genetically altered rats and mice and normal mice in order to achieve the objectives of this proposal. Rats and mice have been proposed to be used in this study as they are the lowest suitable species that can be used.</p> <p>In order to study chronic kidney disease an animal model has to be used as both processes involve several cell types and an inflammatory response which we are unable to replicate in cell culture. We believe that non-coding RNA are involved in CKD and renal/cardiac fibrosis and over expression/knockout of these non-coding RNA should be beneficial therefore use of GA animals should minimise animal suffering.</p> <p>The protocols selected which induce renal damage have been carefully chosen and have clear outputs and humane end-points to maximise animal welfare. The refinements we have introduced to maximise animal welfare include careful monitoring of the animals using special scoring systems to inform on the animals condition. This includes information on the animal's blood pressure, weight and urine. When severe models of renal disease are</p>

	<p>induced pain assessments will be made through careful monitoring of the animals and analgesia given prior to surgery, 24hrs post-surgery and again when necessary. All surgery is carried out using aseptic techniques which limits any infections and animal suffering as a result.</p>
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Project	Novel vaccine development for lyssaviruses and flaviviruses	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<ol style="list-style-type: none"> 1. The development of new vaccines that are able to protect against multiple viruses (lyssaviruses, flaviviruses) following vaccination. 2. Assessment of the ability of existing rabies vaccines to protect against divergent lyssaviruses and recombinant viruses expressing different lyssavirus glycoproteins 	
What are the potential benefits likely to derive from this project	1) Potential identification of novel human vaccines that will go on to further pre-	

<p>(how science could be advanced or humans or animals could benefit from the project)?</p>	<p>registration studies. If successful these vaccines could revolutionise human and animal vaccination strategies the lyssaviruses and flaviviruses. 2) Increase knowledge base on the protection afforded by rabies vaccines, and inform policy on the applicability of different vaccines that target lyssaviruses and flaviviruses.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Potentially up to 800 mice on each of the 3 protocols over the course of 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Protocol 1- Assessment of the pathogenicity of lyssaviruses and relevant flaviviruses in different strains of mice- involves pathogenesis assessments for viruses to use as challenge viruses post vaccination and to simply establish pathogenesis of isolates. Severity is expected to be mild or moderate. Should the outcome be deemed to have been exceeded, for example where an unexpected accelerated disease progression has caused death during the overnight period, the severity limit of the licence will be re-evaluated. Similarly for flavivirus infection, clinical outcomes are used to define humane end points. Protocol 2- Serological assessment of vaccination without challenge- involves assessment of vaccine doses in mice to determine antigenicity and optimal vaccination strategies. This only includes vaccination and serological assessment of animals and as such is rated as mild. No adverse effects of vaccination or the use of monoclonal antibodies to modulate innate immune responses before vaccination are predicted. Protocol 3- Vaccination challenge experimentation with lyssaviruses and flaviviruses- mirrors protocol 1 in that it involves virus challenge with different viruses and although it is expected that all animals will be maximally be moderate. Should this limit be exceeded then the severity rating may be reviewed appropriately. Animals from all 3 protocols are euthanased by a schedule 1 method at the end of the experiment.</p>
<p>Application of the 3Rs</p>	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Initial assessment of vaccine candidates will be done using serological neutralisation of virus in vitro. However, to truly define the antibody response to vaccination and pathogenic virus infection, animals are required. Further, to assess any protection afforded by vaccination, the infection of vaccinated animals is required.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Local ethical review, including by a biostatistician, of proposed in vivo studies will ensure that the minimal number of animals is used. All studies performed for research activities are carried out to ISO9001 quality standards. Our establishment is committed to complying with ARRIVE guidelines. Further, all studies are scrutinised by the AWERB onsite at our establishment.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice and hamsters represent a good choice for these studies as there are established models of lyssavirus vaccination and pathogenesis which results can be validated against. We have a state of the art research facility within which to conduct animal studies to the highest level. Welfare is key to all of our studies and refinements have been made across different experimental platforms to minimise harms including refinement of techniques, improvement in enrichment and environmental elements and increased post inoculation observations and human endpoints.</p>

Project	Nuclear envelope roles in health and disease
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research <input checked="" type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>All cells in an organism share the same DNA sequence (the genetic material); however, the way this DNA is organised in the in the cell nucleus has different patterns for each type of tissue in the body. These patterns play important roles in controlling which specific DNA sequences (genes) are made in each tissue. Much of this patterning is directed by the nuclear envelope, a structure made of proteins and membranes that surrounds the nucleus and separates it from the rest of the cell. The aim of this project is to define the role of nuclear envelope proteins in establishing these DNA organisation patterns, its impact on organism and tissue development, and how its disruption can yield</p>

	<p>human disease. Our data suggests that disruption of this DNA organisation in disease alters metabolism. This research should help understand the basic biology of genome organisation and could eventually yield new treatments for human diseases linked to mutations in nuclear envelope proteins e.g. lipodystrophy, muscular dystrophy, obesity and diabetes.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Mutations in nuclear envelope proteins cause several human diseases including lipodystrophies, muscular dystrophies, heart disease, neuropathies and the premature ageing syndrome progeria. Some genes studied here were also linked to obesity and diabetes. Therefore, there is potential to translate our results into therapies for these major health concerns in addition to our primary target of lipodystrophies and muscular dystrophies. Nuclear envelope-linked lipodystrophies are characterised by local or general loss of fat tissue, with associated metabolic syndromes including insulin resistant diabetes, dyslipidaemia, and non-alcoholic fatty liver disease. Nuclear envelope-linked muscular dystrophies are characterised by progressive loss of muscle tissue and muscle wasting, typically leading to death due to heart failure. We have found mutations in some of the genes being investigated likely cause Emery-Dreifuss muscular dystrophy and other are likely to mediate nuclear envelope-linked lipodystrophies and possibly metabolic syndromes and some obesity. The basic work here on genome organisation will decipher the molecular mechanisms behind disease pathologies and thus direct us towards therapies while some experiments are directly translational.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>To achieve our research goal, we will use genetically altered mice. We use animals carrying mutations in genes that are both designed based on scientific evidence and experiments on cell lines and that were found in human patients. We will also use mice altered to overexpress proteins identified as target candidates for causing nuclear</p>

	<p>envelope linked diseases. Over the 5 years we are planning to use 4000 animals under this licence, most of which will be used only for breeding.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The vast majority of animals will be used for breeding purpose only, and no significant adverse effect are expected in the association with genetic manipulations. Moreover, many experiments are testing treatment methods that are only expected to improve the health of disease model animals. A small number of animals will undergo surgical procedures (transplant of cells for a therapy pilot study) but potential adverse effects will be minimised by using skilled personnel and appropriate anaesthesia and analgesia. All procedures performed under this license are critically assessed by experts REDACTED. There is no protocol in this licence involving prolonged and severe pain for animals. Each procedure will be followed by frequent welfare assessment and animals showing adverse effects expected to breach severity limits will be humanely euthanized. Some short pain will only be cause by glucose and insulin tolerance tests that involve injection of glucose and insulin and during injections of cells in pilot cell therapy experiments. Dietary manipulation will not cause a distress to animals. High fat diet for example was shown to be tastier for animals than standard chow used for regular breeding. Some distress might be caused by single housing necessary for metabolic cages experiment. This is however reduced to 5 days only. All animals will be humanely euthanized by schedule 1 method at the end of the procedures and tissues will be collected for further analysis.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We have extensively researched our hypothesis using mammalian cell culture differentiation systems; however, these approaches cannot test the whole-body metabolism and physiology that depends on a complex interplay of factors in the whole organism for example communication between</p>

	<p>tissues and organs. Our recent studies using a mouse model for one of proteins we study yielded a rare disease phenotype that would not have been visible in the tissue culture systems. Thus, to properly study rare diseases linked to mutations in proteins we are focused on it is necessary to engage these studies in animals. Lower (non-protected) organisms such as fruit fly or worm show limited similarity to mammals on cellular level, they lack many proteins that we research in human and mouse, and some are also significantly different from humans biologically. When ethically possible and scientifically appropriate we will use human tissues donated by patients in our experiments to confirm findings in mouse model.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The animal numbers used in these experiments will be assessed using statistical parameters and compared with similar studies of this type previously published. In addition, we have combined minimally invasive and non-regulated procedures into the same protocols such as dietary manipulation, weighing, behavioural tests, etc so as to minimise the number of animals used. Furthermore, individual blood tests will be used to measure several blood markers from one injection to reduce animal numbers.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mouse is recognised as the best organism to model rare human diseases due to closely matching metabolism and physiology and because several genetically modified mice we plan to use already exist and show phenotype similar to rare diseases we are focused on. Finally, small rodents show common architecture and composition of the group of proteins of our interest with humans.</p> <p>To minimize pain and stress to the animals, the majority of the experiments will be performed on tissue from humanly euthanised mice. Mice will be housed with the appropriate nesting material (for example soft paper or soft wood) as well as material to gnaw (to prevent teeth overgrowing) for example soft wood blocks and chow provided in hard pellets. All</p>

	<p>animals will be housed with cardboard tubes for enrichment, hide and retreat from cage mates, and added cage complexity and additional material for chewing. Single caged animals will be provided with additional nesting material to help regulate body temperature and light levels. Cleaning protocols will be also applied as refinement methods, for example clean cages will be supplemented with some scent-marked nesting material to avoid stress caused by fresh nesting. Procedures on living animals will be performed by experienced research staff and when applicable with the use of aseptic surgical techniques. We will implement health monitoring system to regularly assess distress of animals and wellbeing. Mice will be gently handled from early life (and weight) to adopt and reduce stress from interaction with human. Finally, if any mouse shows symptoms of severe pain or distress at any stage of an experiment, the procedure will be terminated, and animal euthanized by schedule 1 method or referred to the Named Veterinary Surgeon for advice.</p>
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Project	Nuclear receptor regulation of circadian rhythms, metabolism and inflammation	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim to establish the interplay between the body-clock and both energy metabolism and inflammation. Increasing evidence supports the view that energy metabolism and inflammation are fundamentally linked with the same cells (e.g. macrophages), and networks (e.g. glucose metabolism), central to both processes. Our recent work suggests that the body clock plays essential roles in both how the body handles nutrition, and also how the body protects itself against damage, or infection. We can use these insights to develop new treatments for common diseases, by targeting various proteins that can	

	<p>respond to drugs, or drug-like molecules. We think that these are the links between the body clock, inflammation and energy metabolism, and that they may prove useful targets for new medicines in the future.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The benefits arising from this work range from new discoveries into how the fundamental processes of energy metabolism and inflammation are regulated, and how they affect one another. We expect to find new control circuits linking these two super-systems and anticipate that some of the proteins we find that can respond to drugs (receptors) and circadian regulated networks will lie at the centre of these links. Further we will find new targets for drug intervention which we will seek to test in follow on experimental studies in humans, eg asthma. We also expect to improve our understanding of how circadian control systems vary the responses of the commonly used drugs targeting the nuclear receptors across the circadian cycle. This will allow us to find new ways to use the power of our body clock to aid treatment of multiple human diseases eg diabetes. Our results will help design new drug trials in people. As an example, we now have a human clinical trial in asthma, which is testing the importance of the time of day at which a commonly used medicine is given. We are testing if optimising the time of day may result in greater benefits from a common and cheap medicine, which will bring rapid benefit to patients.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The projected duration of the project is five years. We aim to minimise animal usage throughout, and constantly seek new refinements to reduce, replace, and refine the animal studies. We will use mouse as our subject species, as this is the lowest sentient form suitable to model processes relevant to human health and disease; and also because we are able to manipulate gene expression easily in the mouse.</p> <p>We anticipate a maximum number of mice used to be 16,500, but most of these numbers relate to simple breeding of mice for killing in order to</p>

	<p>collect cells and tissues. Such mice will be bred, and maintained in group-housing conditions, with cage enrichment, and ad-libitum food and water. Their well-being will be monitored, and signs of illness, or distress acted upon.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Much of the animal use should result in a very low level of severity limited to breeding, tissue biopsy and humane killing. Many fewer mice will experience greater severity. Typical procedures would include non-invasive monitoring of home cage activity and metabolism following altered diet.</p> <p>Some mice will have altered diet, which will either cause them to gain or lose weight. They will then have injections to challenge their metabolism. Some will also have measurements of their body composition, or imaging of their body organs under general anaesthesia.</p> <p>Some mice will have small devices surgically implanted beneath the skin, or in the abdominal cavity, in order to record temperature, or to deliver a substance over a prolonged period to time. Some will also have a pellet implanted beneath the skin to cause prolonged release of a compound.</p> <p>In addition some animals will have blood testing for glucose concentrations after a glucose challenge, or an injection of insulin. Some animals will have drugs administered by injection, or by oral administration (adding to food, or water, or by gavage).</p> <p>We will make very limited use of low temperature challenges to the mice, which will result in compensatory changes including altered behaviour, nest building, huddling in a group, and the animals will experience being cold.</p> <p>In one protocol animals will have an inflammatory, or immune reaction started by administering a substance which will mimic the effect of an infection. In this way the animal will experience an increase in body temperature, increased need for sleep, loss of appetite, and</p>

	<p>loss of social interaction.</p> <p>For some of the measurements the animals will be singly housed, which will cause some distress, as mice are social animals.</p> <p>At all times animals will be closely monitored, and will be humanely killed if any animal welfare issues emerge. Experimental animals will be humanely killed by an approved method at the end of the studies.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>As far as possible we will use isolated cell and tissues from human donors or humanely killed animals. As mice and humans share great similarity at the DNA level, and share many of the same responses to challenges relating to the immune system, or to excess, or insufficient food the mouse is a good model for aspects of human health and disease. Indeed, elaboration of an inflammatory response, or the adaptation to altered nutrient exposure, or temperature require live animals, and the live mouse is a useful, and accurate model.</p> <p>The systems we study are not well-conserved in lower vertebrates, but where possible we do make use of cell line models, human subjects, and genetic studies in large human cohorts.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We carry out careful experimental design, using our full-time computational biologist and statistician, to ensure clear primary objectives, and consideration of the power of the study. We ensure sufficient animals to reliably test the hypothesis, and avoid studies with excessive variance, or noise, which makes obtaining clear data difficult.</p> <p>We monitor all our genetically altered animal colonies to minimise the numbers of animals generated, and to maximise the efficient use of any animal that is bred.</p> <p>We cryopreserve sperm from GA mice that are not in active use, to prevent breeding of mice</p>

	<p>that are not essential for the scientific purpose.</p> <p>We have a full-time statistician in the group to help with experimental design, and we adopt best practice by avoiding bias, cage effects, and observer effects by use of masking of the scientists as far as possible, eg the scientist performing the in-vivo part will code the animals, so the scientist analysing samples is not aware of group membership until the code is broken after analysis is complete.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use mouse as the lowest sentience mammal on which we can perform the studies. Mouse also offers us ease of transgenic manipulation, surgical intervention to target specific structures, and abundant previous data in the public domain which we can use to minimise new experimental data acquisition.</p> <p>We monitor all experimental studies carefully, and ensure rapid euthanasia is used to handle any animal in distress.</p> <p>We have developed new ways to test inflammation in the mouse, eg by creating an aerosol of an inflammatory stimulant we are able to induce a mild inflammation in the mouse lung without the need to directly inject the mouse.</p> <p>For those mice used in breeding protocols for tissue and cell harvest the animals will not be tested in-vivo, and will not be disturbed before humane killing.</p> <p>Some mice (circa 10%) will have changes to body function and behaviour eg inflammation, altered feeding, and altered ambient temperature. Severity limits will include weight loss, and signs of distress. Some specific examples are included here:-</p> <p>For some of the interventions we will need to use surgery, for example to implant telemetry devices under the skin, which will be used to track variables such as temperature without the need to disturb the animal repeatedly. In the case of surgery we will use general anaesthesia (so the animal does not feel the surgery), sterile technique (to reduce the risk of wound infection), and post operative pain relief (in a</p>

	<p>similar way to post operative care for people).</p> <p>We will try to minimise animal numbers by performing longitudinal studies where possible. For example we can use mice transgenic of a luciferase gene to report gene activity by using photon capture technology. In this way we can track gene expression through time, over many days, to get very high quality, and high resolution data from a far smaller number of animals, and the experience of the individual animal is restricted to the breeding, implantation of the slow-release compound delivery pump, and exposure within the recording cage.</p> <p>Dietary manipulations may cause a weight gain, or weight loss. For this reason we will monitor body weight, and have limits beyond which we will not take the animals. High fat diet can cause issues with coat grooming, and so we will monitor for coat condition during such treatment, and have limits in the event that the animals are seen to suffer.</p> <p>Changes in ambient temperature cause the animals to change their behaviour, and to use energy to maintain body temperature. We will use temperature probes, which transmit, so we can track the animal response. We have limits for body temperature change, which will cause us to return the animal to regular temperature for recovery. We will also use infrared temperature capture to non-invasively monitor animal body temperature.</p> <p>Some animals will have an inflammatory challenge. This will typically be the lowest exposure needed to see a response. Animals will be carefully monitored, and we have limits in place to stop the experiment and to humanely kill the animal in the event that the limit is exceeded. Where possible we use a minimally invasive route for administration, such as generating an aerosol, which the animal can breathe in. This approach results in localised lung inflammation, without further disruption to the animal's routine care.</p>
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Project	Nutrition and lactation in ruminants	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
	<input checked="" type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>To provide novel information on effects of nutrition on physiological and metabolic processes in dairy cattle and other ruminants that will increase understanding of the biology and allow development of strategies to</p> <ul style="list-style-type: none"> • improve efficiency, fertility and health of dairy cattle and their offspring • improve nutritive value of milk for human consumption, • reduce the environmental impact of dairy farming. <p>The objectives of this project are:</p>	

	<p>1. Responses to nutrition - To quantify influences of diet composition, stage of lactation, growth stage and genetics on feed efficiency, rumen function, metabolism, health, reproduction and environmental emissions.</p> <p>2. Lifetime performance – To develop approaches for rearing dairy offspring that optimise health and growth rate of calves, that improve fertility of post-pubertal animals, that increase the proportion of animals calving for the first time within target age and weight ranges, and improve lifetime performance and longevity.</p> <p>3. Genetics - To identify genetic influence and markers for traits associated with between-animal differences in feed efficiency, environmental emissions, microbiomes, fertility and milk synthesis.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This research will develop more appropriate feeding strategies, leading to healthier, more fertile animals, which are more efficient, live longer, and have lower environmental emissions. There will be direct benefits to producers from more efficient use of feed resources, benefits to animals from better nutrition, and benefits to society through reduction in environmental impacts and improved milk composition</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Cattle, age 1 day to adult, up to 2,200 over 5 years. Sheep, adult, up to 20 over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>All proposed procedures (e.g. blood and rumen sampling, manipulation of diets) are mild and cause no more than transient discomfort and no lasting harm. At the end of procedures, animals will be kept alive or discharged from controls of the Act following inspection. Any animal showing adverse clinical signs will be given appropriate veterinary and husbandry treatment. If it fails to respond promptly and effectively, it will be humanely euthanized by a Schedule 1 method.</p>

Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Animals have to be used because of the complexity of digestive, metabolic and synthetic processes in ruminants. In most cases, for example converting feed into milk, there is no substitute for live animals. Some responses to diet, such as changes in feeding behaviour, cannot be predicted or simulated with non-animal alternatives.</p> <p>In vitro systems will be used to supplement or replace some animal studies. For example, we will use mammary cell cultures to study milk synthesis, and fermenters to study activity of rumen microbes (although the latter requires animals as rumen fluid donors).</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>In consultation with our statistician, we will use known variation and predicted responses in power calculations to calculate the minimum level of replication required to provide adequate statistical power for each experiment. When appropriate, we will use covariates and crossover designs to minimise residual variation and reduce the number of animals required.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Lactating dairy cows and their offspring are the target animals for our updated response models, so they are the only animal models appropriate for most of the work.</p> <p>Non-lactating cattle will be used for some studies of rumen function and digestibility because they are easier to maintain.</p> <p>Sheep will be used also, because all feeds in the current national database were evaluated in sheep fed at maintenance. For compatibility, we have to evaluate novel feeds using the same technique.</p> <p>All animals will be maintained to the highest standard of husbandry and care in facilities designed to provide the best possible welfare standards. Procedures will be performed only by suitably competent operatives using appropriate handling facilities to minimise</p>

	stress on animals. In all cases where there are alternatives, we will utilise the procedure that imposes the least harm to an individual animal.
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Project	Nutrition and Management for Sustainable Weaner Production	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Some 10 million pigs are slaughtered annually in the UK. In industry there is a continuous process of evaluating and refining genetic improvements, management techniques, nutritional strategies and novel feed supplements in order to address long term sustainability issues such as; environmental impact, carbon foot print, antibiotic usage, animal health and welfare. By using precision farming techniques a great deal of performance data is collated in a commercial environment on a wide range of nutritional products and management strategies. These types of studies	

	<p>deliver the basic answers to the question “Does it deliver a benefit in a commercial production system?” What is often less clear is the mechanism or physiological differences that might be associated with any beneficial outcomes. These mechanisms may include immune response to a particular set of circumstances, cell function and the response to nutrient supply, gene expression and secretions in the gut lining, changes in the microbiome in the intestinal tract, and blood metabolites and health indicators. The aim of this project is to enhance the scientific understanding of the physiological processes involved in the feeding of pigs for efficient and environmentally sustainable production.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Reducing the use of antimicrobials in pig production requires the application of alternative strategies that are sustainable. This project will enable the scientific evaluation of the physiological effects of innovative nutritional supplements and management strategies and provide guidance on the efficacy and suitability for commercial application. These nutritional supplements may include individual or combinations of ingredients e.g. prebiotics, probiotics, acids, enzymes, flavourings, botanicals, antioxidants, amino acids and novel protein sources. In addition, improvements in performance through traditional genetic selection for example, litter size, growth rate and feed efficiency has the potential to reduce the environmental impact of pig meat production. This project enables us to address the challenge of meeting the nutritional needs of genetically improved stock. Finally, this project seeks to explore the interactions of differing farm management practices on the efficacy of alternative nutritional strategies.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We anticipate that up to four performance trials per year over five years will benefit from additional physiological measurements and understanding. The number of pigs (birth to 12 weeks of age) will be up to 1000 per year (i.e. 5000 pigs over the course of this license).</p>
<p>In the context of what you propose</p>	<p>Nutritional supplements are intended to</p>

<p>to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>enhance pig productivity (e.g. growth rate, efficiency and litter size), health and welfare, therefore are very unlikely to have any adverse effects. However, in the pursuit of understanding the physiological mechanisms, around 15 % of animals will require additional procedures such as blood sampling classified as mild severity. There is a short term stress restricted to the time of sampling and there is only a very small risk that tissue damage will cause bruising and swelling. The risks of this occurring is minimised by competent experienced staff and strict standard operating procedures.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The gut environment of the pig is complex with many processes occurring simultaneously and in response to many independent factors. This is particularly the case during suckling and through weaning when the pig undergoes a rapid dynamic change in biological form and function. The whole gut function changes from digesting milk based diets to cereals and vegetable protein sources. This requires a complex and interactive change in the enzyme excretions, for example, that is not possible to recreate in the laboratory or within computer models.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of pigs in each replicated group (between 2 and 6) is the smallest number which will be statistically valid and properly represent the normal feeding behaviour within the pigs normal social group structure for any particular experimental objective. The number of replications required by each study is minimised by careful control of parameters to minimise variation at the start and the use of statistical tests to predict the minimum replications required for sufficient power given the expected effect size. Factorial experimental designs will be used which will maximise the number of hypotheses to be tested in the least number of treatment combinations.</p>
<p>3. Refinement</p>	<p>Pigs, housed in a standard production environment, are used because the purpose of</p>

<p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>the project is to produce information that will be of direct value to the pig production sector. They will be cared for by competent staff and will be kept in appropriate social groups at all times. In order to statistically identify true physiological differences between treatments it is important that all other influences are minimised. This is achieved by using skilled staff that have high levels of empathy for the animals in their care in addition to strict adherence to well-designed protocols and tried and tested standard operating procedures (SOP's). All protocols are reviewed through the institutes ethical review board.</p> <p>All animals will be observed at least twice a day (more often after a specific intervention procedure). Welfare of the individual animals on studies will be maximised by timely intervention. The facilities and equipment available are specifically designed for pig feeding trials of this nature and all staff are highly experienced. Electronic identification systems will be used to minimise the handling of animals at key measurement points such as weighing and faecal collection. There is a strict blood sampling SOP and trained staff that will be taking blood samples are highly experienced, which will minimise the time from restraint to release. Should any experimental procedure have an adverse effect on an individual, this animal will be removed from the study and treated appropriately. Specialist veterinary support is available on demand and will be referred to if required. All housing and management practices fall within the British Quality Assurance (QA) standards which is include as part of this project proposal. The QA standards include quarterly veterinary visits.</p>
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Project	Nutrition of growing & mature ruminant livestock	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
	<input checked="" type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this programme is to increase our understanding of the mechanisms that contribute to the inefficient use of feeds by cattle and sheep, and therefore affect the output of pollutants such as nitrates, ammonia, and methane. For example, dairy cows use only approximately 25% of the feed protein they consume to produce milk protein. Ruminant livestock, and dairy cows in particular, contribute significantly to the UK agriculture's emissions of greenhouse gases and other pollutants. Livestock contribute approximately 50% of UK's methane emissions, about 85% of which comes from enteric fermentation, the remainder coming from manures. At the same time, there is a	

	<p>need to maintain the home-grown production of dairy foods in the UK, to support the agricultural industry and reduce the reliance on imported foods.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This project will allow an improved understanding of the interactions between feed and rumen microbes (colonisation, degradation, fermentation, and microbial protein synthesis) that will enable feeding to improve the efficiency of diet utilisation. This will improve the output of ruminant products (meat and milk) and help reduce the environmental impact of ruminant agriculture. For example, an improvement in the use of feed protein for meat and milk production will reduce the amount that is excreted, thereby reducing nitrous oxide (a greenhouse gas) and ammonia release into the atmosphere.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Cattle – approximately 400 over 5 years Sheep – approximately 400 over 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>A small number of animals, approximately 20, will be fistulated at the rumen (cattle and sheep) and/or small intestine (cattle only), and the surgery for this is a moderate level of severity. Following surgery, these, and other animals used by the project, will be subjected to regulated procedures such as restraining them in stalls to measure feed intake, taking blood samples, and collecting outputs of faeces and urine using harnesses and chutes. Some animals will be used in short-term research that may last for between 2 to 4 months, whereas other animals will be monitored for years as part of their normal growth and development. These procedures carry a mild level of severity. At the end of the procedures, those animals that have not been surgically modified will be re-homed in the establishment's herd or flock, or will be sent to slaughter as part of the normal supply chain for human consumption. Those animals that have been surgically modified are a valuable resource, and if their general health and well-being is good they will be transferred to another project licence. Cannulated animals</p>

	often live for longer than a cow or sheep on a commercial farm, and will be euthanised when their general health and well-being starts to deteriorate, for example with the onset of arthritis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	For some of the work, measurement of growth or milk production of animals fed particular diets is required. In addition, whole-body utilisation and partitioning of nutrients between productive and non-productive (pollutant) outputs may be measured. These data cannot be collected using <i>in vitro</i> techniques, and therefore animals must be used for those parts of the work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All work carried out will follow protocols of work that ensure the maximum amount of information is obtained from the minimum resources required to be statistically valid. Use of changeover design experiments, where appropriate, efficiently controls random variation and therefore fewer animals can be used. Where changeover designs cannot be used (e.g. growth studies) more animals may be required and/or the technical constraints of the experimental design have to be accepted. Some work combines <i>in vitro</i> and <i>in vivo</i> measurements, e.g., initial screening done using lab-based models of the rumen, followed by field trials of the most promising treatments.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Nutrition studies of farm livestock need to be applicable to farming conditions, and therefore work investigating milk or meat (growth) production in cattle and sheep requires the use of those animals housed under normal commercial practises. Similarly, the highest standards of animal health and welfare must be maintained throughout any experiment in order for results of the work recognised by other scientists and farmers to be transferable to productive farms. The welfare of the cannulated animals used in

	<p>this work will be safeguarded using aseptic techniques and refined methods of anaesthesia during surgical operations. Following surgeries, animals will be monitored closely and given pain-killers during their recovery. Cannulated animals will be washed daily to ensure they are kept clean, their skin is not irritated by leaks of digesta, and flies are not attracted to the cannulae.</p>
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Project	Nutrition of poultry
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input checked="" type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input checked="" type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The objectives of this project are:</p> <ol style="list-style-type: none"> 1. To determine the efficiency of utilisation of feedstuffs, including unconventional feedstuffs, by poultry species 2. To elucidate the effect of the use of feed additives in improving the utilisation of feedstuffs by poultry species 3. To examine the effect of different dietary interventions on growth, productivity and nutrient utilisation by poultry species 4. To understand the various factors that cause ill-health in foot and hocks of poultry species and dietary interventions to prevent such

	<p>5. To establish proper procedures for determining efficiency of nutrient utilisation by poultry, and</p> <p>6. To understand the interactions between nutrition and poultry health, with particular emphasis on gut health, and ascertain how different dietary interventions influence ability of poultry species to resist infection</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Some of the potential benefits from the project are: 1. Understanding of variability, and causes of such, in feedstuffs (conventional and unconventional) with the objective of reducing competition between man and animal for food resources 2. Development of strategies, using feed additives, to reduce possible negative effects of intensive animal agriculture on the environment, as for example the use of phytase to reduce phosphorus excretion to water bodies or in the manure 3. Improving efficiency of utilisation of finite resources by studying of alternatives that meet animal need without jeopardising animal growth and productivity 4. Understanding of alternatives to antimicrobial growth promoters to ensure optimum growth of birds and reduce subclinical growth performance issues</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Species to use are broilers, ducks and turkey Nutrition efficacy studies: 25,407 Feed evaluation – gavage: 300 Feed evaluation – raised floor: 11,000 Foot and hock studies: 2,000 Standardised digestibility studies – 5,000 Nutrition and gut health studies – 11,000</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Some of the birds in the project will receive diets that are not meeting nutrient requirement (vast majority of the birds will receive diets with adequate nutrients) and such birds will be expected to gain weight more slowly. In establishing the standardised digestibility, some of the birds in the experiment will be provided with diets with very little protein or mineral for a very short period, not exceeding five days. The potential negative effect is reduced growth, but in order to reduce this effect, the treatment will only be applied to</p>

	<p>birds that have received diets that are adequate in nutrients for at least 7 days. Oral gavage, administration via feed or water or oral inoculation of birds with campylobacter or coccidia may produce reduced growth rate. However, this is to mimic what may happen in a typical poultry farm, and the negative effect will be minimised by ensuring that birds that are challenged with the organisms are kept only up to the age at which relevant useable data can be obtained after induction. The maximum severity limit in the project is moderate but most of the birds to be used in the project will have mild severity level. At the end of the experiments, some of the birds will be euthanised using humane methods. Some of the procedures do not require euthanasia of birds as part of the experiment and such birds will be signed off the Act.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Studies that require effect of treatments on growth can not be done in non-animal substitute. Digestibility studies are usually preceded by in vitro proof of concepts but ultimately because the feed additives will be incorporated to feed for actual animals, it is a requirement that such products are fed to animals.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of replications and animal per replications are determined based on statistical analyses and previous experience. Each experiment is individually set up to maximise ability to test for treatment effect combined with every effort to use the minimum number of animals. Experimental protocols are reviewed before each experiment to ensure that animals are not used unnecessarily. In addition, there are monitoring exercises after experiment to see what lessons are learnt. The 3Rs is part of the monitoring exercises. Part of the review of the experiment include the input of expert statistician REDACTED</p>
<p>3. Refinement</p>	<p>The objective of this project is to provide information that is relevant to the poultry</p>

<p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>industry, and hence poultry species are the choice of animals for the project. There are instances where nutritional effects studied are applicable across species (i.e. what is studied in broilers may be applicable to turkey) and in such cases, studies are not repeated for all the poultry species. Most of the protocols in this project are mild in severity level. Birds used in this project will be monitored on a daily basis to ensure that birds wellbeing is not compromised. There is also on-site specialist (avian) veterinary support who helps ensure birds wellbeing is maintained.</p>
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Project	Nutrition-parasite interactions	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	Drug resistance hampers disease control in animals and we need alternatives to control disease. Improved nutrition can reduce worm infections. Here, we study plants with anti-parasitic properties and protein nutrition to reduce gastrointestinal disease levels in rodents as model for farm animals.	
Why is it important to undertake this work?	Drug resistance hampers disease control and we need alternatives such as nutritional strategies. Many plants may have anti-parasitic properties, and we aim to identify their efficacy. Feeding extra protein reduces worms and we also asses effects of protein quality. We will also assess how hosts	

	<p>respond to parasitic challenges in the presence of other pathogens and how nutrition improves host resistance under these circumstances. parasitism and nutrition effects across generations. Results will benefit informing nutritional strategies to achieve worm control with minimal use of drugs in animals and potentially humans.</p>
<p>What outputs do you think you will see at the end of this project?</p>	<p>This work will provide novel information about the beneficial effects of macronutrients, such as protein and plant secondary metabolites (PSM), such as tannins on health and welfare. It will advance our knowledge of how these nutritionally mediated effects have an impact on hosts' immune responses. Factors that interact with nutrition, such as host and pathogen genetics, may be identified and molecular pathways will be studied when appropriate. The dissection of the molecular interactions between nutrition and immunity to parasites is of strategic importance to predict the risk of infection, define disease predisposition and develop sustainable measures for parasite control. Consumption of certain types of PSM has been reported to occasionally cause negative effects on digestibility and ultimately performance; the work here will increase our understanding on the trade off between the positive effects of PSM (antiparasitic) and possible negative effects (antinutritional) when PSM are offered at high quantity. The association of types of PSM with health and performance traits will enable the effective, efficient and safe use of these compounds for health and disease in animals.</p> <p>The work will increase our knowledge on the molecular basis of host responses to disease and will help developing tools useful in disease diagnosis and prognosis. The characterisation of the molecular interactions between host's nutrition and immunity to parasites will help towards identifying novel biomarkers for nutritional imbalance, disease susceptibility/predisposition and novel therapeutic strategies.</p>

<p>Who or what will benefit from these outputs, and how?</p>	<p>Findings will be made available to other scientists through publication in peer-reviewed journals and presentations at scientific conferences and meetings. Under the previous project licence we have published more than 12 papers (and 4 more are currently in draft form) and presented our findings at national and international scientific meetings.</p> <p>It would be expected that the outcomes here will be tested and validated in ruminants and will be combined with other non-chemical measures to improve satisfactory disease control with greatly reduced reliance on medicines. This improvement is expected in the duration of this PPL. Results obtained will have relevance for controlling disease in not only farm animals but also humans, including hospitalized patients and in developing countries, where for example protein-energy malnutrition is more often the rule than the exception. Although this is a possible development from the work described in the PPL it is unlikely that it will happen in the duration of this PPL. In developing countries, such as Indonesia, medicinal plant extracts are already in use to treat people and animals (http://www.gbgindonesia.com/en/manufacturing/directory/2015/javaplant-natural_extracts/interview.php).</p>
<p>Will this work be offered as a service to others?</p>	<p>No</p>
<p>How will you look to maximise the outputs of this work?</p>	<p>The work undertaken is funded by national and international funding bodies, which will facilitate dissemination and knowledge transfer of the approach taken and the outputs. It is also cross disciplinary, underpinning parasitology, nutrition, animal science, immunology and molecular biology and as a consequence has the potential to</p>

	<p>impact on a variety of scientists. It is expected that this work will result in scientific publications in peer reviewed journals.</p>
<p>Explain why you are using these types of animals and your choice of life stages.</p>	<p>Drug resistance hampers disease control in farm animals and we need alternatives such as nutritional strategies. Although the fundamental questions we aim to answer are relevant for farm animals, in this project we aim to use rodent animals as models for sheep. Imposing large nutritional variation in sheep would seriously affect their welfare, insight in immune responses to sheep worms is limited, and large generation interval makes sheep trans-generational studies difficult. However, rodents have short generation intervals, tolerate much larger variations in nutrient supply, there is detailed knowledge on responses to aforementioned worms, and models exist for testing anti-parasitic plants in mice and protein effects in rats. Whilst their guts differ considerably, metabolism of anti-parasitic plant compounds and digested protein, and worm immune expulsion are remarkably similar in sheep and rodents.</p> <p>To study the impact of protein supplementation on periparturient parasitism, we need to use periparturient animals. To study the antiparasitic properties of plants we are using growing animals, which are the most susceptible as their immune system is still undeveloped.</p>
<p>Typically, what will be done to an animal used in your project?</p>	<p>Typically, mice will be infected with worms and/or another pathogen or left uninfected, and then dosed or fed with plant extracts. Rats will be infected with worms, and fed different levels and types of protein during pregnancy and/or lactation. Mice on different foods will be infected during pregnancy, and offspring response to worm infections will be monitored. Animals will then be subjected to different nutritional treatments, which all have the potential to impact on the level of disease and the ability of the host to cope with the disease. At the end of the experiments animals will be euthanized and sample collection will take</p>

	place to determine the impact of our treatments.
What are the expected impacts and/or adverse effects for the animals during your project?	Our refined infection and nutrition protocols cause little or no harm, and the experimental foods used have high quality ingredients. Depending on the experiment, animals may be deliberately fed a little bit below their nutrient requirements, in which case they simply grow slightly less or produce slightly less milk without suffering. Animals are daily observed, and quantifiable, clear end-points have been established to ensure that animals do not exceed a mild severity limit. Animals are not expected to show any signs of abnormal behaviour as a consequence of experimental treatments.
What are the expected severities and the proportion of animals in each category (per animal type)?	The expected severity for the rat protocols is mild, whereas the expected severity for the mouse protocols is moderate, with up to 5% of mice expected to experience that harm.
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	This program of work aims to assess the impact of host's nutritional environment towards sub-clinical disease and in particular gastrointestinal parasitism. Disease affects the host animal in a range of ways, including through its nutrient ingestion, digestion, production and behaviour. It can therefore be anticipated that interventions affecting (the outcome of) sub-clinical disease do so through the involvement of multiple mechanisms of the host's physiology, including its immunology, endocrinology, digestive physiology and neurology.
Which non-animal alternatives did you consider for use in this project?	Mathematical methodologies to study some aspects of sub-clinical disease have been developed to test in silico a range of management scenarios that could impact sub-

	<p>clinical disease.</p> <p>In vitro methodologies, such as egg hatch and larval motility assays, will be used as much as possible to inform animal studies. In particular they will be used to screen a large number of plant extracts and the most active extracts will then be used in animal experiments.</p> <p>Additional in vitro studies, such as cytotoxicity tests, will be used to assess toxicity of the extracts prior to their use on animals, to reduce the possibility of side effects.</p>
Why were they not suitable?	The involvement of such a large range of host bodily functions in their response to sub-clinical disease reduces the possibilities to use non-animal experimentation, and thus justifies the use of animals. In addition, a small number of animals are needed as helminth donors, which can not be produced in vitro.
Enter the estimated number of animals of each type used in this project.	mice: 1600 rats: 300
How have you estimated the numbers of animals you will use?	<p>The number of replicates required will be informed through a combination of experience and statistical tools. For example, our experience is that variation in performance measures like weight gain is usually higher in parasitized animals than in control animals, and we therefore often allow for less replicates in the control animals. Resulting unbalanced data sets can readily be analysed through statistical methods like REML.</p> <p>Power calculations will be used when the expected or desired effect size is known. Where possible, this will be derived from our earlier studies and from the literature.</p>
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	Each individual animal experiment under this project requires approval from the establishment's Ethical Review Committee. This includes assessment of appropriate number of replicates and use of statistical tools. To this effect, a statistician sits on the establishment's ERC, who is also consulted

	prior to experimental design submission
<p>What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?</p>	<p>The program of work on anti-parasitic plant extracts will test only those that show strong in vitro activity and possibly in vivo activity from preceding rodent studies. This significantly reduces the number of target animals needed to study plant extract impacts on parasite establishment</p>
<p>Which animal models and methods will you use during this project?</p>	<p>For our rat and mouse studies, we focus on the <i>N. brasiliensis</i> and <i>H. bakeri</i> infection models as our host/parasite systems, respectively, although other infection models may be explored as and when required for pursuing our hypotheses. These species are the ones in which response to the parasite and the course of infection have been well characterised, bearing considerable similarities with parasitism in small ruminants. We build on our previous studies that have confirmed and refined the infection levels required to induce sub-clinical rather than clinical disease. The nutritional protocols developed over the last decade or so are highly repeatable in demonstrating nutritional sensitivity of resistance to parasites, with very few animals having to be removed from trial due to unexpected side-effects.</p> <p>Our earlier studies have refined our lactating rat model to a low severity and highly repeatable model, and will form the starting point for our studies on nutritional sensitivity of breakdown of immunity. We have chosen to use mice for the objectives related to screening anti-parasitic plant extracts, as the proposed <i>H. bakeri</i> infection is relatively long-lived, which thus provides a window of time to assess impact on resilience, resistance and possible immune responses. Infection levels have been refined REDACTED to levels that do not show mortality, even on low protein foods. For the mixed infection protocols we propose to use avirulent strains of viral pathogens to limit host response to avoid</p>

	inducing clinical disease.
Why can't you use animals that are less sentient?	It is not possible to use immature, less sentient or terminally anaesthetised animals. The project aims to increase our understanding on how the nutritional environment of the host impacts on subclinical disease in farm animals and rodent animals are used as models.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	Attend relevant seminars organised by the establishment and other local establishments and looking at updates from the NC3RS website
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	The protocols described here are already refined as a consequence of our experience over the period of the last 15 years or so, that are in use by our team. For example, to minimise the possibility of accidentally overdosing the animals, we have introduced an extra baermanisation step to distinguish between dead and alive infective larvae. Infective larvae often appear to be dead (and thus are not counted in the infective larvae dose), which increases the risk of overdosing, but this extra step mitigates this risk. Infection levels have been refined in previous licences to levels that do not show any mortality even if animals are on low protein foods, a refinement particularly relevant for lactating animals. Protein scarcity levels have been refined at levels that demonstrate nutritional sensitivity of lactational resistance to parasites without litter body weight losses
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	The National Centre for the Replacement Refinement and Reduction of Animals in Research.

Project	Object recognition and visual memory formation in zebrafish	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The main goal of this project is to understand how what we see with our eyes is recorded in the brain. Brain consists of billions of specialised cells called neurons. When memories are formed in the brain, these neurons change in a process called synaptic plasticity. We know a great deal about how these changes happen but much less is known about how synaptic plasticity actually helps us to form visual memories. In other words, we try to understand the language used by the brain to record memory using synaptic plasticity.</p> <p>To address this question, we will first develop</p>	

	<p>special molecules that will become bright when synaptic plasticity occurs. We will then create transgenic animals that have these molecules synthesised in each individual neuron in the brain. This will allow us to find which parts of the brain and which neurons change when the memory is formed.</p> <p>For this project we chose to use zebrafish, a small striped fish, living in shallow waters. Contrary to the popular belief, fish do form memories that can last long. However, unlike other animals (such as monkeys and humans) their brains are simple, which will help us to understand the basic principles of memory formation better. We will make fish to memorise certain objects or get used to specific environment, then find neurons that change during zebrafish brain forms memories and study the properties of these neurons in details.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Our results will contribute to several important areas of scientific knowledge. Firstly, we will study general principles of how zebrafish vision works. This is important because zebrafish becomes very popular in animal research and better understanding of how visual system works in these animals will make it possible to study vision more productively. Secondly, we hope to understand how fish brain achieves object recognition. Understanding this in simply organised brains, such as that in zebrafish, will be important in understanding how object recognition works in more complex brains, such as that of humans. Finally, this project proposes to study visual memory in the zebrafish is achieved through changes of connections between nervous cells. This may help to understand how memory is formed in the human brain.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>For our experiments we will use young zebrafish larvae. We chose zebrafish for several reasons. First, they are transparent which allows us to study the activity of neurons using microscope rather than electrodes. Thus, we will not use any surgical preparation, so we do not expect to cause much harm to the animal. Second, zebrafish nervous system is</p>

	relatively simple, which will simplify our task to study the way it works. We will use around 5000 fish in the whole project, 3000 fish for production transgenic zebrafish animals and 1500 larva for experiments.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We propose methods that will limit harm caused to the animals. In contrast to established and widely used electrode techniques, we will use advanced microscopes that will let us to avoid performing any surgery on live animals. We will also perform behavioural experiments – observations of the fish larvae while they memorise new objects or environment. This will not cause pain to the animals, although some degree of stress may be expected. After the experiments, the animals will be humanely killed and their brains and other parts used for further analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Unfortunately, we have no choice but to use live animals for our research. This is because computer simulations can only <i>propose</i> how the memory is formed; they cannot answer the question <i>directly</i> . Neither can we use cultured cells or brain slices because they will no longer connect to the eye.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will carefully examine the number of animals required for each experiment and experiments will be first proposed using mathematical modelling, done either by us or statisticians.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have chosen to work using zebrafish, which has a relatively simple nervous system. This animal has less complex emotional and behavioural reactions compared with other animals that are closer to humans. We will use microscopes on live animals, a technique that does not require any surgical operation. During our procedures we will carefully monitor the condition of the animals and will apply immediately stop our experiments should if we find that animals

	suffer from a notable pain or distress.
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Project	On the mechanisms of risk for psychiatric illness	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Psychiatric disorders like schizophrenia, autism and depression remain poorly understood. Treatments for these conditions are either lacking have limited efficacy and significant side effects. Recent findings in genetics and epidemiology have advanced our understanding of the causes of psychiatric disorders. These findings are of great importance, but on their own they will not advance the treatment of these conditions. To enable us to advance treatment for neuropsychiatric disorders we need to understand the impacts of these risk factors (genetic and environmental) on the brain, so we can develop reliable markers of disease and</p>	

	<p>develop new treatments. The overall aim of this licence is to fill this need. Specifically, we will use animal models as part of an overall integrated research programme to:</p> <ol style="list-style-type: none"> 1. Identify biological markers associated with risk for neuropsychiatric diseases. 2. Identify novel targets for the treatment of neuropsychiatric diseases.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Our work will help to pave the way for the development of a new understanding of mental disorders based on biology. Advances in biological understanding of risk for mental disorders will take us beyond current understanding and classification of psychiatric disorders and, we anticipate, will lead to the development of new approaches for the treatment of these disabling conditions.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We anticipate using up to 9000 rats and mice in total over a 5 year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We will be breeding genetically altered animals including both rats and mice. These animals will be bred to understand the effects of genetic changes associated with risk for human neuropsychiatric disorders. These genetic changes are not typically associated with adverse health consequences in rodents but one of the strains we use has some background physical health vulnerability so we will take additional care to avoid stresses in this line. In some studies we will also investigate the effects of early life factors such as immune activation, which are known to be important in risk for psychiatric disorders. We will assess the behaviour of these animals using a range of measures. Examples include: measures of simple reflexes and basic motor skills; learning ability as assessed by maze and lever-pressing tasks and tests of anxiety and emotional reactions. These tests are typically only mildly aversive. For some of these tasks the animals will have a safe restriction in either their food or</p>

	<p>water access to maintain motivation to complete the tasks. All animals undergoing such restrictions of food or water access will be carefully monitored to ensure their overall health. Some animals will also undergo MRI scanning under anaesthesia. MRI scanning is a safe repeatable procedure which can give extensive information about brain structure and function using measures that can be translated into humans. Animals will be scanned under anaesthesia to minimise stress and movement. The main risks associated with this procedure relate to the anaesthesia and animals will be carefully monitored during the procedure and the recovery phase. Overall MRI scanning is a good approach to gaining extensive information about brain structure and function in a longitudinal manner which limits aversive impacts on the animal. In order to investigate the causes of brain alterations and potential new treatments it is at times necessarily to directly manipulate brain function. A limited number of animals will therefore undergo brain surgery to allow us to administer either compounds or genetic vectors (viruses) into the brain. These procedures may produce some temporary pain or discomfort in the treated animals. However we will take a number of steps to minimise any suffering including the use of anaesthesia and careful surgical methods, as well as close monitoring of the animals for any signs of distress. Some animals who have had such surgery will then go on to complete behavioural testing to examine the effects of the treatments on their behaviour. All the animals used in this licence will, at the completion of testing, be killed by a humane method (typically under sedation). In many cases we will then use the brain tissue for further molecular or physiological analysis.</p>
Application of the 3Rs	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>It is necessary to use animal models to study the mechanism through which genetic (and environmental) risk factors for psychiatric illness operate because: (i) we cannot directly access the relevant tissue in patients (i.e. the brain) AND (ii) cellular models and more basic systems cannot fully recapitulate the complexity and</p>

	<p>function of brain circuits. However, we conduct our work in animals in the context of parallel work in patients and human cellular models, enabling us to use methods for replacement as and where appropriate.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use appropriate statistical and experimental approaches to optimise the number of animals used in each study. We will only generate animals when needed and will avoid excess breeding. Where possible we will use techniques that allow multiple measures to be taken in a non-invasive manner (eg MRI scanning). We will also where possible without adding to severity gain additional data measures from experimental animals (for example conducting molecular analysis on post-mortem brain tissue). The extensive experience of the investigators in the techniques used in this licence will also help minimise the number of animals required.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rodent species are chosen as they provide a combination of genetic flexibility, a sufficient and established behavioural repertoire and suitability for physiological/molecular investigation. Rats are used in addition to mice as their larger brain size and extensive experimental behavioural repertoire is advantageous for some studies (eg MRI imaging).</p> <p>In all studies we will work to use the most refined methodology available. For example in behavioural testing we will only use food and water restriction to motivate behaviour where necessary and will keep the duration of restriction to the minimum required for the studies to be completed. For physiological studies we will wherever possible use methods that minimise animal welfare costs, for example non-invasive MRI imaging. Where more invasive techniques such as surgery are required we will use adequate analgesia to avoid pain and aseptic techniques to avoid post-operative complications. Across all our work, animal welfare will be maximised by close liaison with animal support and veterinary staff.</p>

Project	Oncology Pharmacology Studies	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Cancer is a complex disease and despite an increase in the number of new drugs available to treat patients, not every patient will respond to existing treatments and be cured.</p> <p>It is therefore important to continue to support the discovery and development of new anti-cancer drugs.</p> <p>This service licence will be used to determine whether potential new anti-cancer drugs can stop tumours from growing and seek to understand how the drug works.</p> <p>This service licence will also be used to develop</p>	

	new cancer models for use in future research into new cancer treatments.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Increased knowledge of cancer biology is leading to the discovery and development of new drugs. Some of this success has resulted in improved cancer survival rates seen in recent years (Cancer survival rates have doubled in the last 40 years. However benefits are still limited and are better for some cancer types than others. In addition, the side effects of many existing drugs have a significant impact to the patient's quality of life. There is therefore a clear need for more effective drugs that can be used either alone or in combination with existing or other new drugs. The work carried out under this licence will provide benefits:- <ul style="list-style-type: none"> • In the short term by supporting cancer drug discovery • In the medium term by sharing cancer research data which will increase the understanding of cancer biology • In the longer term by supporting the clinical development of new drugs for the treatment of cancer. It is expected that a number of drugs research programmes supported by this service licence will be successful in developing new cancer drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	Only rats and mice will be used on this project. Approx 93% of the total usage will be mice and ~7% will be rats. The total number of animals used over the duration of the licence will be approximately 19,850
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals will be kept in a purpose built facility. Animals will be housed in groups. Tumour growth in animals will be started by injecting tumour cells or pieces either: <ul style="list-style-type: none"> • Under the skin • In breast tissue Injections require the use of needles and in some cases minor surgery. • The use of needles will cause brief discomfort or pain. • Where minor surgery is required, animals are expected to recover quickly and will be given painkillers during and after the surgery and post-operative care just like people recovering in hospital. The majority of animals will be given the experimental drug every day, using the same drug delivery method as used in patients. In the majority of cases this will involve the use of needles only, which will cause

	<p>Only brief pain or discomfort. • In a small number of cases, brief local irritation of the skin where the drug has been injected. A small number of animals will have minor surgery to implant a device under the skin which can slowly release the test substance. Animals are expected to recover quickly and will receive painkillers during and after the surgery and post-operative care, just like people recovering in hospital. Animals will be regularly monitored for weight loss and general condition. Animals may become unwell as a result of the test drug. Signs that the animals are starting to become unwell can include: • Weight loss • Deteriorating coat condition • Reduced movement • Reduced social interaction. Animals will be humanely killed if these signs of being unwell persist. Blood samples will be collected during the experiment, which will cause brief discomfort or pain. The majority of animals will undergo experimental procedures which are classified as moderate severity. At the end of the study it is necessary for the animals to be humanely killed and tissues taken for analysis after death.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Non-animal alternatives are used in the identification and selection of potential new drugs before testing in animals.</p> <p>However, non-animal testing cannot predict how experimental drugs stop tumours from growing.</p> <p>Cancer development is a complex process. It involves lots of different cell types including the immune system, which cannot be recreated in non-animal alternatives or non-protected species.</p> <p>Therefore protected animals are needed for the studies proposed in this licence.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>To obtain good quality data and to use the minimum number of animals, statistical expertise will be applied to work undertaken on this licence.</p> <p>The following guidelines will be used to minimise the number of animals required:</p>

	<ul style="list-style-type: none"> • The number of animals required in each group will be determined using previous experimental data, historical databases, pilot studies or published data • Appropriate statistical tests will be used. • Studies will be designed to enable at least an 80% chance of finding a meaningful result. • Wherever possible, multiple test drugs or doses of test drug will be compared against one control to reduce the number of studies performed.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The overall plan of the work is to support the discovery and development of novel cancer drugs to benefit human health in the treatment of cancer.</p> <p>Using non-mammalian species is not possible because they lack relevant tissue physiology and therefore cannot replicate human physiology</p> <p>Only rats and mice, including strains which lack an immune system, are used on this licence.</p> <p>Mice will be used in the majority of studies unless there is a scientifically relevant reason that mice cannot be used.</p> <p>The most appropriate species and strain of mice and/or rats will be chosen based on previous data and choice of tumour model.</p> <p>To study human tumour growth in rats or mice, strains lacking an immune system are required. The least immune-deficient strain required to promote good, reproducible tumour growth will be used.</p> <p>Best practice and use of the most refined methods will be applied to all experiments</p> <p>Animals are observed by trained staff, with referral to the Named Animal Care and Welfare Officer, Named Veterinary Surgeon and Project Licence Holder as necessary.</p> <p>All animals will be regularly monitored for weight loss and general condition.</p>

	<p>For anaesthetic protocols the optimal anaesthetic regime relevant for the species/strain will be developed and used in conjunction with the Named Veterinary Surgeon.</p> <p>Where necessary, painkillers will be used under the guidance of the Named Veterinary Surgeon.</p>
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Project	Oocyte chromatin determinants of offspring health
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Basic research
	Translational and applied research
	Regulatory use and routine production
	Protection of the natural environment in the interests of the health or welfare of humans or animals
	Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
What's the aim of this project?	We wish to understand a newly-discovered route by which information from the egg is transmitted to the embryo, which depends on chemical tags (epigenetic marks) added to genes in the egg. We aim to understand whether these epigenetic marks are modified by maternal factors such as age and diet, and whether these effects persist to influence the development of the embryo or cause longer-term physiological outcomes.
Why is it important to undertake this work?	Our genes are wrapped by proteins to form chromatin to help condense the genetic information in the cell nucleus. How the chromatin is organised differs on genes that are active from those that are silent in any cell. In some cases, these different states can persist over the lifetime - a process referred to as 'epigenetic

	<p>memory' - but it is also known that some chromatin states can be modified by factors such as nutrition or the environment.</p> <p>The extent to which chromatin states in the egg or sperm are sensitive to extrinsic factors in a way that influences, or programmes, the development of the next generation is still poorly understood. Recent work has identified a new way in which epigenetic memory is passed between generations – a new form of ‘imprinting’ that depends on chromatin states in the egg – but very little is known so far about this new mechanism. We believe we have found a molecular explanation for this form of imprinting, finding that the controlling elements of these genes are of a class that could be sensitive to extrinsic factors. Our work will provide fundamental understanding of this newly described form of imprinting, answering questions such as the nature of the genetic elements involved, why it becomes restricted to the placenta, whether it is likely to be conserved, whether it is sensitive to extrinsic or physiological factors, what impact it could have on offspring development and health. This will provide important underpinning information from which to explore the existence and significance of this form of epigenetic memory in humans, and whether it is sensitive to maternal nutrition, age or procedures employed in assisted reproduction.</p>
<p>What outputs do you think you will see at the end of this project?</p>	<p>The key outputs will be a detailed understanding of a newly-described form of epigenetic inheritance in terms of how it is established in the female germline, maintained during preimplantation development and then restricted to the placenta; the functional impact of these genes on the placenta and through the placenta on the fetus; whether these genes have lifelong consequences in offspring; and whether these genes are particularly sensitive to deregulation from maternal factors, such as age, diet. All these outputs will help evaluate whether this form of imprinting could be conserved and what factors should be evaluated to test its existence and significance for healthy development in humans. These findings will translate into peer-reviewed publications, as well as public engagement and dialogue about the new science we discover.</p>
<p>Who or what will benefit from these outputs, and how?</p>	<p>(i) The academic scientific community, particularly in relation to developmental biology, epigenetics and reproductive biology. Our research will provide a detailed evaluation of the mechanism and</p>

	<p>developmental significance of a new mechanism of intergenerational epigenetic inheritance. Our research will also contribute to future studies: a number of new datasets will be identified and made available to other users to advance future research.</p> <p>(ii) Staff and students supported by our lab, who will receive exemplary research training. Gaining technical skills and expertise to carry out research, in addition to other transferable skills, personal and professional development, will prepare them for careers in the academic or commercial research sectors or other related careers.</p> <p>(iii) REDACTED Our research underpins the delivery of strategic priorities for these funders.</p> <p>(iv) Our research will equip policy makers with critical understanding of the impact and value of basic research, providing scientific rationale for the influence of diet and lifestyle on healthspan, reproductive biology and disease mechanisms.</p> <p>(v) The general public including, but not exclusive to, students, teachers, patients, the local, national and international community. Benefits to these groups will include increased knowledge, understanding and awareness of our research on epigenetics, including the impact of environmental and nutritional exposures during development and its potential social and economic relevance.</p>
<p>Will this work be offered as a service to others?</p>	<p>No</p>
<p>How will you look to maximise the outputs of this work?</p>	<p>We have many mechanisms in place to do this in addition to the immediate academic route of publishing research papers and presentations at international conferences. This includes a well-appointed and trained Knowledge Exchange and Commercialisation (KEC) team, and a similarly advanced and engaged Public Engagement (PE) team. We expect to develop collaborations in evaluating placenta physiology and programming. And with the principles of non-canonical imprinting established in mice, we would expect to develop collaborations with human geneticists interested in establishing whether an analogous epigenetic mechanism exists in humans.</p>
<p>Explain why you are using</p>	<p>The aims of the project are to understand epigenetic</p>

<p>these types of animals and your choice of life stages.</p>	<p>control of genes from the egg to the developing fetus, with a particular focus on how epigenetic memory from the egg controls the action of genes in the placenta. It is necessary to carry out this investigation in animal models because these genes affect organismal function in a complex way. For example, there is no cellular model yet for events start in the developing egg, are perpetuated in the early embryo before implantation, but have their effects mostly in how the placenta develops and controls growth and health of the developing embryo. We have been able to move a substantial proportion of our work into <i>in vitro</i> cell systems, thus reducing the number of animals used and refining the experimental approaches before applying them to mouse models.</p>
<p>Typically, what will be done to an animal used in your project?</p>	<p>The most common procedure in this project is the breeding and maintenance to produce adult or pregnant mice that will be killed via Schedule 1 to supply tissue for the aims described in this project.</p> <p>We shall also be generating new genetically altered mouse strains using highly refined genetic modifications that will selectively affect genes in the placenta.</p> <p>Smaller numbers of mice will be fed altered diets, such as high-fat diets, to evaluate the effect of diets on how the genes we are interested in are controlled.</p>
<p>What are the expected impacts and/or adverse effects for the animals during your project?</p>	<p>The general type of genetically altered mice produced under breeding and maintenance in this project will be the type that use conditional gene ablation, which allows us to remove a gene specifically in target tissues (the placenta or egg) rather than in whole animals where constitutive ablation could have a severe phenotype, thereby avoiding adverse effects.</p> <p>Mice fed altered diets, e.g., high-fat diet over a period of two to three months are expected to become mildly obese and diabetic, but these will be monitored to avoid development of harmful side-effects.</p> <p>For mice that undergo surgery, mostly for transferring embryos, the duration of anaesthesia and surgery is short and the animals are expected to make a full and unremarkable recovery, although analgesia will be administered to mitigate short-lived pain.</p>
<p>What are the expected severities and the</p>	<p>Overall, the expected severity of this project licence is Mild, with fewer than 5% of animals expected to</p>

proportion of animals in each category (per animal type)?	experience a maximum severity of Moderate.
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	The aims of the project are to understand epigenetic control of genes from the egg to the developing fetus, with a particular focus on how epigenetic memory from the egg controls the action of genes in the placenta. It is necessary to carry out this investigation in animal models because these genes affect organismal function in a complex way, which is not possible to recapitulate in purely cell-based systems. For example, there is no cellular model yet for events start in the developing egg, are perpetuated in the early embryo before implantation, but have their effects mostly in how the placenta develops and controls growth and health of the developing embryo. In addition, the impact of altered physiological states in the female, for example as caused by high-fat diets, on the development and quality of the egg and then into the offspring depend upon multiple cellular and tissue interactions that cannot be fully reproduced in cell-based systems. We have been able to move a substantial proportion of our work into <i>in vitro</i> cell systems, thus reducing the number of animals used and refining the experimental approaches before applying them to mouse models.
Which non-animal alternatives did you consider for use in this project?	Inherent in our experimental strategy is the exploration of aspects of the regulation and cellular function of non-canonical imprinted genes in relevant cell culture systems, such as trophoblast stem cells (TSCs) or 2C-like cells that can be obtained from embryonic stem cells (ESCs), and this provides the information for the design of the <i>in vivo</i> genetic models. We are keeping fully aware of developments in cell-based systems, including organoids, and would adopt them where we can, if they prove reproducible and representative of the <i>in vivo</i> situation.
Why were they not suitable?	It is recognised that TSCs cells in culture do not faithfully maintain epigenetic states, they only partially mimic the full differential potential into extra-embryonic lineages, and cannot fully recapitulate the transition from before the fertilised egg to the development of a functioning placenta, and how these transitions could be

	influenced by physiological factors such as maternal diet.
Enter the estimated number of animals of each type used in this project.	mice: 14,600
How have you estimated the numbers of animals you will use?	From experience of similar experimental designs in previous projects. With advice from the Institute statistician in relation to the minimum number of animals (data points) necessary to achieve statistically robust results in any procedure with a quantifiable outcome, including use of power calculations.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	We have been able reduce the numbers of animals needed for these investigations because, for example, we have been able to develop highly sensitive methods for profiling the location of epigenetic tags in very small numbers of cells. We can also reduce animals numbers by making multiple measures from the same animal or sample, wherever possible. For example, current protocols for molecular profiling of tissues <i>ex vivo</i> enable us to obtain measures of gene expression, DNA methylation, and chromatin state in the same assay. As well as reducing the total number of samples, thus animals, needed to obtain these measures, obtaining multiple data from the same sample is a refinement in experimental design.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	Use of power calculations of optimized animal group sizes based on comparable data from previous experiments and advice from the Institute statistician. Minimising inter-group variability using controls of matching age, sex and genetic background. Cryopreservation of strains when no longer required. Use of colony management software that helps avoid overproduction.
Which animal models and methods will you use during this project?	We use mice in these studies because in this species we understand the most about where and how epigenetic tags are placed in the DNA to control the activity of genes, and because we are able to follow the fate of epigenetic mistakes during development in this species in a way that is not possible in other mammals,

	<p>especially humans. We believe that the processes that put epigenetic tags in place and how they control genes in offspring in the mouse are very similar to those in humans, so the mouse is a very informative model.</p>
<p>Why can't you use animals that are less sentient?</p>	<p>We are studying a form of gene regulation that is unique to mammals (imprinting) and its effects in tissues that are unique to mammals (the placenta). Much of the analysis will be done at an immature life stage, i.e., in tissues from mid-gestation conceptuses (<i>ex vivo</i> analysis of placenta), or will be done under terminal anaesthesia (e.g., placenta function assays).</p>
<p>How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?</p>	<p>We keep fully aware of developments in cell-based and organoid systems and would adopt them where we can, if they prove reproducible and representative of the <i>in vivo</i> situation.</p>
<p>How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?</p>	<p>Harm to animals is minimised by using sterile conditions, anaesthetics, humane methods of killing, and by targeting genetic mutations to the cells of interest (e.g., eggs, placenta) to avoid the possibility of whole-animal suffering.</p> <p>Housing, husbandry and care conditions are REDACTED staffed by highly-trained animal technicians and overseen by experienced supervisors and NACWOs. The BSU enjoys permanent veterinary cover.</p> <p>If, in rare circumstances, an animal has an unexpectedly severe response to a drug or operation, or where an infection develops, treatment is given where possible and, if necessary, the animal is humanely killed.</p>
<p>What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?</p>	<p>NC3Rs Arrive Guidelines.</p>

Project	Optimising lung therapy using large animal models	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Lung disease is one of the three biggest killer disease areas in the UK, alongside heart disease and non-lung cancers. It kills 115,000 people each year, the equivalent of one person every five minutes and it places a huge burden on our health care services, accounting for over 700,000 hospital admissions and over 6.1 million hospital bed days in the UK each year.</p> <p>Research is urgently needed to improve the way we prevent, treat and care for people with lung disease, from the earliest to the end stages of life.</p> <p>Ordinarily new treatments are developed through a process that involves early testing in preclinical</p>	

models – and in most instances these models involve the use of mice. Unfortunately this system is largely failing to deliver the new drugs necessary to keep pace with the upward trends in lung disease. New models are urgently required that improve on existing systems.

Sheep and pigs have been used as models for many years to predict the safety and efficacy of drugs for lung disease. Recently the potential benefits of studies in large animal models were realised in translation into a large clinical trial involving people with cystic fibrosis in the UK, which demonstrated that repeated doses of gene therapy had a meaningful effect on the disease, slowing its progression. The use of sheep was prompted by the realisation that mice were not predictive of humans in this context and a larger species with more relevant physiology and anatomy was required to model the delivery of new treatments to the lung and the assessment of benefits due to those treatments. We believe that this model can extend to benefit other lung diseases.

We believe that the extension of our translational models to include piglets will help to tackle neonatal human diseases such as Surfactant protein B deficiency, a rare but lethal condition which currently depends on organ transplantation as the most effective form of correction. Due to lack of donor organs and the invasive nature of transplantation surgery we contest that gene therapy of these newborns is an efficient alternative. Again, the larger and more physiological relevant piglet animal model will allow for therapeutic efficacy development and may overcome the limitations of cell line and rodent models. Our overall plan is to identify new treatment targets through studying large animal models of lung disease and to assess the treatment benefits of new drugs or gene therapies directed at those disease targets.

REDACTED. Diseases will also include pneumonia, an inflammation of the lung usually caused by an infection, as well as radiation-induced lung injury (RILI). Both are important with pneumonia and lung infections accounting for over 325,000 hospital admissions and over 3 million bed days each year, and RILI affecting 1 in 10 of the

	<p>patients that receive radiotherapy for lung cancer every year. The risk of developing RILI is a well-recognised dose-limiting consideration in radiotherapy planning meaning that the majority of patients may have potentially less efficacious modifications made to their treatment plans based on the perceived risk to a minority.</p> <p>Developing new treatments or ways of preventing lung disease is an urgent clinical priority. Much of what we learn from our studies will also be directly relevant to lung disease in domestic and farmed animals. Research into lung-directed gene therapy may help in developing vaccines relevant to lung disease in animals, better understanding of normal and abnormal bacteria in the lungs could lead to more effective methods to control respiratory disease in animals, and any radioprotective strategy developed for humans could potentially also be used for domestic animals.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>In the context of patients, this project will potentially furnish strategies and policy guidelines to help the healthcare professionals responsible for their care to make the most appropriate decisions in relation to their management. Ultimately, that benefit will likely be realised in the form of improved quality of life, and increased life-expectancy.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use 150 sheep and 80 pigs over a period of 5 years. These animals will be commercially sourced from farms and/or markets in the UK and EU.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Typically the animals will be anaesthetised to allow disease to be induced. They will thereafter recover from anaesthesia and the progress of disease, with or without modulation by a proposed treatment, will be assessed by anaesthetizing animals periodically and sampling tissues and/or fluids from the areas under study. In all of our approaches, we are able to limit the proportion of the lung that becomes functionally abnormal. Because the lung has tremendous reserve capacity, it is our experience that the animals do not experience adverse effect in the form of shortness of breath, coughing, or difficulty in breathing, hence appearing essentially unaffected when compared to control animals. The fact that we have to anaesthetise animals to</p>

	<p>perform bronchoscopy or related procedures, renders most of the procedures outlined in this license moderate severity. However, it is our experience that sheep and pigs tolerate anaesthesia very well and recover to standing within 10-15 minutes after cessation of anaesthesia, and experience no apparent adverse effect. The animals will be killed at the end of these experiments in order to allow us to examine the lungs at post mortem and take samples that would otherwise be unavailable.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The way in which the lung responds to injury and disease is complex and involves the interplay of multiple systems and factors including the circulation, and the nervous and immune systems. Whilst facets of the response may be modelled using alternatives, it is currently not possible to use these systems in isolation to predict how the human lung will respond. However, where possible we do use alternatives to animals in our studies. For instance, in relation to gene therapy we employ human, mouse and sheep cell culture systems to screen for efficacy in gene transfection. Such studies can prove valuable in screening potential gene transfer agents prior to their initial assessment in mouse, and then in sheep. The latter studies are essential, because only in living animals can the influence of an intact immune system be properly assessed – an important aspect of gene therapy using viral vectors. In relation to identifying systems that could replace animals in modelling the effects of radiation on the lung we are actively involved in using precision cut lung slices for this purpose. These studies will parallel our progress under this license and we will naturally seek to establish the benefits and limitations of this system as a model of in vivo events.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>Every experiment is carefully evaluated by a statistician to determine whether its design is capable of answering the question that is being asked. This involves using prior data to estimate the variability of the phenomena we intend to measure, and our estimation of what would be a clinically useful, or relevant, result. From this it is</p>

	<p>possible to ensure that the minimum number of animals are used in each instance. This process is part of an ongoing process throughout the project that subjects every experiment to rigorous evaluation by experts in statistics, ethics and animal care.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>In the context of gene therapy rodent studies always precede and inform upon their subsequent application in the sheep or pig. However, both the sheep and pig are recognised as being a more appropriate translational model to address issues especially relating to delivery. In the context of studying the effects of infection and radiation in the lung we have carefully considered the option of studies in rodents. However, it is inevitably extremely difficult to limit injury to a small proportion (<10%) of the total lung volume in small animals – and for example in the context of radiation, almost all investigators simply expose half, or indeed all of the lungs to radiation. These approaches are generally not relevant to commonly used radiotherapy regimes in clinical medicine and the impact on the animal is correspondingly higher, leading to obvious respiratory symptoms. By using larger animals it is possible to limit the volume of lung involved to the extent that respiratory symptoms do not occur. It is also possible to return to the same animal at intervals thereafter to assess the response to radiation relative to unaffected parts of the lungs. These considerations shape our contention that studies in large animals for these purposes are more ethically sound and we do not advocate the suitability of piloting studies in rodents for that reason. However, we will keep a watching brief on developments in the field and where appropriate will use such information to refine our own research. The rationale behind the choice of sheep and pigs as our animal models includes the fact that they are large animals whose lungs are anatomically and physiologically similar to humans, and that they are amenable to both bronchoscopy and radiotherapy, and tolerate repeated interventions in this manner with no discernible clinical effect. Measures of toxicity relating to bronchoscopic interventions show consistency with related work involving human subjects.</p>

Project	Oral Microbiology in Health and Disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Many diseases of the mouth and intestines are not caused by a single type of bacterium but instead are a result of an upset or an imbalance in the numbers and types of our normal health associated bacteria that live in these parts of the body. This imbalance is referred to as dysbiosis. The aim of this work is to understand what factors are responsible for changing our normal health associated bacteria to disease associated bacteria. In addition, the work also intends to investigate ways in which the disease associated bacteria may be changed back to the health associated type</p>	

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Although many of the diseases of the mouth and intestine are not life threatening they are very costly in terms of treating the diseases and also in terms of the quality of life of the patients. Understanding what leads to changes in our normal bacteria and, conversely, what we could do to reverse disease associated bacteria back to the normal health associated bacteria will help in both the prevention and treatment of these diseases.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The project will use up to 6000 laboratory mice that will be bred for this work over the five-year progression of these studies</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In these experiments, the mice will get gum disease. However, this is not a painful disease in humans and the mice are not expected to have any, or only minimal adverse effects. Other experiments will lead to the development of inflammation in the intestines which can lead to weight loss and some bleeding. The effects in some animals in these studies would be considered to be moderate severity, though the majority of them would be of mild severity. All animals will be killed by a humane method at the end of the experiment.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The objectives of the research are to understand the role of commensal bacteria on the maintenance of oral health and the development of disease. This is a complex interplay involving the interaction of multiple bacterial species in the oral environment with the highly specialised tissue architecture of the tooth supporting tissues and immune responses. The use of an animal model is therefore the only practicable way to define this interaction.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>Our previous studies have demonstrated that animal group sizes of 5-10 animals provide sufficient statistical power to obtain significant results. We routinely obtain statistical advice and guidance from academic statisticians in order to ensure that our experimental numbers</p>

	are kept to the bare minimum.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse periodontal bone loss model and the DSS model of inflammatory bowel disease are very well established and are considered the gold standard of rodent models of destructive periodontal disease and inflammation of the digestive tract.</p> <p>Periodontal disease is a painless condition and we anticipate no or very limited harmful effects of experiments involving manipulation of the oral commensal microbiota. Animals involved in the DSS experiments will be monitored daily for weight loss and discomfort and also clinical measurements and severity will be noted based on IBD scoring.</p>

Project	Organ Transplantation and Replacement
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	Regulatory use and routine production
	Protection of the natural environment in the interests of the health or welfare of humans or animals
	Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project aims to advance our group's understanding of alternatives to conventional transplantation, to enable and inform further human clinical trials. Our particular objectives are to:</p> <ol style="list-style-type: none"> 1. Assess in vivo safety, biocompatibility, durability, and function of organ replacements made from natural, synthetic and hybrid materials manufactured by our group; 2. Assess strategies for improving blood vessel ingrowth within these scaffolds and integration into surrounding host

	<p>tissues;</p> <p>3. Assess cell harvest, culture and scaffold seeding techniques to determine optimum cell type, combination and delivery systems;</p> <p>4. Assess and refine optimal surgical strategies for deployment of personalised organ replacements.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Organ failure or dysfunction affects millions of people worldwide and frequently necessitates the replacement of such organs with new ones. Conventional organ transplantation is not possible for all organs, due to the poor quality of harvested donor organs. The need for new organs is also comparatively high amongst babies and children, where there is a low supply of donor organs of a suitably small size. Additionally, conventional transplantation come with a life-long need for immune system-lowering medication, which can have terrible side-effects including an increased susceptibility to life-threatening infections. Great leaps have been made in the fields of organ regeneration and personalised therapies over the last decade. The prospect of being able to build individualised tissues and organs is a hugely attractive one and would get rid of the need for human tissue donors. However, although animal work has been pivotal in the previous refinement of our clinical programmes, many scientific and safety questions remain to be addressed before the technology can be universally accepted and widespread clinical trials can be set up. This project will allow us to build on our group's extensive in vitro and in vivo work, and preliminary clinical work, to focus on making second generation scaffolds with improved cell survival, as well as optimising which combinations of cell types, scaffolds, seeding techniques and surgical techniques are likely to be most successful clinically for maximal cell survival, tissue integration and functionality of grafts. It will also allow us to work out the best ways to ensure a new and functional blood supply connects the scaffolds to the recipient. We have collaborations with the leading clinical groups</p>

	<p>working in this area. This data will therefore form an integral part of the pre-clinical justification for full-scale clinical trials.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use the following approximate numbers of animals over the course of the 5-year licence: • Mice - 600 • Rats - 850 • Rabbit – 375. Actual numbers used in any individual year of the project will vary, with a progression from smaller animal trial of materials and/or cells to larger animal studies where organ scaffolds are tested in the correct anatomical (orthotopic) position.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Our animals will typically undergo a 60- to 90-minute procedure, where they have the organ of interest exposed. A portion of this is removed and replaced with a graft seeded with the animal's own cells (harvested at an earlier date) and the skin is re-sutured. Animals may have the grafts implanted many weeks before into a section of muscle, or abdominal lining (omentum), to start the process of growing a new blood supply before it is needed at the time of surgery – this procedure will also take around 60- to 90-minutes. In cases where human cells are to be tested, animals will have been given immune system-modifying medication to help the seeded cells to survive. Animals recover well with good painkillers. In the majority of surgeries, animals are often active, eating and drinking on the same day. Animals tend to remain active throughout the experiments, but their breathing can become laboured if grafts develop scarring. Animals are put under sedation regularly to check that the grafts are acceptable and they are treated using surgical instruments to stretch or remove scar tissue or secretion plugs. However, there may be times where these problems develop too suddenly for treatment under anaesthesia and animals may die as a result. Animals who have had digestive organ surgery will take longer to feed on oral food (grafts are at risk of blockage in the early days) and so will be implanted with a feeding tube directly into the stomach to enable them to be given nutrition in the first week (they will always be allowed to drink water). These tubes will have to be kept</p>

	<p>clean and flushed regularly to avoid blockage. In addition, animals with digestive organ surgery may have to wear Elizabethan collars at the start to prevent grafts blocking with ingested fur and be kept in a barren environment without bedding, and so will be unable to groom themselves initially. Single housing may be required on a temporary basis to look after grafts under particularly close attention are for the immediate post-operative period. If grafts block despite these measures, instrumentation of the blockage will be done under general anaesthesia to see if the blockage can be cleared – if this is not possible, the animal will be killed by deepening the anaesthetic. At the end of the experiments, animals will be killed by a humane method and tissues taken for analysis after death.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Neither in vitro nor ex vivo systems model the in vivo milieu with sufficient complexity to evaluate complex tissue-engineered organs or the in vivo interplay of cells and scaffolds with the host's tissue and ensure such transplants can function effectively when in their desired position within the body. It is vital to ensure ongoing investigation into scaffolds and cells for continued improvement of the scientific understanding and safety profile of second and third generation implant candidates, the results of which form mandatory parts of a dossier to the MHRA to allow clinical trials.</p> <p>Alternatives of testing cell-seeded scaffolds ex vivo in bioreactors will be performed prior to animal experiments to inform in vivo experimental conditions, as well as limit experimental groups and sizes to those that show sufficient in vitro and ex vivo promise.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>Statistical analysis will be planned at the conception of each experiment to ensure minimum numbers of animals are used. Multifactorial randomized block designs will be employed to reduce group numbers as far as possible whilst maintaining statistical significance (at 90% power and significance of</p>

5%). Paired grafts will be comparatively evaluated for statistical differences (e.g. using Student's paired T-test), whilst experiments with more than one group will be analysed using ANOVA analysis or similar. All animals will be included in statistical analysis to minimise attrition bias in survival data (analysed via Kaplan Meier survival curves). To account for 5% morbidity requiring early termination from causes related to immunosuppression/immunodeficiency (such as increased susceptibility to wound infections), this number might need to be increased in experiments where immunosuppression/immunodeficiency is used. [L]
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For pilot studies (e.g. Protocol 2), 3-5 animals will be used, depending on the likely size of variation in principal outcome measure, to obtain estimates of effect size for formal power calculations as well as refine technical aspects.

To reduce bias, animals will be ordered in batches from the same suppliers according to a specified weight and sex. Pairing of experimental and control grafts in each animal is an example of how we plan to use multifactorial designs to minimise bias due to individual variability. Animals will be randomised to surgery arms at the start. Prior to surgery, grafts will be randomised to cell seeded/non-seeded groups and prepared for surgery by a separate researcher, such that surgeons do not know the seeded status of a given graft. Where possible following surgery, animals will be reassigned identifiers to blind surgeons during postoperative endoscopic follow-up and post-mortem analysis. Video footage will be taken during endoscopic procedures to enable repeat assessment by a second blinded surgeon, and to enable the same animal to receive longitudinal follow-up at multiple time points. Each animal will be analysed in as many ways as possible without increasing animal suffering to reduce the numbers needed for experiments (e.g. physiological monitoring, imaging in vivo, analysis of organs).

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice and rats are appropriate species for the screening of combinations of scaffold materials, cells and growth factors, as they are economical and are the lowest animals on the evolutionary scale suitable for the modelling of some human conditions. We will use rodents for early phase experiments, whilst rabbits are the appropriate species for organ replacement modelling in babies and young children as they are an excellent size and geometric match and have low individual variation in organ size. Longer follow-up is achievable in rabbits than in rat or mouse surgery, as commonly-available veterinary equipment can be used to reduce unnecessary mortality from graft scarring.</p> <p>Animals will receive regular monitoring several times a day, particularly in the first month after surgery, and will be monitored continuously if/when problems arise. Painkillers will be given routinely following any painful procedure, and local anaesthetic will also be given generously to aid comfort in the immediate recovery period. Animals undergoing digestive organ surgery will be given supportive care with their nutrition via a tube directly into their stomach (implanted under the skin at the time of surgery) – our previous experiments have found this to be well-tolerated.</p> <p>Even with frequent observations and supportive care, the risk of sudden severe breathing difficulties in some animals with respiratory grafts, or the potential risk of them being found dead in the morning despite appearing completely well at their evening checks, cannot be completely removed. The unpredictability of this secretion plugging is why we feel a ‘severe’ rated severity limit is justified on this protocol, in order to prevent premature termination of animals who are well at the time of assessment, or who may be improved following treatment. End points for animals who develop clinical signs of respiratory problems will be at a default of 36 hours following the first discovery of symptoms in animals who continued to decline, as we have seen that by 48 hours this moderate respiratory effort would usually either resolve, stabilise or worsen to the point of requiring early termination. Assessment of</p>
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	these animals under anaesthesia will help to further tailor endpoint timings to individual animals (i.e. less than 36 hours).
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Project	Organisation of neuronal dynamics in cortical microcircuits and related structures	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Neuroscientists seek to explain how brain activity gives rise to perceptions (e.g., recognising pictures, sounds and smells), movements (e.g., getting up from a chair), and memories (e.g., recalling your yesterday's whereabouts). These cognitive processes occur in a fraction of a second, so most neuroscience research is focused on neural signalling that happens on this and faster timescales. Some neural processes, however, happen on much slower timescales. Sleep is one such example: one cycle takes about 24 hours, a duration that	

	<p>is controlled by endogenous neuronal processes and persists even in complete darkness.</p> <p>In fact, neural dynamics is not limited to one or just a few specific timescales but has prominent features on all timescales - seconds, minutes and hours included. Unlike fast timescale neural activity, the functions and mechanisms of the slower changes in neuronal activity received very little attention and are not well understood. The aim of the present project is to understand the mechanisms regulating the activity of individual cortical neurons on timescales of tens of seconds to several minutes, as well as the ways in which the activity of individual neurons is interrelated with the activity of large ensembles of cortical neurons. This will give us new insight into cognitive processes that happen on these timescales, such as changes in attention, motivation, introspection, vigilance, as well as how these slow processes modulate the fast cognitive processes.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This work will answer fundamental questions about nerve cell functions, however there is an additional important reason for needing to understand the slow changes in activity of cortical neurons. This reason concerns 'functional magnetic resonance imaging' - the most advanced method for non-invasively observing neural activity, which is widely used in both science and medicine. Functional magnetic resonance imaging (fMRI) measures neural activity indirectly, via its effect on the blood supply to the brain, and is consequently limited to revealing only the slowest components of neural activity. As a result of fMRI's widespread use, much is known about slow changes in activity of cortical areas, and this information is beginning to be used for diagnosis and treatment of neurological conditions. What is sorely lacking, however, is an understanding of how individual nerve cells give rise to the high level activity patterns. Our study will help filling this gap in our knowledge, and thus provide a better understanding of the origins of fMRI signals.</p>
<p>What species and approximate</p>	<p>Mice: 100-200 / year Rats: 15-20 / year</p>

<p>numbers of animals do you expect to use over what period of time?</p>	
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The main experiments in this project involve monitoring or influencing their neuronal activity using electrophysiological or imaging techniques. To allow access to the brain, mice undergo one or two surgery procedures. During surgery they are fully anesthetised (and do not experience pain). The animals are also implanted with small bars that allow to keep their head in a fixed position for a duration of a few hours (mice can still adjust the position of their body with respect to their head). This is necessary because some of the equipment used to look at the neural activity (e.g. a microscope) weighs many kilograms and cannot be used otherwise. The experiments themselves are performed several days after the animals recover from the surgery and are not painful. After the experiments are complete, the animals will be humanely euthanized. We need to record in drug-free (awake or naturally sleeping) animals because anaesthetics significantly modify neuronal activity, therefore it is impossible to fully understand the mechanisms of brain activity in humans and other mammals if one only studies brain activity under anaesthesia. In addition to experiments in healthy animals, we will conduct these experiments in transgenic mouse models of motor neuron disease, such as Amyotrophic Lateral Sclerosis (ALS). These animals will only be used at early and intermediate stages of disease progression, where the disease does not cause excessive discomfort. The severity of the above procedures is classified as 'moderate'.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Measurements of neural activity are currently only possible in live animals. Non-invasive approaches, such as fMRI, cannot be used to address the questions we are studying. In fact one of the main aims of the project is to better understand the physiological</p>

	<p>mechanisms behind the fMRI signals.</p> <p>Computer simulations of neural networks also cannot give us the information we seek, as the understanding of physiological mechanisms which have to be simulated is lacking. In fact furthering our understanding of such mechanisms is the very goal of our research.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We strive to use the latest technology for recording from large numbers of neurons simultaneously (tens to hundreds of neurons). This makes it possible to use much fewer animals than in previous studies.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Among other reasons (such as genetically altered strains), rodents were chosen out of the attempt to use the least sentient animals in which the scientific questions can be studied. Furthermore, their needs can be more easily met in laboratory environment.</p> <p>Some of the methods for recording the activity of large ensembles of neurons (a reduction approach mentioned above) are only available in genetically altered strains of mice.</p> <p>The health and wellbeing of the animals will be closely monitored. Appropriate anaesthetic and analgesic procedures will be used. The animals will be provided with environmental enrichment and when possible they will be group housed.</p>

Project	Orthotopic Tumour Models for Therapy of Advanced Cancer and Fibrosis Models	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to utilise the most clinically-relevant animal models to understand the role of specific genetic abnormalities causing cancer development and progression and the role of fibrosis within the local environment of cancer cells in mice. The main objective is to evaluate novel therapeutic approaches for advanced cancers.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit)	Once cancer spreads (metastasis), cure rates significantly diminish and over 90% of cancer deaths are due to secondary cancers at sites in the body other than the origin. We are	

<p>from the project)?</p>	<p>developing therapies targeting genetic alterations associated with tumour growth, tissue invasion, cancer spread and also the cancer's blood supply through new blood vessel growth (angiogenesis) on which sustained growth and the opportunity to disseminate via the blood stream depends. We need to model both common and rarer cancers which are hard to cure and also their major sites of metastasis to ensure that our new drugs are capable of tackling these unmet clinical needs. While targeted therapies have shown some promise, the development of drug resistance and the need for rationally-designed combinations of a drug is becoming a major issue which will be addressed in this licence. We measure the efficacy of an agent (on primary tumours and/or metastases) in relation to its levels in the blood and/or tumour to inform the optimum starting dose and schedule in man. In parallel, we develop quantitative biomarkers of response which help us to understand determinants of sensitivity or resistance and to confirm that efficacy is tightly linked to the desired mechanism of action. This knowledge and the technology we develop in our models is directly transferrable to the clinic. We also check normal tissues at autopsy, and aim to define the 'biologically effective dose': the minimum dose of the drug that gives therapeutic benefit without significant adverse effects. The most promising compounds proceed to clinical development and trials in cancer patients.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We use immunocompromised mice (weak immune system, not able to respond normally to an infection) -the simplest species suitable for such complex pathophysiological studies in which human tumour cells can be grown in a mouse. Over a 5-year period, we expect to use no more than 8500 mice.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The vast majority of mice in the project will carry some form of tumour – injected under recovery anaesthesia into specific organs, such as the breast, pancreas, liver and under the skin; whilst some mice will have the cells injected into a vein to allow spread to the lungs, or in organs where metastatic tumour cells spread to naturally. Up</p>

	<p>to 90% of mice will be treated with new drugs (typically orally, or by injection) appropriate to the cancer types under study. Where possible (up to 80% of mice), imaging will be used to track tumour size and location in the body, and comparisons made between control and drug-treated mice. Mice will be injected, by one of a number of routes, with potentially novel drugs that have been shown to be effective in the laboratory (cell cultures), and then need to see how they act in a whole animal. To allow us to make these measurements we will use similar imaging methods that are used clinically, whilst the mice are briefly anaesthetised to monitor effects on tumour growth in 80% of the mice. Furthermore, 40% of the mice will also be injected with chemicals that aid the imaging process. We will also take blood samples from the tail vein for measurement of blood-borne chemicals and concentration of the novel drugs in 10% of mice. A further 10% may also be killed humanely whilst anaesthetised in order to obtain larger blood volumes and tissue samples for microscopic observation. In approximately 90% of cases, mice would be expected to experience only moderate levels of discomfort, as the tumours they carry would not make a significant impact on their general health and wellbeing, and the majority of other procedures (non-invasive imaging, injection of therapeutics), will generally result in no more than transient discomfort and no lasting harm. Up to 1600 of the mice will be used to study the processes of lung and liver fibrosis, which influences the implantation of circulating cancer cells. In the liver fibrosis model however, it is likely that the agent used to induce the fibrosis will cause transient pain, which will be managed by local anaesthesia, and local inflammation which will be managed with advice from the vet. All the mice will be killed by humane methods at the end point of the experiments.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-</p>	<p>Human cancers develop in 3-dimensional (3-D) space within specific tissues in the body. Each tissue provides a unique growth environment which cannot be adequately modelled in 2-D cell</p>

<p>animal alternatives</p>	<p>cultures grown on plastic dishes in the lab. Cells grown in the lab are provided with constant, optimal levels of oxygen and nutrients, and are all growing at the same rate. This is rarely the case in the body, and this variability can significantly influence responses to therapy. Metastasis in particular (the major cause of treatment failure) is exclusively an <i>in vivo</i> phenomenon, as tumour cells from a primary cancer must access the blood circulation to spread around the body and colonise new organ sites. Similarly, the effects of drugs must be tested <i>in vivo</i> to determine that adequate levels are achieved in tumour tissues, that adverse effects on normal tissues are minimised and that efficacy tracks with effects on indicators of tumour growth.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>All compounds are first tested in tissue culture for suitability for testing in mice using both simple tumour cell monolayers and more complex 3-D functional assays (e.g invasion). Failure at any of these stages, limits the number of compounds going forward for testing in animals. As far as possible we use cells in which we express luminescent or fluorescent markers that emit light, enabling detection of tumours inside mice using optical imaging, which is quick and requires only light anaesthesia. Otherwise we use methods such as magnetic resonance imaging or ultrasound to locate and follow the development of internal tumours and their response to therapy. Thus fewer mice are required and studies can be terminated before the animals experience significant symptoms. We ensure that we obtain the maximum possible information from each tumour, assessing not only tumour growth, but also correlating efficacy with drug levels and biomarker responses to give statistically robust data in proof of concept trials.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to</p>	<p>Mice are the lowest sentient species that are appropriate for <i>in vivo</i> drug development studies and are widely used for this purpose. Most of our work is carried out using well-characterised human tumour cells, grown in the appropriate anatomical site in naturally immunodeficient adult mice to avoid tissue rejection. This enables us to study human cancers in the correct tissue</p>

the animals.	microenvironment. The animals are maintained in individually ventilated cages using sterile food and bedding with enrichment of the cage environment and all procedures are carried out in special cabinets using strict aseptic techniques to avoid infections. Suffering will be minimised by keeping tumour sizes within tolerable and acceptable limits and according to recognised guidelines. Compounds are delivered using previously determined well-tolerated doses and schedules, and are generally of low toxicity (e.g. agents targeted to molecules selectively overexpressed or mutated in human cancers). Due to these types of models, we will be able to make the most of all non-invasive methods to study stroma, fibrosis, with BIL, MRI, CT-Scan, Radiography, optoacoustic, ultrasound. These Refinements will be applied to all the protocols described.
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Project	Osteoblast-endothelial interactions regulate bone formation and remodelling	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>To work properly bones need to be healthy and strong. To maintain their strength the bone tissue itself is continually replenished. The cells that produce new bone are called osteoblasts. In disease like arthritis and osteoporosis the osteoblasts are not working properly and therefore do not produce enough new bone.</p> <p>We have discovered a group of genes that work together to control the movement of osteoblasts along blood vessels to sites of new bone formation. REDACTED We think that understanding how these genes function could</p>	

	<p>provide a new way to treat musculoskeletal diseases by boosting osteoblast movement to sites of new bone formation and therefore improving bone strength and repair.</p> <p>This licence will test this idea by assessing the role of the REDACTED pathway in:</p> <ol style="list-style-type: none"> 1. Normal bone development and turnover 2. Bone turnover in osteoporosis and arthritis <p>Ability of drugs that target the REDACTED access to increase bone formation in osteoporosis and arthritis</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Musculoskeletal diseases such as arthritis and osteoporosis affect > 10 million people in the UK, costing the NHS ~£4.7 billion per year and accounting for over 30 million working days lost per annum. There is still no effective treatment that is capable of reversing the loss of bone that occurs in these diseases. This project builds on new findings that a specific type of blood vessel in the bone can drive bone formation. Understanding this process has the potential to provide the next generation of treatment for osteoporosis and bone repair in arthritis – two of the largest bone disorders affecting our ageing population. The earlier we can treat patients with age-related or arthritis-induced bone diseases - the better the long term prognosis will be for the individual in terms of disability and mortality.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This project will run over 5 years and will use mice as the experimental animal. In this project, the majority of the mice used will be genetically modified with a non-harmful phenotype (protocol 1). These mice will be used to maintain breeding colonies or will be culled by a schedule 1 method for tissue collection. Over the duration of the project we expect that the number of mice used for this part of the project will not exceed 6000. A further 2000 mice will be bred under protocol 2 - genetically modified with a harmful phenotype. The majority of these animals will be used to maintain the colony of which half will carry the</p>

	harmful transgene. Over the 5 year period we expect that approximately 500 mice will be used for bone turnover experiments (mild), 500 for ovariectomy (moderate) and approximately 1000 for polyarthritis models (moderate) and 200 for blood collection (non recovery)
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The maximum severity limit in this licence is moderate. Possible adverse effects include pain from surgery, irritation from local injection, pain from joint inflammation, weight loss or mobility problems. Any animal showing deviation from normal behaviour as judged by daily monitoring of food and water intake, body weight, general and coat appearance, gait or behaviour will be treated with pain relief and food supplements. Before pain and inflammation exceed a moderate severity level, animals will be killed to prevent any on-going pain or suffering. All animals will be sacrificed at the end of the protocol with the exception of protocols 1 and 2 (Breeding and maintenance of genetically modified mice).
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The process that we are studying is that of osteoblast (bone-forming) cells moving along blood vessels and forming bone at sites of damage. There are no non-animal (<i>in vitro</i>) methods that mimic this process. By definition, to study bones, we must use vertebrate organisms</p> <p>Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace <i>in vivo</i> animal use.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Where pilot data exist we will always perform statistical power calculations to ensure that we use the minimum number of animals needed to obtain statistically significant results.</p> <p>In designing our experiments we will ensure randomisation of animals to different treatment groups across the different cages. Researchers performing the analysis will always be are</p>

	<p>blinded to animal treatment groups to prevent confirmation bias and ensure the accuracy of results.</p> <p>Where new routes of administration or new therapeutics are being examined, pilot studies will first be established in 2-3 mice prior to full experiments.</p> <p>We will use the NC3R's experimental design tool to aid experimental design and consult trained statisticians before using any new protocols. All staff performing animal experiments will attend appropriate training on key aspects of experimental design. We will publish in open access journals that support the ARRIVE guidelines for reporting.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>It is vital to utilise experimental models that most closely mirror the human disease so that effective therapies in these models are likely to translate in novel treatments in the clinical setting. The mouse is the choice of species for our experimental models as they have been well characterised. In mice the induction of different bone diseases that mimic human disease has been demonstrated by many researchers and these models have been used to develop new therapeutic options.</p> <p>Each experimental model will be monitored daily following intervention and mice will be assessed for any signs of distress such as pain and inability to feed. Wet food will be given for mice with diarrhoea and barrier cream will be used prophylactically to prevent skin irritation. Surgical interventions will be undertaken using the most appropriate anaesthetic and analgesia will be given. The mode of substance administration will be chosen to cause the least harm and distress to the mouse. Any new substances or route of administration will be tested in a small pilot study and the mice monitored daily for signs of distress. Humane endpoints will be strictly adhered to at all times.</p> <p>We have extensively refined the scoring system for each individual polyarthritis model to capture the specific aspects of each arthritis phenotype, ensuring clear and consistent use of analgesia and humane endpoints. We have also made</p>

	<p>refinements to the housing of the animals to cater for any disability arising from arthritis.</p> <p>We will also systemically review each experiment on completion to see what lessons can be learned from the study in terms of endpoints (scientific and humane) and any animal welfare issues that may have arisen during the experiment that could then guide any subsequent experiments.</p>
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Project	Overlapping mechanisms of sleep and anaesthesia	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project seeks to understand how general anaesthetics and sleep-inducing drugs act with the aim of developing safer, more efficient or more selective agents. We also hope to shed light on the fundamental mystery of why we need to sleep and why the lack of sleep is harmful.</p> <p>Modern surgery would be impossible without general anaesthetics, yet the underlying mechanisms by which they produce unconsciousness and pain relief are only just beginning to be discovered. Understanding the molecular actions and neuronal pathways involved presents a major intellectual challenge</p>	

	<p>for basic neuroscience, and research into anaesthetic mechanisms can be expected to provide fundamental information on neuronal excitability which has broader applicability. It is widely recognised that the anaesthetic drugs presently used in clinical practice are far from satisfactory. Currently used anaesthetics are relatively “non-specific”, mostly act at high concentrations, and affect many targets. Consequently, many patients suffer from undesirable side-effects from the anaesthetic and analgesic drugs used for their perioperative care. Serious morbidity (e.g. cardiovascular side effects) can be provoked in already compromised patients, which is an issue of increasing concern in an ageing population. Our Programme seeks to understand which molecular targets are responsible for the desirable effects of the anaesthetics and the neuronal pathways that are involved.</p> <p>Because we have determined that sedatives and anaesthetics act, to some extent at least, on natural pathways of sleep and arousal, our work is also directed towards understanding why the need for sleep is so powerful and why the lack of sleep can be so damaging. For example, it is possible that dementia is exacerbated by the long-term lack of sleep, and our research might provide novel approaches towards treatment of this and other neurological disorders. Our work may also provide new approaches towards the development of more selective sedatives and anaesthetics.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The clinical relevance and potential benefits of our work is four-fold. First, the prevalence of sleep disorders is large and growing rapidly with approximately 15% of the UK population affected, with the elderly being particularly susceptible. This results in a substantial societal and economic burden. The need for novel drugs to treat insomnia, daytime sleepiness, shift-work sleep disorder, and other sleep disorders is growing. Second, in the hospital, sedative drugs may contribute to the development of delirium. When patients are sedated in intensive care for prolonged periods, delirium keeps patients on mechanical ventilation, thereby prolonging their stay and increasing the risk of complications</p>

	<p>from infections. Application of sedative drugs during intensive care that produce a more “natural” sleep may promote the restorative benefits of sleep, prevent delirium, and minimise the deleterious effects of sleep disruption which may, in itself, significantly increase morbidity and mortality. Third, during many procedures, for example endoscopy, patients must be both sedated and relatively immobile yet remain compliant. Therefore, depending on the patients’ needs and the specific procedure, drugs that highlight different elements of the sedative-hypnotic continuum are needed. Understanding the neuronal networks involved in the production of the sedative/hypnotic response by different classes of agents will permit rational selections tailored to the patient’s needs and could result in major healthcare benefits. Fourth, there is a growing appreciation that certain neurological disorders such as Alzheimer’s disease may be exacerbated, or even caused, by long-term bad sleep. If this link can be established, it may be possible to slow the onset of such diseases by paying specific attention to sleep regulation. In summary, by exploiting what is known at the molecular level, our research should provide insights into both how natural sleep is regulated, as well as how and where general anaesthetics act at the level of neuronal networks. This will inform strategies for the development of new drugs, including those to treat sleep disorders, and these strategies may be applicable to currently intractable neurological disorders.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Because we are studying sleep and loss of consciousness induced by anaesthetics, the use of animals is unavoidable. We use mainly mice. We use mice because genetic engineering allows particular mice to be bred which carry putative anaesthetic targets or circuits that have been modified genetically. This allows us to test specific hypotheses regarding the importance of particular molecular targets or the importance of particular brain circuitry. Thus we can investigate the roles of individual anaesthetic and sleep drug targets in the response on the whole animal. We use, typically, 4000 mice over 5 years. Approximately 200 mice are killed humanely and their brain</p>

	<p>tissue used immediately. The remainder of the animals are used in procedures that last only a few hours for individual experiments. Occasionally we use tadpoles to assess anaesthetic potencies. Approximately 200 tadpoles will be used for the project.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Animal suffering is minimal in our experiments because the drugs we study are anaesthetics. By their nature, these drugs render animals insensible to pain. In some experiments where we investigate specific neuronal pathways, we introduce electrodes or fine tubes into the brain to deliver chemicals, or temperature-recording devices in the abdomen. Then, anaesthetics or sleep-inducing drugs are applied to specific parts of the nervous system, animals are closely monitored; the experiment is terminated, if suffering is evident. Such events are rare, however.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>To understand how anaesthetic receptors in the brain and neuronal subtypes contribute to brain physiology and generate sleep, or respond to anaesthetics to produce unconsciousness, it is essential to use native brain tissue. Although properties of individual channel types or receptors are best studied by using cell lines that are able to express certain brain receptors, to study how the channels/receptors work <i>in vivo</i> and in their native membrane environments requires the intact animal. The complexity of how receptors and ion channels influence brain physiology and how the channels interact with each other can only be appreciated by using mouse lines (or tissue derived from them) with deleted or modified ion channel genes.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>1. Reduced animal numbers and appropriate statistics: we maintain mouse lines at the minimum needed. To reduce the number of individual mice needed we plan the experiments (e.g. injection of substances and agents) by applying appropriate statistics so that the minimum number of animals are used to give</p>

	<p>statistically meaningful data.</p> <p>2. Reducing the numbers of mice needed: a genetic Refinement to identify subtypes of neurons in slices of brain taken after death by visually guided recording of genetically fluorescent cells To understand how particular brain circuits function, we need to know the electrophysiological properties of the neuronal types. We use a technique whereby specific types of neuron can be made fluorescent so that they can be easily visualized under a microscope. This means that these neurons can be efficiently identified in living brain slices, so requiring far fewer animals to get the same results.</p> <p>3. Computer-controlled injections reduce the number of animals needed We are using computer-controlled devices ('Angle 2' Stereotaxic frame manufactured by Leica) that allow for highly improved accuracy of stereotaxic injections/electrode placement and this greatly reduces the number of animals that need to be used to get a useful experimental results. In addition, these devices allow us to store target coordinates for each animal. This reduces surgery time, and thus speeds recovery.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use tadpoles and mice. Tadpoles are useful to study the potencies of anesthetics that are rapidly metabolised in mammals. This allows true potencies to be determined without being affected by the complexities of metabolism. For many anaesthetics, however, potencies in mammals need to be determined because these better reflect potencies in humans. To get close to the human condition, we study how neurotransmitter receptors, ion channels and neuronal subtypes influence brain physiology using mammals. Mice are the only mammals that have easily modifiable genetic systems. Hence the mouse is a model organism for our work.</p>

Project	Parental Genetic Effects in Blue tits	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of the project is to understand the genetic basis of parental care, using blue tits as a wild model system. Young blue tits differ a lot in how fast they grow and how long they live. We know that the most important factor in driving these differences is who raises them. However, we don't know whether parents differ in how good they are because of the genes they carry or because the environment they have experienced.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	Measuring the impact of parental genes on offspring growth and survival is hard, and the three wild/feral species in which it has been tried (all mammals) have provided very	

project)?	uncertain results. By employing cross-fostering, where parents raise foster offspring, on a large sample of birds we will be able to provide definitive answers to this important question.
What species and approximate numbers of animals do you expect to use over what period of time?	The 250 nest-boxes we have erected are typically occupied between 100 and 150 pairs, each of which have approximately 8 eggs that go onto hatch. Consequently, we expect 800-1200 chicks and 200-300 parents each year for the duration of the study (5 years). This would result in up to 7500 birds in total across the 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Each pair will raise half their own offspring and half of someone else's and these foster offspring will be fostered into their host nests as eggs. Because the eggs hatch en masse it is not possible to assign a chick to an egg and hence know whether they are a foster chick or not. To circumvent this we will take a small blood sample (less than a drop) which we can use to obtain DNA and hence determine if they are related to their foster parents or not. Blood sampling probably entails minor discomfort but the adverse effects are not long lasting, and the chicks are placed back in their nest immediately after the sample is taken.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to study parental care, and we need to study it in the ecological setting where it evolved. It is therefore necessary that we work on a species that shows extended parental care and that we can work on in the wild. Blue tits are one of the most tractable and safe to work with species in this regard.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The project licence holder is an expert statistician and has conducted analyses to work out what is the minimum number of families required in order to get sufficiently good answers.

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Blue tits were chosen because they are a common species that readily uses man-made nest boxes to breed and is very robust to disturbance at the nest site. The handling and bleeding protocol causes minimal suffering to the animal, and alternatives, such as feather plucking do not yield DNA of sufficiently high quality. We keep all chicks warm during the procedure and work in teams of three in order to minimise the amount of time the birds are out of the nest.</p>
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Project	Paternal epigenetic inheritance in zebrafish	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We inherit our traits from our parents through genes embedded in the DNA of the sperm and the oocyte. However, other components of the egg apart from the DNA also contribute to the offspring. In recent years it is becoming clear that the environment of the father (including diet or stress) can also be inherited by the offspring and even passed on to the next generations resulting in altered traits. Increasingly, scientists consider the possibility of inheritance of environmental effects through chemical tags which influence the way DNA is condensed inside the nucleus of the cell. These tags are referred to as epigenetic marks. This DNA packaging will influence on how genes get	

	<p>switched on and off during the embryo formation and can have lasting impact by changing the traits of the offspring.</p> <p>The objectives of this project are:</p> <ol style="list-style-type: none"> 1. Establishing a model epigenetic inheritance of the paternal experiences using zebrafish 2. Determining how long these paternal experiences last across several generations 3. Identifying the epigenetic marks involved in transgenerational inheritance and how they are linked to specific environmental stressors
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>In this project we shall answer whether common substances of abuse impact on the offspring and to what degree. This work will provide crucial insight into the type and degree of risks of environmental exposures and how they impact on the offspring not only in fish but also in humans. In the longer term, these observations will have impact on how advice is given to parents to be on their lifestyles and preparing them and their offspring for potential consequences of those exposures.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Zebrafish, 300 adults, 3 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The exposure to addictive substances will be chronic meaning that we expect a gradual adaptation of the fish to these stressors and the effects will be minimum. We will monitor possible behaviour alterations as well as any kind of injury or distress. We will also perform blood analysis from fish after schedule 1 to ensure that markers related to these substances of abuse correspond to their previously described levels in a chronic exposure. Regarding these possible side effects, we will apply schedule 1 the moment that humane endpoints have been reached.</p>

Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>It is not possible to study complex progress such as how traits in father are inherited by offspring with non-animal alternatives. Within animal models, invertebrates such as <i>Drosophila</i> and <i>C. elegans</i> are other popular model systems for studying inter- and trans-inheritance. However, invertebrates show differences in epigenetic mechanisms. For example <i>C. elegans</i> presents an evolutionary loss of DNA methylation.</p> <p>However throughout the course of the project we will look for non-animal alternatives to parts of our work by constantly reviewing the literature</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>General considerations:</p> <p>In this study, sperm collection will be done in the same fish before and after the exposure to nicotine and ethanol in order to decrease the number of fish used and the variability within our experiments. Zebrafish embryos and larvae, which are not regulated animals, will be used wherever possible. .</p> <p>The protocols included in this project will be revised by the AWERB (Animal welfare and Ethical Review Body) to ensure robust design of the experiments.</p> <p>The ARRIVE guidelines will be considered when conducting our studies and communicating results to the scientific community to ensure the best reporting of our studies .</p> <p>Environmental stressors: exposure to ethanol and nicotine.</p> <p>We will use a pre-test work to determine and prevent unexpected adverse effects and control the logistics of the set up. In this experiment a small number of fish will be exposed to an increasing concentration of the substances until reaching the final dose proposed in this project. This way we will avoid repetition of experiment or exposure which would lead to use a larger</p>

	<p>number of fish. The amounts of ethanol and nicotine here chosen are the lowest between those previously reported by other groups working with zebrafish.</p> <p>During the exposures, we will control variability and bias by using the experimental designer provided by the 3Rs website.</p> <p>The sample sizes determined for the exposures to environmental stressors are based on our previous experiments in collaboration with collaborators</p> <p>Larvae will be randomly and blindly allocated in the experimental well plates. Sample size has been determined based on literature and advice of statistician</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Zebrafish are non-mammalian vertebrates that have been shown to have stress response and have been extensively utilised as a genetic model for the study of development. Therefore, a large number of resources are available and laboratory protocols are well established.</p> <p>For our experiments with environmental stressors, we have chosen some of the mildest forms which are however robust and well document to have distinct effects. We avoid using adverse stimulus that may have lasting impact in the adults.</p> <p>For DNA extraction, we plan to switch to mucous swabbing when possible which has been demonstrated to be less disruptive for the fish than fin clipping. This method has been recently standardized in our facility and members of our group are well trained in the procedure.</p> <p>All animals will be killed at the end of a protocol unless they were only subject to control treatment that consists merely of placement and lay eggs with specific markings. These fish may be returned to the breeding programs but will not be used for further behavioural analysis. Transgenic and mutant families shall only be kept if there is no evidence of morphological abnormality and no sign of distress (abnormal breathing, difficulty in swimming, failure to eat)</p>

in the adults.

Only untreated fish carrying non-harmful mutations or transgenes will be kept alive for breeding .

Project	Pathogenesis and Control of Mycobacterial Infections	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The objective of this license is to develop and evaluate new drugs and vaccines for the treatment or prevention of tuberculosis disease. In order to achieve this, new vaccine/drug candidates will be assessed in a sequential series of pre-clinical evaluations in appropriate animal models of the disease e.g. mouse and guinea pig.</p> <p>Tuberculosis is one of the leading causes of death of humans from a single infectious agent worldwide responsible for one and a half million deaths each year. The largest challenge to the successful control of TB is the detection and successful treatment of individuals with latent <i>M.</i></p>	

	<p><i>tuberculosis</i> infection who are at a high risk of relapsing with active, contagious disease. Many of the fundamental aspects of the host-pathogen relationship between <i>M. tuberculosis</i> and humans are poorly understood and there is an urgent need to define clear correlates of protective immunity and immunological biomarkers of disease.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The development of a safe, effective and affordable new TB vaccine or drug regimen would have a huge global impact upon human health. The studies proposed in this licence will have a direct impact upon the progression of novel candidates to early stage clinical evaluation. The need for new interventions (improved diagnostics, therapeutics, and vaccines) has been recognised as a priority by international agencies including the WHO and this programme of work will have a direct impact upon meeting targets laid out in the WHO global plan to stop TB.</p> <p>The benefits of treating TB using drugs and vaccines has already saved 54 million lives globally between 2000 and 2017. This programme of work will have a direct benefit of progressing the most promising new therapeutics to human clinical trials, will reduce the financial burden of TB disease control and improve the lives of tens of millions of people.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>A total of approximately 1500 guinea pigs and 1200 mice will be used in this five year project, in order to study the pathogenesis of tuberculosis, and develop and evaluate new vaccines and drugs to prevent or treat global tuberculosis disease.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Drugs and therapeutics will be administered to animals and will be closely monitored for the unlikely event of an intolerance to treatment. Microdialysis probes will be implanted under anaesthesia and closely monitored for signs of distress. Animals will experience transient distress during induction of anaesthesia. This will be minimised by the use of sedative pre-medication if appropriate. The animal may feel a level of discomfort following implantation of the</p>

	<p>microdialysis probe and therefore, if possible, appropriate post-operative analgesia will be used to minimise severity. Animals may experience distress when individually housed as necessitated by the use of implanted cannulae. Individual housing will be necessary to prevent damage to externalised microdialysis probes from conspecifics. The effects of individual housing will be minimised through maintenance of auditory, visual and olfactory communication with other animals and through the use of modified enrichment and refuge shelters wherever possible.</p> <p>All studies will have clear strict indicators of disease progression ensuring the lowest level of distress possible. Following aerosol challenge, <i>M. tuberculosis</i> infection progresses slowly and animals remain clinically well for long periods. Early time-points therefore, allow assessment of progression of infection in the absence of adverse clinical events. Signs of severe infection include significant weight loss, loss of appetite and laboured breathing. These adverse effects are minimised by using early readouts and careful monitoring of weight and eating habit that measure the progression of disease before the onset of severe adverse events. The expected severity level is moderate. Animals will be euthanised at the end of each study.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Where alternatives to the use of animals exist or are developed such approaches will be used. Use of animals in pre-clinical evaluation of vaccines and therapeutics is needed to determine important safety and efficacy performances prior to introduction of these products to clinical or field trials. In addition, vaccines are targeting specific states of tuberculosis disease that cannot currently be replicated <i>in vitro</i> due to complex infection processes with many unknown mechanisms of evading the immune system.</p>
<p>2. Reduction</p> <p>Explain how you will assure the</p>	<p>This strategy will ensure that only the most promising candidates reach clinical/field trials thus reducing the numbers of animals used in</p>

<p>use of minimum numbers of animals</p>	<p>these studies. Study group sizes will be determined using power calculations to ensure the minimum number of animals are used in studies that will allow inferences to be made about significant differences in efficacy between groups.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Vaccine and drug targets will be generated from a series of non-animal studies using defined culture conditions that mimic environments that <i>M. tuberculosis</i> may experience in humans during TB infection. The candidate vaccines will then be evaluated in a step wise progression of <i>in vivo</i> experiments increasing in model complexity, primarily assaying for immunogenicity in the mouse. Those vaccines showing significant immune responses in the mouse will proceed to guinea pig or mouse efficacy studies. The most efficacious candidates in short-term guinea pig studies, showing reduced bacterial burden in tissues compared to controls, will proceed to long term survival studies in the guinea pig. Similarly, the drug candidates will be evaluated in a series of <i>in vivo</i> experiments. Mice and guinea pigs are widely recognised as being suitable species for the early stages of screening of TB vaccines in order to demonstrate safety, immunogenicity and protection against virulent challenge. Mice may be used to enable detailed immunological analyses, which are not currently feasible in guinea pigs. Guinea pigs are the favoured model of TB disease because pathology and immunological responses are more similar to human disease compared to those seen in mice. In most cases, TB is a relatively slow chronic infection and the majority of the studies will end at an early infection time point before animals will succumb to the disease. An absolute humane-endpoint of 20% loss in maximal body weight will minimise welfare cost to the animals.</p>

Project	Pathogenesis and management of pancreatitis	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Acute pancreatitis and chronic pancreatitis are common diseases that cause much human suffering and premature death for millions of people worldwide every year. Chronic pancreatitis can lead to pancreas cancer, one of the worst cancers that people can develop. Both diseases lack accurate methods to tell how ill patients will become, and both diseases are without licensed drugs to treat them. The aim of this project is to develop new methods of diagnosis and treatment for acute and chronic pancreatitis. Our objectives are to identify the disease mechanisms, to improve the animal models, to test new diagnostic methods and to test new drugs for both acute and chronic	

	pancreatitis. This work will build on our progress in the laboratory using isolated cells and patient samples.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits from this project are greater understanding of how acute and chronic pancreatitis develop, improved animal models that will help to find out what will work in patients, more accurate measures that tell us how ill patients will become, and new, much needed drugs to improve the outcome of acute and chronic pancreatitis in patients. We will share our findings with others working in the same field to ensure maximum benefit is gained through further research to achieve our aim.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 3600 mice and 400 rats over five years. This is in the context of millions of people worldwide who have very much suffering and premature death from acute and chronic pancreatitis.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We propose to breed and house mice that have had careful changes made to inherited genes. Most of the gene changes have been described and unexpected adverse effects are unlikely. We will keep a close eye on growth, development and bowel habit. When acute or chronic pancreatitis is induced, animals will be given pain relief and will be regularly checked. When chronic pancreatitis is induced, animals with fatty stool will be given pancreatic enzyme granules to help them digest their food. Surgery, needed for acute pancreatitis caused by bile (similar to gallstone pancreatitis in patients, the commonest cause worldwide), will be under anaesthesia using sterile techniques with a warming blanket and fluid to maintain the water in tissues. When surgery is done, recovery is normally expected within two hours. The risk of animal death is likely to be very low, but animals with very abnormal behaviour will be humanely killed to avoid suffering. All the work in this project is up to a moderate level of severity; at the end of the work all animals will be humanely killed.
Application of the 3Rs	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Most of our work is done on cells from humanely killed animals or from patients who have agreed to this. We also use computers to help find solutions, using '<i>in silico</i>' and '<i>big data</i>' methods. Only when it is very likely that we will prove a mechanism or effects of a drug will we test these in living animals. We have to do these tests to develop new drug treatments for any disease, as for acute and chronic pancreatitis, which are diseases that cause very great human suffering, lifelong morbidity and untimely death.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Our work will be step-by-step with go-no-go points to use the fewest animals for our scientific aims. We will use isolated cells to work out how to do the live animal work, and how much of a drug to give. This will help reduce the number of animals needed to find the right dose. We will use careful design of our work with proven statistical tests so our results will be reliable and use the smallest number of animals. We will measure as much as we can from each animal to avoid repeating work. We will test gene changes or drugs in one model of disease. If results are negative, usually no further tests in other models will be done. If results are positive, tests in other models may be needed to confirm what may occur in patients.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We propose mice and rats (rodents) for this work. The rodent pancreas is very similar to the human pancreas. The toxins that cause acute and chronic pancreatitis in humans are the same in rodents. The work we have done so far shows that disease develops in rodents is very similar to humans. Mostly mice and rats have been used for animal studies of acute and chronic pancreatitis in the past, far more than any other animal. We will refine the models further to make these more like human disease, with measures of the amount of oxygen in the blood, the speed of the heartbeat and how often animals draw each breath. We will follow best practice in animal care, as required by the Home Office, to cause animals as little pain and suffering as possible. Animals will be given pain relief, kept warm and given extra fluid as needed, with careful checking. The risk of animal death is very</p>

	<p>low, but animals that are suffering badly will be put down humanely. Previously we have used death as a measure of how well drugs work (the fewer deaths the better), but we have removed death as a measure as this is no longer required. As a result of our previous research in animal models of pancreatitis, we have been able to refine our methods in this programme of work in order to reduce suffering and distress while still being able to achieve our scientific objectives.</p>
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Project	Pathogenesis and prevention of infections by respiratory pathogens	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project will use mouse models of infection to investigate lung infections caused by respiratory pathogens such as the main cause of pneumonia, <i>Streptococcus pneumoniae</i> . The ultimate aim is to identify novel ways of treating or preventing these infections. Lung infections are a major clinical problem worldwide – they are the commonest cause of death due to bacteria, and one of the major causes of death in children under 5. They are also very common in the UK, with pneumonia causing about 65000 deaths per year. Pneumonia is particularly common in the elderly affecting about 1 in every 100 person	

	<p>over the age of 75 each year. However at present we do not have good vaccines that prevent the common bacterial causes of pneumonia in adults, and resistance to antibiotics is becoming commoner amongst the bacteria that cause lung infections. It is not clear why some bacteria such as <i>S. pneumoniae</i> often cause pneumonia whereas other closely related bacteria such as <i>Streptococcus mitis</i> do not. This project will further our understanding of what allows severe infections with respiratory pathogens to develop and how they can be prevented, and will eventually lead to improved preventative (including vaccines) or treatment strategies against these pathogens.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>1. Improved understanding of why and how serious lung infections can occur 2. Identification of potential targets for new antibiotics 3. Further development of new vaccines that are able to prevent respiratory tract infections or pneumonia</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Solely mice - we will use between 200 to 500 mice per year over the 5 years of the project.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Mice will be infected usually via inhaling micro-organisms under general anaesthetic or by injection into the blood or peritoneal cavity. Depending on the experiment the mice may have been vaccinated with potential vaccine candidates or treated with a drug therapy prior to or after infection. In the majority of experiments, mice will then be culled before severe infection develops and the response to infection analysed in target organs such as the blood, lungs and spleen. These timepoint experiments only require small numbers of mice per group (5 to 10) and, because mice are culled before severe infection develops, minimise any distress caused. Rare experiments will monitor whether a vaccine, bacterial mutant, or a drug effects the development of infection over time by watching for physical signs that infected mice have developed severe infection. The main adverse effects of these experiments are failure to recover from a general anaesthesia, local discomfort and very rarely significant trauma necessitating immediate culling after injection,</p>

	<p>and the signs and symptoms of infection (weight loss, piloerection, reduced mobility, and depending on infection site possibly cough, respiratory distress, diarrhoea, local tenderness, erythema and swelling). All animals are culled humanely at the end of the experiment using a schedule one method or exsanguination (terminal bleed from the heart under deep terminal anaesthesia).</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The incredibly complexity of lung anatomy, immune responses to infection, and bacterial / host cell interactions prevent these experiments from being done without using animal models of infection. For example, the lungs have a three dimensional structure consisting of a mucosal layer with multiple cell types, and during infection several different types of white cells are recruited to the lungs with their relative proportions varying in a very dynamic way. A vaccine will alter the response to the infection in multiple ways. In addition the bacterial infection may spread from the lungs elsewhere within the body. This highly complex process can not be fully replicated in laboratory cell culture models, nor by insect or fish infection models.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We minimise the number of animals needed by:</p> <ol style="list-style-type: none"> 1. Doing laboratory tests using models of specific bacterial host interactions to fully define how a given bacterial mutant or component interacts with the host before moving to animal models - that way we can be very specific about the information needed from an animal model and thereby reduce the number of mice required. Recently we have expanded the laboratory testing to use more human cells and even slices of human lung to further refine the data we may need from animal experimentation. 2. By only looking for large biological effects using animal models - these need fewer mice than subtle effects to obtain a statistically significant result. 3. For the majority of experiments using infection

	<p>models that can lead to important data with small numbers of mice eg competitive infection experiments that only need 3 to 5 mice, and timepoint experiments that need 5 to 8 mice per group.</p> <p>4. Using technical developments that means we can monitor bacterial numbers in the same mice repeatedly over time i.e. using nasal presses to assess bacterial colonisation of the upper respiratory tract, or imaging of fluorescent bacteria in live animals for infections affecting the lung or blood.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Our experiments are performed in mice as infection in mice usually closely mimics human infection and because genetically modified mice provide a powerful tool for identifying important host factors involved in the development of infection. We minimise welfare costs of the infection experiments by: (a) using for the majority of the infection experiments pre-selected timepoints for culling mice – this means that most mice will not develop severe evidence of disease before being culled; and (b) close monitoring of mice over the experimental period to identify any that may develop evidence of unexpected</p>

Project	Pathogenesis of REDACTED recurrent laryngeal neuropathy	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Many REDACTED are affected by a disease of their nerves that supply the larynx (voice box) which affects their athletic performance: consequently, they are less successful athletes. Many affected REDACTED undergo invasive surgical procedures by vets to tie the airway into a permanently open position or they are euthanased. However, this surgery is invasive and sometimes not successful and in some REDACTED it results in secondary lung problems relating to food material entering the windpipe, because the airway is not protected	

	<p>during swallowing.</p> <p>The cause of this nerve disorder is not understood, even though it has been recognised for 100s of years. Whilst up to 15% of REDACTED have clinical involvement of the nerves, there is good evidence that most if not all large breed REDACTED suffer the disease to some extent but in these animals it is only possible to detect the disease post mortem. Unfortunately, because the disease's cause is not understood, it is not possible to identify specific and logical treatments.</p> <p>In this project we are evaluating use of a method that should allow us to study the disease in living REDACTED based on a technique that is already utilised clinically by vets in client-owned REDACTED. It involves non-painful magnetic stimulation of nerve pathways and measurement of the conduction velocity within nerves. Our aim is to determine whether the technique is reliable so that it can be used by vets in the future in the clinical diagnostic setting and also to determine whether other nerves are affected in this disorder.</p> <p>The second objectives of this project are to study the effects of the disease directly, by detecting the precise cells in the brain that are affected and the transport processes that occur in the nerves themselves, using fluorescent markers that are injected into the muscles of the larynx, which get tracked back to the brain within the nerves. These studies should allow us to examine the processes that are occurring which lead to nerve cell death, with a view to identifying specific treatments that might be less invasive than the current surgery.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<ul style="list-style-type: none"> • An improved method for diagnosis of this condition for vets in clinical practice • Improved understanding of the disease's cause and the mechanisms that lead to nerve cell death in affected REDACTED
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Up to 20 REDACTED over 5 years</p>

<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The protocols are moderate severity because they will involve general anaesthesia, however the procedures are of an equivalent level to those that are currently used by vets for treatment of this condition. At the end of these experiments animals will be euthanased with an overdose of an anaesthetic drug allowing us to examine tissues to confirm the severity of the disease and efficacy of the techniques used.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The disease of REDACTED that we are investigating is dependent on the length of the nerves that supply the larynx and it is not possible to model the disease in cell or tissue culture, because nerves that are over 2m long cannot be maintained or grown in culture.</p> <p>Also, because we do not know what causes this disease, it is not possible to model the disease in other species.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>REDACTED varies in its severity and to test the application of the diagnostic technique we are proposing, we need to examine REDACTED with a range of severities of disease. Vets grade this disease from grade 1 (least severe) to grade 4 (most severe). We aim to test 3 REDACTED of each grade, meaning that we will examine 12 REDACTED overall. This number should give us meaningful, and statistically valid results.</p> <p>For other studies examining movement of substances within the nerves themselves and the tagging of neurones in the brain, we will utilise as few animals as possible. No more animals will be used, than we require to show the technique is successful and reliable.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms)</p>	<p>Since REDACTED is a naturally-occurring disease of REDACTED with unknown cause, and since we are studying this disease, we can only use REDACTED with varying severity of this disorder. Unfortunately, currently many REDACTED or other performance REDACTED are euthanased with this condition: our studies are designed to study this disorder with a view to</p>

<p>to the animals.</p>	<p>reducing the disease's high prevalence and identifying improved treatments and reducing the need for euthanasia.</p> <p>For our studies, many of the techniques we will use are already used by vets on client-owned REDACTED in the clinical setting. For other techniques, we are using approaches that are very similar to the surgical techniques used for treating this condition in REDACTED. We will always use painkillers and REDACTED will be housed, fed and maintained in the same way that REDACTED are normally kept and cared for.</p>
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Project	Pathogenesis of influenza infections in murine models	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Influenza (flu) is a seasonal virus that affects everyone and can cause pandemics.</p> <p>Our risk of getting sick from infectious disease is determined partly by our genes.</p> <p>The aim of this project is to understand how our genes contribute to our susceptibility or resistance to infection.</p> <p>Also, we want to develop new ways of treating viral infections that take advantage of the body's natural defense mechanisms.</p> <p>We will use computer programs and lab work to identify new genes and new antivirals to help us</p>	

	in the fight against flu.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits of this project will be the identification of new targets and treatments for influenza infections. This will provide us with new specific targets to generate novel antiviral molecules and so enable us to treat and prevent infection.
What species and approximate numbers of animals do you expect to use over what period of time?	All the animals in this project will be mice, a total of approximately 3,500 mice are expected to be used over the 5-year period of this licence however there will be continued efforts to minimise these numbers
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In all cases the animals used within these studies will be closely observed and monitored to ensure their health. Many animals will be anaesthetised for viral administration reducing the stress/discomfort. Substances will be administered to prevent or treat the symptoms of infection, thus testing the effectiveness of new antivirals. Some of the mice we use in this study will get very ill and we expect a high severity in these cases. We will closely monitor these animals and have set humane endpoints. Where possible animals will be removed from the study at the earliest time points where the science can still be achieved.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although we can use computers and cells in the lab to investigate and test new antivirals and the effects of changing genes in the mice during infection, they cannot replace the complexity of the infection response in a mouse or a human which uses lots of different cells at the same time.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have developed infection models that allow us to investigate multiple aspects of viral infection and the host response in a single animal. This greatly reduces the number of animals required to observe many changes at once.

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Our infection models permit the assessment of multiple aspects of infection in the same animal and so, we can look at the host/virus interaction in great detail. We have clearly defined end points, with experimental protocols that follow regulated standard operating procedures and are performed by trained staff. We are continually refining the experimental protocols we use.</p>
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Project	Pathology of chronic inflammatory disorders	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Our project aims at understanding the causes underlying the development of long lasting (chronic) inflammatory diseases.</p> <p>We are primarily, but not exclusively focusing in our research on Rheumatoid arthritis (RA) and Sjögren's syndrome (SS). We have been researching how each of each of these conditions occurs and finding new methods for treating them.</p> <p>We are in particular interested why cells that belong to the body's immune system, which normally protects against infections, turn hostile towards their own body and attack their own</p>	

	<p>tissues (this aberrant behaviour of the host's immune system is termed auto-immunity). These cells accumulate in the joints (in RA) or in the glands (in SS) causing severe inflammation. The role of the microbiome (the bacterial flora which colonise our guts) in the development of these auto-immune diseases will be scrutinised.</p> <p>In addition, we plan to investigate and develop new drugs capable of correcting the abnormal behaviour of the immune system cells in order to switch off inflammation and achieve long-term cure.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>As a model of long-lasting inflammatory diseases that we focus our research on, RA is one of the most disabling joint inflammations worldwide. It affects approximately 1% of adults, reduces quality of life, increases mortality and results in large medical costs. National-Audit-Office figures indicate that 45% of RA patients are of working age and within 1 year of suffering the disease 30% are unemployed. RA costs the NHS/society £560 million annually and £4.8 billion in work-related disability. Several new drugs provide good results in treatment of 60-70% of RA patients, still 30-40% of patients do not benefit from these drugs. By understanding the underlying causes of the abnormal behaviour of the immune system in long term inflammatory disease, we seek to:</p> <p>(1) Better utilize current drugs by giving the right drug to the right patient. For example, by confirming that a specific target protein or cell is involved in causing the disease in our animal models, and finding that this target is abnormally elevated/decreased in the patient; then this patient can benefit from a category of drugs that specifically target this protein or cell. This would provide: i) better care as it would avoid delay starting a more effective drug; ii) prevent unnecessary exposure to potentially toxic drugs and iii) avoid wasting NHS money on drugs which are not going to work. This work has the potential to benefit patient in the near future as it uses approved drugs which have been modified to target diseased tissue and decrease systemic damage.</p>

	<p>(2) Develop new drugs that will target specific cells and mechanisms contributing to the development of long lasting inflammation.</p> <p>(3) Explore the interaction of the gut bacteria with the immune system and its role in the development of disease. Establish the benefits of patient stratification for personalised medicine. Determine if the gut bacteria can be manipulated by drugs/compounds or “good” bacteria.</p>
What species and approximate numbers of animals do you expect to use over what period of time?	We will use around 5000 mice within 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	<p>Most methods used in this project are expected to involve mild to moderate distress for the animals. Some studies involve induction of joint inflammation which will be associated with mild to moderate joint pain, redness and swelling. Only where joint pain and swelling has been induced to mimic RA are these disease manifestations expected to be severe e.g. causing difficulty for the animals to get access to food and water as normally presented. In such cases, special care will be provided. If the condition of animals deteriorates they are killed to avoid further suffering. Drugs that induce reversible loss of sensation (Anaesthetics) will be used where possible and relevant during the studies e.g. when examining animals with painful joints. Drugs that relieve pain (Analgesics) will be used whenever it's possible. At the end of the protocols animals are killed and their joints, glands, and tissues of the defence (immune) system (lymph nodes, spleen, blood) are analysed under the microscope and biochemically to assess disease manifestations and confirm whether the treatment had any effects.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-</p>	We utilise several in vitro models that in many cases replace in vivo experiments or in some cases reduce their size. For instance, we have recently developed an organ culture in order to

<p>animal alternatives</p>	<p>tests compounds penetrance and efficacy in modulating complex structures which are found in tissue and would be otherwise lost in traditional cell culture. However, diseases are complex and involve a great number of interactions between cells leading to the disease manifestations that cannot be assembled in the test tube. We therefore use animals to look at how cells, tissues and gut bacteria work together, interact with each other in the whole organism to produce disease, and how they may be corrected to stop the progression of the disease.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>A range of in vitro testing using cells and tissues obtained from humans or animals will be set up first to define targets that may play a role in inflammation. This strategy makes a vital contribution towards minimizing animal usage. Careful optimization of models allows us to reduce variability and consequently allow smaller treatment groups. Statistical advice will be sought to ensure that the experiments are powered up in order to reduce chances of having to repeat them. Longitudinal monitoring (collecting sequential samples from the same animals) will reduce the number of animals necessary for a statistically meaningful cohort size.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Experiments on induction, progression, and management of inflammatory disorders require in vivo (in animal) studies where the immune responses can be assessed over a period of time. Mice are animals of choice because reagents and genetic variations needed to analyse mechanisms of inflammation are available in mice. Every invasive procedure will be performed with the animal unconscious (under general anaesthesia) and painkillers (analgesics) will be given pre-emptively and afterwards to relieve pain and ensure minimal distress and discomfort is caused to the animal. New methodologies assessing disease development have been implemented, such as in vivo imaging for accurate measure of mechanisms involved in the disease. This reduces experiment size as animal are followed</p>

through the disease.

We have expertise with multiple different models that vary in severity; we will be able to match the appropriate model to the question asked so to avoid excessive suffering of a severe model.

In models where limbs are inflamed special care will be taken to minimise suffering such as soft litter and soft food and water gel. Pain relief will be provided whenever possible.

Project	Pathophysiology of the Cardiovascular System	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Coronary Heart Disease (CHD) is a chronic disease that causes more than 7 million deaths per annum worldwide, while non-alcoholic steatohepatitis (NASH) is a chronic liver disease that is on the rise. The main risk factors (co-morbidities) for both include poor diet resulting in high blood cholesterol, Type 2 diabetes and obesity, which together are termed Metabolic Syndrome (MetS). The complexity of these factors working together presents a significant need to identify new approaches to both prevention and treatment of this CHD and HASH in order to reduce the global burden of disease. The key scientific objectives of the project are to identify new biochemical markers in the blood that will	

	<p>indicate the level of risk of people with metabolic syndrome developing CHD and NASH and to identify new drug targets that can be used to prevent the development or progression of these diseases.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The results from these studies will lead to a greater understanding of how MetS contributes to the development and progression of atherosclerosis, worsens the outcome of a heart attack, and leads to the development of NASH. This will be of benefit to researchers within the field of cardiovascular science, researchers working in the field of NASH and liver cancer, and to the pharmaceutical industry undertaking R&D programmes to develop novel ligands targeting the receptor systems under study. In addition, identification of biomarkers that are associated with MetS-related disease development could inform clinical trials that could ultimately inform personalised treatment strategies for individuals with MetS.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>All experiments will be carried out using either rats (approximately 750) or mice (approximately 2,750) over a 5 year period</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Most of the dietary interventions are classified as mild severity and have little or no adverse effect on the health of the animals. However in animals that are given zinc free diet there is loss of appetite and reduced weight gain, although the animals do not show signs of ill health. In some (very few) experiments there may be a need to restrict food intake in normal fed rats to match that in Zn deficient rats to ensure that the results can be directly attributed to Zn deficiency; this will mean that these animals will also experience slowed weight gain. These experiments would be of moderate severity. In studies where prolonged drug administration that cannot be given via the food or water is required, some animals may have osmotic mini pumps implanted under the skin under anaesthesia; animals normally make a rapid recovery from this procedure due to care measures that include analgesia and heat loss prevention. These experiments would be of moderate severity. At the end of any dietary or</p>

	<p>drug intervention the animals will be either euthanized by a Schedule 1 method, or will undergo procedures under terminal anaesthesia, from which they will not regain consciousness; this is an unclassified level of severity. Genetically modified animals bred and used under this licence are similarly of mild severity.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The overall objective is to identify (i) biomarkers and (ii) novel interventions that prevent progression of pre-disposing dietary risk factors for CVD. A significant part of the programme of work will employ a range of dietary interventions in mice or rats, each of which is used to induce a different pathological state. Since the aim is to study the impact of these interventions on whole body physiology there is no alternative to using live animals.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Procedures involving animals will not be carried out if the data obtained is already available, with the exception of replication of work in order to validate the study under our own conditions or if we have reasonable doubts as to the veracity of the data. Measures taken to avoid unjustified duplication of procedures will include close monitoring of the literature and conference attendance. Exact numbers of animals required for any study will be determined by the experimental design which will, wherever possible, allow assessment of a combination of interventions against the same contemporary controls. To ensure sufficient statistical power estimates, power calculations will be made with the advice of the university statistician.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>All of the animal models to be employed in the project are long established and well characterised. For example rodent (rat and mouse) models of MetS have been published widely in the literature (rats- 2,936 original articles; mice 3,337 articles). The choice of species will depend upon the nature of each individual study and will be informed by the wider literature. General measures to minimise welfare costs will be to closely monitor body weight, food intake and</p>

	check general health on a daily basis. For the majority (<95%) of studies animals will be group housed and for any surgical procedures peri-operative and anaesthesia care measures will be taken following consultation with the Named Veterinary Surgeon.
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Project	Pathophysiology of the Cardiovascular System
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Coronary Heart Disease (CHD) is a chronic disease that causes more than 7 million deaths per annum worldwide, while non-alcoholic steatohepatitis (NASH) is a chronic liver disease that is on the rise. The main risk factors (co-morbidities) for both include poor diet resulting in high blood cholesterol, Type 2 diabetes and obesity, which together are termed Metabolic Syndrome (MetS). The complexity of these factors working together

	<p>presents a significant need to identify new approaches to both prevention and treatment of this CHD and HASH in order to reduce the global burden of disease. The key scientific objectives of the project are to identify new biochemical markers in the blood that will indicate the level of risk of people with metabolic syndrome developing CHD and NASH and to identify new drug targets that can be used to prevent the development or progression of these diseases.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The results from these studies will lead to a greater understanding of how MetS contributes to the development and progression of atherosclerosis, worsens the outcome of a heart attack, and leads to the development of NASH. This will be of benefit to researchers within the field of cardiovascular science, researchers working in the field of NASH and liver cancer, and to the pharmaceutical industry undertaking R&D programmes to develop novel ligands targeting the receptor systems under study. In addition, identification of biomarkers that are associated with MetS-related disease development could inform clinical trials that could ultimately inform personalised treatment strategies for individuals with MetS.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>All experiments will be carried out using either rats (approximately 750) or mice (approximately 2,750) over a 5 year period</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Most of the dietary interventions are classified as mild severity and have little or no adverse effect on the health of the animals. However in animals that are given zinc free diet there is loss of appetite and reduced weight gain, although the animals do not show signs of ill health. In some (very few) experiments there may be a need to restrict food intake in normal fed rats to match that in Zn deficient rats to ensure that the results can be directly attributed to Zn deficiency; this will mean that these animals will also experience slowed weight gain. These experiments would be of moderate severity. In studies where prolonged drug administration that cannot be given via the food or water is required, some animals may</p>

	<p>have osmotic mini pumps implanted under the skin under anaesthesia; animals normally make a rapid recovery from this procedure due to care measures that include analgesia and heat loss prevention. These experiments would be of moderate severity. At the end of any dietary or drug intervention the animals will be either euthanized by a Schedule 1 method, or will undergo procedures under terminal anaesthesia, from which they will not regain consciousness; this is an unclassified level of severity. Genetically modified animals bred and used under this licence are similarly of mild severity.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The overall objective is to identify (i) biomarkers and (ii) novel interventions that prevent progression of pre-disposing dietary risk factors for CVD. A significant part of the programme of work will employ a range of dietary interventions in mice or rats, each of which is used to induce a different pathological state. Since the aim is to study the impact of these interventions on whole body physiology there is no alternative to using live animals.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Procedures involving animals will not be carried out if the data obtained is already available, with the exception of replication of work in order to validate the study under our own conditions or if we have reasonable doubts as to the veracity of the data. Measures taken to avoid unjustified duplication of procedures will include close monitoring of the literature and conference attendance. Exact numbers of animals required for any study will be determined by the experimental design which will, wherever possible, allow assessment of a combination of interventions against the same contemporary controls. To ensure sufficient statistical power estimates, power calculations will be made with the advice of the university statistician.</p>
<p>3. Refinement</p>	<p>All of the animal models to be employed in the project are long established and well</p>

<p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>characterised. For example rodent (rat and mouse) models of MetS have been published widely in the literature (rats- 2,936 original articles; mice 3,337 articles). The choice of species will depend upon the nature of each individual study and will be informed by the wider literature. General measures to minimise welfare costs will be to closely monitor body weight, food intake and check general health on a daily basis. For the majority (<95%) of studies animals will be group housed and for any surgical procedures peri-operative and anaesthesia care measures will be taken following consultation with the Named Veterinary Surgeon.</p>
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Project	Patient derived tumour xenografts as improved preclinical tools with clinical predictive power	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Breast cancer is a group of different diseases, each with variability both between and within individual womens' cancers, therefore with variable effectiveness of treatment. Our group has developed one of the largest and most comprehensive biobanks of breast cancer models, derived by growing patients' cancer tissue in mice, for which we determined the molecular characteristics. These models, known as patient-derived tumour xenografts (PDX), and the corresponding short-term cultures of PDX cells, retain most of the original cancer's variability and capture the diversity of inter-patient responses to	

	<p>treatment. We will build on this success to explore the use of PDTXs as an anticipatory tool in precision cancer medicine (giving the right treatment to the right woman) by investigating whether drug responses in the mouse model predict the original patient's drug responses. We hope to use this information to develop a dataset to enable us to predict future patients' responses accurately.</p> <p>We also aim to further characterise these state-of-the-art PDTXs by investigating the interactions between the cancer cells and the normal cells alongside them, their microenvironment. Understanding the model better will further the development of knowledge of the different trajectories of drug responses as the cancer evolves, ultimately giving much better predictions of patients' responses.</p> <p>Cancer is a moving target, it evolves as any other ecosystem in nature, fuelled by its intrinsic intra-tumour diversity. The evolutionary processes usually responsible for killing the patient are resistance to anti-cancer therapy, recurrences and metastases. PDTXs are the only laboratory tools that capture cancer's intra-tumour diversity, allowing the study of cancer evolutionary processes at unprecedented resolution.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>By continuing the work achieved on my previous licence, we aim to expand our unique collection of PDXs to model the intra- and inter-tumour heterogeneity of breast cancer. Our previous success positions our lab as world-wide leaders in this field. Moreover, we have provided the community with a larger biobank of living breast cancer related samples linked to highly annotated molecular and drug-response data, serving as a reference resource for the research/clinical community. Because PDXs capture cancer's diversity, we will model evolutionary cancer trajectories to assess them as anticipatory clinical tools. In summary, the proposed work will advance personalise cancer treatment.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We may use around 13,500 mice over 5 years, mainly mice lacking an intact immune system to avoid the rejection of the implanted tumour. We try to implant clinical specimens from consenting patients from a nearby hospital. This varies between 0 and 4 new samples weekly. Each sample will be implanted</p>

	<p>in individual mice. From the ones that successfully engraft, we will expand cells from that individual patient by serially transplanting PDX tissue into more mice. Large numbers of engrafted tissue samples are needed to 1) capture the clinical diversity to understand which breast cancer subtype will benefit from our investigations and 2) to have enough material to achieve our aims. Additional mice will be used for specific projects, co-clinical trials or other pre-clinical trial approaches.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We will follow the guidelines for the welfare and use of animals in cancer research to minimise the adverse effects if any and use appropriate humane endpoints when needed. For tissue implanted subcutaneously or in the mammary fat pad, animals will be killed before the tumour size exceeds 1.5cm³ or if the tumour is restricting the normal movement of the animal. Humanising procedures might induce a graft versus host reaction which might induce weight loss, hunched posture, fur loss, reduced mobility. Adverse effects might result from surgical procedures, as mice will experience some short-term post-operative discomfort. Other possible adverse effects could be due to toxicity from the anti-cancer therapeutic approaches. However all animals will be monitored daily for signs of ill health and assessed for clinical signs that necessitate intervention. Animals will be killed if they show any signs of ill health likely to exceed the moderate severity limit and showing adverse effects that cannot be ameliorated by mild veterinary interventions.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Patient derived tumour xenografts (PDXs) are the current state-of-the art preclinical models. Cell lines have been proven to be very useful to provide genomic : phenotype associations yet are a completely artificial system with little/no resemblance to the tissue of origin. Due to the well accepted shortcomings on the use of cell lines, the ideal scenario for preclinical drug testing would be to use primary cells from each individual patient to personalize each treatment decision. We have tried to expand human breast cancer cells in vitro but find that they drift from their original nature. By expanding human breast cancer tissue in mice through the generation of PDX models we have found that much</p>

	<p>of the genomic and functional heterogeneity is maintained. PDX models therefore represent the individual tumour much more accurately and hence provide the first step towards personalized treatment avenues.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>When appropriate ex vivo/in vitro growth of tumour cells and other animal free approaches will be used in the initial steps of the project. These approaches will be used to generate proof-of principle data. They also allow maximisation of the number of tests done on a given patient's samples without the need of in vivo work. This initial step can be used as a pre-screen to help us design a pre-clinical study with the appropriate mouse numbers</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The animals used will usually be immune compromised, to optimise engraftment rates. These severely immunodeficient mice are sensitive to infection by a wide range of normal pathogens, opportunistic pathogens, and commensal organisms. To avoid such infections, we will implement strict barrier practices and aseptic technique to maintain a pathogen-free mice environment. Engraftment will be performed under asepsis following LASA guidelines. Our past experience has informed us on when we should consider ending a procedure due to old age of the mice (we currently set up 12 months of age as a limit for having mice alive because our usual choice of mice strain starts to develop signs of ill health after that time). We have also become experienced in identifying signs of rapid tumour growth, which ultimately can impact on the health of the animal because for example, of tumour ulcerations. We have also further refined the framework and have established SOPs which include a watch list and emailing/data-base/sample tracking/actions protocol for a tighter monitoring of tumour growth, possible adverse effects and any other effects arising in our mice.</p> <p>Perturbation experiments might be performed for example by implementing an anti-cancer therapy. When possible, such compounds will be used to affect exclusively tumour growth and hence unlikely to cause systemic and/or severe phenotypes. We will however monitor very closely all mice enrolled in perturbation-based experiments. I am confident the measures we will use over the next 5 years of</p>

	<p>research to minimise the cost on mice reach the highest quality standards. We have improved, and we will continue to do so, our scientific procedures through these past years. We have furthermore trained a dedicated team of animal technicians that are now world-experts in preclinical in vivo work, and we will continue to have their support over the next 5 years.</p>
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Project	Peripheral gate in somatosensory system	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Peripheral nerves respond to changes at the surface or inside our body, the brain then interprets these responses in terms of tactile or visceral sensations, such as heat, touch, or pain. Until now, accepted scientific theory has held that only the central nervous system could interpret and analyse such sensations. The peripheral nerves were seen to be a mainly wiring network, relaying information to and from the central nervous system by delivering messages to the 'control centre' (the brain), which then tells the body how to react. Our recent findings challenge this view and suggest that peripheral nerves could be capable of interpreting their environment and modulating pain. My overarching goal is to	

	develop a comprehensive mechanistic understanding of how peripheral nerves can regulate and control pain. These studies will change current view on the principles of pain processing and will provide new ideas for the treatment of pain.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	I believe that this programme of work will lead to the development of improved, novel means by which both acute and chronic pain can be controlled; these new means may reduce side effects of traditional analgesics (e.g. addiction, tolerance). Thus, the ultimate impact of this research should be with patients suffering from acute or chronic pain. The economic costs associated of chronic pain reach tens or even hundreds of billions annually. Enabling individuals to return to work more promptly, or indeed to avoid absences, through the development of new approaches to pain control and treatment, may have a tremendous positive impact on national economy and, therefore, the nation's international competitiveness, which in turn should further enhance individuals' quality of life.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice (including transgenic mice). We estimate usage of approximately 175 mice and 150 rats p.a. Mice are needed as extensive transgenic approaches that are suggested here are only available in mice. On the other hands, rats as larger animals, allow better success rates for some surgical approaches suggested. The research that has led up to this proposal has been performed on mice and rats.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Some of the experiments (pain models) will be of moderate severity. Lesions to peripheral nerves or peripheral inflammation may result in moderate hyperalgesia and in some distress associated with it. At the end of each experiment animals will be humanely sacrificed using the Schedule 1 procedure.
Application of the 3Rs	
1. Replacement State why you need to use	Pain management is an unmet clinical need as many types of pain (i.e. neuropathic pain) cannot be successfully treated with current medications.

<p>animals and why you cannot use non-animal alternatives</p>	<p>Therefore the experiments with mammals are necessary. However, our programme does involve a large body of mathematical modelling of pain processing as per our earlier published work. We also abundantly use experiments with the in vitro systems, such as expression systems, to replace animal tissue.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Our aim is to reduce the number of animal experiments whenever possible. A large share of our experiments is done with cultured neurons. This is a very efficient way of animal usage since a culture from one animal usually provides enough material for up to a week of experiments. In the in vivo experiments we will keep the group size to a minimum sufficient to detect significant changes between the groups. Mathematical modelling will also be extensively used.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The species of choice for this work are rat and mice. These species offer a well characterised models that are used widely in pain research. Mice are needed because they are currently the species of choice for gene manipulation, which will be used in this programme. Rats are larger animals and some surgical procedures used in this programme have better success rates on rats because of this. Moreover, confirmation of findings in two mammalian species ensures broad applicability of these findings. Moreover, the part of the nervous system responsible for pain sensation is well conserved between rodents and human. We will only use pain models that are well established in the field. In most cases in these models animals only experience relatively mild distress, close to the threshold of feeling discomfort. As animals are checked daily, signs of significant discomfort will result in immediate sacrifice of the animal with humane schedule 1 procedure.</p>

Project	Perivascular clearance in dementia and neurodegenerative diseases	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Neurodegenerative diseases such as dementia and Alzheimer's disease (AD) affects more than 800,000 people in Britain and 35 million people worldwide. Old age, genetic factors and having high levels of cholesterol are risk factors for the development of dementia, but it is still not understood why. One of the pathological characteristics of dementia is the build-up of toxic proteins (such as amyloid beta (A β)) that kill brain cells. This build-up occurs as a result of the failure of the brain's capacity to remove these proteins. A β and other proteins are normally removed from the brain by drainage along membranes that are present in the walls of blood	

	<p>vessels, called basement membranes. Therefore, increased deposition of toxic proteins may result from changes in the health and/or structure of the basement membrane. The objectives of this project are to understand how Aβ and other proteins drain from the brain normally, how risk factors for neurodegenerative disease affect the basement membrane and Aβ removal, and to identify possible routes of improving the drainage of toxic waste as well as vaccination against toxic proteins such as Aβ accumulating in the brain.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Old age is the strongest risk factor for the development of dementia. As the proportion of people over 60 years old is growing faster than any other age group, it is predicted that over 131 million people will be affected by dementia by 2050. Current therapies do not stop, reverse or even slow the disease progression. Understanding how Aβ and other peptides are removed from the brain under normal and pathological conditions is essential to understanding how dementia develops. The findings from this project will a) give a better understanding of how factors such as age and cholesterol affect the efficiency of solute clearance from the brain and b) identify possible routes of vaccination against toxic peptides such as Aβ accumulating in the brain. This will provide a new direction for effective preventative and therapeutic treatments for dementia to be developed.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Approximately 3800 mice will be used over the 5 year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Our proposed research involves breeding mice under mild, moderate and severe categories that have been genetically modified to develop adverse effects of neurodegenerative disease. We will also test wildtype mice for the role of a modified diet in the development of neurodegenerative disease. Mice will be used to either investigate how the brain deals with removing waste, undergo vaccination regimes against the toxic proteins that accumulate in the brain in neurodegenerative disease or be assessed for behavioural abnormalities. We do not expect to have adverse effects in the mild breeding protocol. However, mice bred under moderate and</p>

	<p>severe breeding protocols have the potential to show adverse effects. These will be inspected carefully for and closely monitored daily. Animals exhibiting abnormal features that cause signs of suffering that is greater than minor and transient or any unexpected harmful features will be killed (Schedule 1). We have chosen to use some strains that are bred under a severe protocol, as the advantages related to their pathological features that replicate the disease outweigh the disadvantages related to their phenotype. In the experimental protocols the level of severity is not expected to exceed moderate. Mice being vaccinated will receive injections of substances via different well characterised routes, in the tail vein or intramuscular. These mice may display chronic low levels of discomfort. Mice receiving a stereotaxic injection will require surgery in which a small burr hole is drilled through the skull to expose the brain. The majority of mice will undergo this protocol under non-recovery so will feel no discomfort or pain. Some mice will be allowed to recover and are expected to show a transient higher level of discomfort. These mice will be administered pain control before and after surgery and will be monitored closely. Some behavioural tests may cause low levels of stress and anxiety. We will monitor these closely. After exposure to the water maze test, animals will be dried off before returning them to their respective cages. Mice will be closely monitored and will be removed immediately from the tank if they appear panicked or distressed. We have designed our experimental endpoints to ensure that animals will be used before developing any phenotype that would cause long lasting pain or suffering. At the end of the experiments, animals will be killed humanely.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The complex nature of the brain makes it difficult to study using non-living models. The rodent brain functions in many similar ways to that of the human brain. Many aspects of neurodegenerative disease can be accurately modelled using genetically altered rodents and can be used to test potential new treatments.</p>

<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of animals to be used in the project has been calculated by power analysis to provide the minimum number of mice sufficient to support robust statistical analysis by standard methods such as Analysis of Variance, Students t-test and linear regression.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the most refined model organism of choice to study neurodegenerative diseases such as AD, because the structure and function of the rodent brain is similar to that of the human. Further, the mouse genome can be easily used to make genetic alterations that replicate features of human neurodegenerative disease. Unfortunately, the causes of neurodegenerative disease are complex and multifactorial and it is highly unlikely to find one animal model that will represent the condition as a whole. We have therefore refined our use of animal models by carefully identifying models that display specific characteristics comparable to that seen in humans that we feel will enable us to generate clinically relevant data and achieve our objectives successfully.</p> <p>Rodents also breed easily, with a short generation time, facilitating multigenerational and ageing studies. Finally, protocols for rodent husbandry and health management are well established.</p> <p>To minimise suffering, all animals will be assessed daily for signs of distress or ill health. Vigilant monitoring will be done in animals following surgical procedures. Any animals showing signs of distress and/or pain will be killed by a Schedule 1 method. Handling will be minimised to routine husbandry and procedures required for the project.</p>

Project	Pharmacodynamics and pharmacokinetics of novel compounds	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>There remains a large unmet need for new medicines to treat a large variety of disorders. However, drug discovery efforts within the UK have been hit particularly hard by the reduced investment in Research and Development by major pharmaceutical companies. We aim to exploit the UKs world renowned basic science and drug discovery expertise by translating our increased understanding of disease processes in areas such as cancer and neuroscience into new drugs. Although great advances have been made in computational (<i>in silico</i>) aspects of protein (drug target) structure and drug design that have reduced</p>	

	<p>the usage of animals in the drug discovery process, there nevertheless still remain large gaps in our knowledge that necessitate <i>in vivo</i> testing in rodent species. The key aspects of the drug discovery process that this license address are: 1) how rapidly is the drug broken down (metabolised) by a live animal? This is a question applicable to all disease areas and is important because there is little point in developing a drug which is rapidly broken down in animal species (and therefore by extrapolation man) since such drug will need to be administered multiple times a day, which is very inconvenient; and 2) to what extent does a drug designed to treat diseases of the brain actually get into the brain and interact with the protein of interest (so-called “target engagement”)? A secondary aim of this license is to understand the biochemical changes that occur in animal models of a disease or following the application of certain drugs.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The benefits of this project are the identification of improved treatments for patients suffering from a variety of different forms of cancer as well patients with disorders of the central nervous system, including Alzheimer’s, Parkinson’s and Huntington’s diseases, schizophrenia, anxiety, depression, epilepsy. Additional indirect benefits of new drugs include a reduction in emotional strain of families and loved ones as well as a reduction in financial costs to care-givers and society in general. Aside from the drug discovery aspects, studies of disease mechanisms that are also covered by this Project License may provide insights into the disease mechanisms of, for example, cancer and disorders of the brain.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Rats = 5000 Mice = 2500 These animals will be used over a 5-year period</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The adverse events from the procedures themselves are expected to be mild. However, given the nature of the studies to be conducted (i.e., the <i>in vivo</i> testing of completely new drugs that have not previously been tested in animals), then it is possible that more severe adverse effects (e.g. lack of mobility and/or grooming) may be observed and therefore animals will be monitored closely and if</p>

	adverse events are observed then animals will be humanely killed. At the end of each of the studies covered by the Protocols described in this license, animals will be killed and where appropriate, tissues (e.g., brain, blood) will be harvested for subsequent biochemical and/or bioanalytical analyses.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	Although <i>in vitro</i> (test-tube) studies of the mechanisms that break down drugs are possible (and will be an integral part of our generic drug discovery efforts), their predictive validity is variable and accordingly there are as yet no <i>in vitro</i> assays or computational models that can simulate the complexities of the <i>in vivo</i> system. Furthermore, there is also no current substitute for <i>in vivo</i> assays to determine if a drug can get into the brain.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	The primary objective of the studies described in this project are to select and prioritize compounds suitable for further studies and to screen-out those that are not. Accordingly, we will use sufficient animals to permit us to make reliable judgments as to whether the <i>in vivo</i> properties are good, bad or intermediate rather than to power our studies (<i>i.e.</i> , increase the group size) to demonstrate that the <i>in vivo</i> properties of compounds are statistically significantly different from one another.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Our species of choice will be the rat since any data that are generated as part of this project are then consistent with subsequent safety and toxicity studies that are required by the regulatory bodies to be conducted in rat. However, where necessary, we will use mice to generate data that are consistent with other studies showing the effects (efficacy) or side-effects of drugs in mice (<i>e.g.</i>, efficacy studies in particular strains of transgenic mice).</p> <p>As regards animal welfare, pre-meetings between researchers and animal care staff will take place to evaluate and put in place monitoring systems to identify potential welfare issues arising from specific protocols. Environmental enriched housing is provided to all of the animals throughout these procedures.</p>

Project	Pharmacodynamics of Antibacterial and Antifungal Agents	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)		
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits are to provide patients with life-threatening infections new therapeutic options. Currently, these patients have few if any options and die as a result. This project will allow academic groups, small-medium enterprises (SMEs), biotechnology companies and larger pharmaceutical companies to develop promising new compounds in an accelerated	

	<p>manner. The project will also prevent compounds with limited or suboptimal efficacy being tested in humans. The project will involve examining the efficacy of approximately 20 new antibiotics and antifungal agents with ultimate aim of identifying safe and effective dosages for humans.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice and rabbits are the only species being used. We plan to use 18,000 mice and 1150 rabbits over a 5-year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Most animals in this project have treatment that damage their immune systems prior to being infected with a bacterium or fungus. These models closely mimic human infection of skin and soft tissue infection (e.g. surgical site infection), pneumonia (bacterial and fungal), and meningitis (bacterial, fungal and tuberculous infection). The laboratory animal models are mimics of neonatal and adult infection. Animals then receive new antibiotics and antifungal agents that are being developed for humans. Most of the animals used in this project have mild to moderate symptoms. Nevertheless, most protocols in this project are graded as severe reflecting the fact that we expect to see (on occasions) animals that are significantly unwell. The grading reflects the fact that we are modelling severe human infections where the mortality is generally 50% and on occasions higher. Most the adverse effects are related to the infection rather than drug related toxicity, which is screened out in early testing.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We use a range of non-animal models (i.e. in vitro) of infection to help understand how antibiotics work. A wide range of experimental models that include both laboratory animals and in vitro models of infection are required to obtain a complete understanding of the potential benefits of new antibiotics. In vitro models are especially useful for understanding how resistance to antibiotics develops. However, in vitro models cannot completely replace laboratory animal models for several reasons that include: (1) the inability to understand how antibiotics work in complex infections within tissues such as the lung and brain. The pattern of infection in those body sites may have an important bearing on the activity of an antibiotic in humans (e.g. sometimes antibiotics just will not work in the</p>

	<p>lung despite working elsewhere in the body); (2) the inability to assess the additional benefit of the immune system over and above the effect of the antibiotic; (3) the inability to understand how an antibiotic penetrates into tissues (e.g. parts of the brain) where some infections may reside; and (4) some infectious processes simply cannot be modelled using an in vitro system. For these reasons, a wide range of model systems are required to fully assess the potential benefit of a new antibiotic.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We will minimise the numbers of animals in several ways. We design experiments collectively so they yield the maximum amount of information with the fewest animals. We plan experiments precisely so that we minimise technical error and therefore minimise the number of experiments that need to be repeated. We seek statistical advice and input when necessary to ensure experiments are adequately powered to address the scientific question that is being posed. We progressively learn from experiment to experiment so that we maximise the use of previously obtained knowledge. We use all the data that is obtained. This means that all animals used in the experiments contribute information and allow the primary scientific question to be addressed in an efficient manner.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use the most refined models possible that enable us to obtain a realistic estimate of drug activity and an insight into the dosage that is likely to be effective for humans. We use analgesia routinely. We perform invasive procedures under general anaesthesia. We use catheters if appropriate to minimise the number of needlesticks that are required to administer drugs or obtain blood samples. We inspect and care for animals very carefully and are available around the clock. We use the shortest possible models to obtain the necessary information. We minimise the number of invasive procedures through careful experimental design.</p>

Project	Pharmacodynamics of Antibacterial and Antifungal Agents	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)		
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits are to provide patients with life-threatening infections new therapeutic options. Currently, these patients have few if any options and die as a result. This project will allow academic groups, small-medium enterprises (SMEs), biotechnology companies and larger pharmaceutical companies to develop promising new compounds in an accelerated	

	<p>manner. The project will also prevent compounds with limited or suboptimal efficacy being tested in humans. The project will involve examining the efficacy of approximately 20 new antibiotics and antifungal agents with ultimate aim of identifying safe and effective dosages for humans.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice and rabbits are the only species being used. We plan to use 18,000 mice and 1150 rabbits over a 5-year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Most animals in this project have treatment that damage their immune systems prior to being infected with a bacterium or fungus. These models closely mimic human infection of skin and soft tissue infection (e.g. surgical site infection), pneumonia (bacterial and fungal), and meningitis (bacterial, fungal and tuberculous infection). The laboratory animal models are mimics of neonatal and adult infection. Animals then receive new antibiotics and antifungal agents that are being developed for humans. Most of the animals used in this project have mild to moderate symptoms. Nevertheless, most protocols in this project are graded as severe reflecting the fact that we expect to see (on occasions) animals that are significantly unwell. The grading reflects the fact that we are modelling severe human infections where the mortality is generally 50% and on occasions higher. Most the adverse effects are related to the infection rather than drug related toxicity, which is screened out in early testing.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We use a range of non-animal models (i.e. in vitro) of infection to help understand how antibiotics work. A wide range of experimental models that include both laboratory animals and in vitro models of infection are required to obtain a complete understanding of the potential benefits of new antibiotics. In vitro models are especially useful for understanding how resistance to antibiotics develops. However, in vitro models cannot completely replace laboratory animal models for several reasons that include: (1) the inability to understand how antibiotics work in complex infections within tissues such as the lung and brain. The pattern of infection in those body sites may have an important bearing on the activity of an antibiotic in humans (e.g. sometimes antibiotics just will not work in the</p>

	<p>lung despite working elsewhere in the body); (2) the inability to assess the additional benefit of the immune system over and above the effect of the antibiotic; (3) the inability to understand how an antibiotic penetrates into tissues (e.g. parts of the brain) where some infections may reside; and (4) some infectious processes simply cannot be modelled using an in vitro system. For these reasons, a wide range of model systems are required to fully assess the potential benefit of a new antibiotic.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We will minimise the numbers of animals in several ways. We design experiments collectively so they yield the maximum amount of information with the fewest animals. We plan experiments precisely so that we minimise technical error and therefore minimise the number of experiments that need to be repeated. We seek statistical advice and input when necessary to ensure experiments are adequately powered to address the scientific question that is being posed. We progressively learn from experiment to experiment so that we maximise the use of previously obtained knowledge. We use all the data that is obtained. This means that all animals used in the experiments contribute information and allow the primary scientific question to be addressed in an efficient manner.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use the most refined models possible that enable us to obtain a realistic estimate of drug activity and an insight into the dosage that is likely to be effective for humans. We use analgesia routinely. We perform invasive procedures under general anaesthesia. We use catheters if appropriate to minimise the number of needlesticks that are required to administer drugs or obtain blood samples. We inspect and care for animals very carefully and are available around the clock. We use the shortest possible models to obtain the necessary information. We minimise the number of invasive procedures through careful experimental design.</p>

Project	Pharmacokinetic evaluation of novel therapeutic agents	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	For new medicines to be effective, we need to ensure that enough of the medicine will reach the right place in the body to have a positive therapeutic outcome. Pharmacokinetic (PK) studies enable us to test potential new medicines and determine how long they last in the body, and which organs they reach.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This work will enable the discovery and development of new medicines across a wide range of diseases that affect human patients. This could include: cancer; lung diseases such as asthma and chronic obstructive pulmonary disease (COPD); cardiovascular and metabolic diseases	

	including kidney fibrosis and diabetes. This project will also enable us to investigate new ways of delivering medicines to patients in the future, that are more convenient or safe, such as inhalation or delivery of DNA to cells so that they can make the medicine directly inside the body.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use adult rats and mice, and we estimate that over the 5 year period of the licence up to 4700 mice and 1300 rats will be used. The majority of these will be in pharmacokinetic studies where animals are dosed with a potential new therapeutic agent and then have blood samples taken over a period of hours to days, typically less than 1 week.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The typical experiment would be to dose the animals with a substance and then take blood samples over a period of days to weeks and measure the substance in the blood over that time. Blood samples are usually of a small volume and are taken from a vein in the tail. A smaller number of studies could involve more complex designs, for example giving an animal an additional dose of a substance that will stimulate the immune system so we can measure how levels of a potential new medicine can change the inflammatory response. Other studies may look at technologies that will enable to us to deliver medicines more effectively in the future, which could require injections directly into muscles or skin under anaesthesia. The majority of studies performed are expected to be of mild or lower severity, but some studies using surgical techniques to inject into muscle cells r skin, or using animals with surgically implanted tubes for sampling blood or bile will be in the moderate band. No studies will be in the severe category. At the end of the studies all animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although we do many experiments using cells and computer modelling, living animals are still needed for some studies because isolated cells and organs do not reproduce the complex nature of in vivo biology. For example, the interactions between immune cells, nerve cells, complex

	hormonal systems and organ-specific cell changes cannot be recreated outside of living animals or simulated using computers.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Studies on this licence will generally use serial sampling techniques, where a small blood sample is taken from each animal on multiple occasions. Usually only three replicates are needed at each sample point. These practices reduce the total number of animals used. We use statistical methods to ensure that the correct number of animals are used in studies where the objective is to test the effect of a new substance in modulating a biological system, which reduces the need for studies to be repeated in the future. Other measures such as random assignment of animals to treatment groups and elimination of observer bias also increase the robustness of studies.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rats and mice are the most appropriate animals for these studies as they are mammalian species with many features in common with humans. They have been studied for many years and there is good understanding of their biology and how this relates to humans. Animals are kept in high quality facilities, free from pathogens and with access to food, water and environmental enrichments.</p>

Project	Pharmacokinetics of novel therapeutic agents and disease modification in oncology
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research <input checked="" type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The overall aim of this project is to develop novel therapeutic compounds to combat human diseases, including cancer. This involves identification of proteins that are important in causing the disease, understand its chemical structure and function, then designing novel compounds that can control them.</p> <p>The first aim of this project is to provide animal tissues and cells from freshly-killed animals to test novel compounds in the laboratory, where relevant cell lines for in vitro testing are not available. Each animal can provide sufficient material to test multiple compounds, and this helps reducing the</p>

	<p>number of subsequent experiments with live animals.</p> <p>The second aim is to understand how the novel compounds behave and are processed by the body (ADME profiling), which can lead to better design of drug-like compounds. We aim to find compounds that are able to reach the target disease tissue without significant health risks. The most promising compounds are then tested in disease models at REDACTED (cancer) or externally (cancer and other diseases).</p> <p>The third aim is to test the activity of the potential anti-cancer compounds in the animal models. The overall effects we desire is the killing of cancer or slowing of its growth. Our investigation also requires studying of key molecular events that are important in cancer cell survival, then the impact of our novel compounds on these events.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Our investigation will lead to identification of novel therapeutic drug candidates which can be tested in human patients. If testing in clinic is successful, these will widen the therapeutic options for patients. Our investigation will lead to further knowledge of the diseases and builds experience of drug-discovery. Early studies, such as the ADME studies, are used to identify the areas of molecules that should be improved. Building data like these improves the efficiency of novel drug designing.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Majority of our studies will use mice. We expect to be using up to 39,300 mice in total during the 5-year period of this licence. We will perform smaller number of experiments with rats, with the total of up to 3,850 animals in the same period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Majority of the animals will experience mild to moderate adverse effects due to non-surgical intervention (blood sampling, injection, oral dosing), therapeutic drugs, tumour growth or the combination of all. For the early phase of compound development, each animal will be given injection or oral doses of novel compound at low levels that is not expected to have any therapeutic effects, and blood samples taken to determine the compound level in circulation. These procedures should only cause momentary discomfort</p>

immediately after. The therapeutic dose levels and regimen are estimated for the promising compounds and their tolerability tested in pilot experiments. Upon repeat dosing of an anti-cancer compound, we expect that the animals experience mild to moderate side effects such as weight loss, transient diarrhoea and changes in normal behaviour. Since the compounds are novel and despite our effort to predict toxicity risks in vitro, it is impossible to avoid rare events where the animals experience severe adverse effects or die due to toxic effects. We monitor animals on this type of pilot studies daily or more frequently in order to stop suffering as soon as possible. The studies are repeated with lower doses until a tolerated dose schedule is found. Efficacy of anti-cancer compounds is tested, initially, in subcutaneous xenograft models in which mice are growing human cancer under the skin. In these, each animal receives tumour implantations, which is minimally invasive and can be monitored externally. Any animal, on or off drug treatment, with its tumour approaching a set maximum burden (14 mm in average length in mice, and 25 mm in average length in rats) or appearing to be breaking up will be killed. Therapeutic compounds are administered at the dose that was found to cause adverse effects of moderate levels or less. Treated animals will be monitored to measure efficacy or killed to investigate the drug effects in tumours ex vivo. With most models, some animals (typically 5-20%, depending on the cell line) fail to develop tumours. These may be killed or re-used to investigate the drug exposure and tolerability of novel therapy. Only the most promising compounds may be tested in leukaemia or mammary tumour models which are more invasive and complex than subcutaneous models, and requires extra tumour monitoring methods. Hollow fibre assay offers a means to test multiple cell lines in parallel, where animals receive implantation of multiple fibres containing cells under the skin followed by treatment. We also aim to improve in vitro screening by using fresh tissues which this programme provides. Animals are humanely killed without any treatment, or receive procedures under general anaesthesia and killed before recovering from anaesthesia. Some animals may receive prior injection of non-therapeutic compound(s) which may cause mild and transient discomfort. Any animals that suffer adverse

	effects likely to exceed stated severity will be killed. All animals used in the experiment will be killed on completion of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	<p>We have built multiple ways to test novel compounds, including an extensive list of in vitro biology, ADME and toxicity studies which replaces some of the animal work. Drug properties and efficacy are, however, dependent on the complex system involving multiple organs which cannot be mimicked sufficiently in vitro, so the overall effects of the compounds can only be tested reliably in animals.</p> <p>For some diseases, cell lines that best represent the target organ and diseases are rare. Primary cells and tissues from limited number of freshly-killed animals can provide materials to test compounds prior to proceeding to in vivo studies. This replaces testing using many live animals.</p>
2. Reduction Explain how you will assure the use of minimum numbers of animals	<p>We will follow the in vitro compound selection system to avoid testing compounds that are sub-optimal in studies involving large groups of animals.</p> <p>Where blood collection is needed, we use multiple micro-sampling from each animal, reducing the overall number of animals.</p> <p>We will re-use animals that failed to develop tumours (typically 5-20% of mice subcutaneously implanted with tumours) in tolerability and ADME studies.</p> <p>Ex vivo assays reduce overall usage of animals as organs from each animal provides sufficient material to test several compounds.</p>
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	<p>We will use primarily mouse for screening. Established protocols for testing drugs and historical data are widely available in the literature for this species. Ways of testing treatment effects on human cancers in mice are also well-established, allowing investigation of clinically-relevant disease tissues. We will perform the screening using subcutaneous tumour models as it is the least invasive and causes the least</p>

	<p>discomfort to the animals.</p> <p>Rats are another well-characterised species for which many study protocols are established. These are also useful in predicting the drug-like properties and activity of compounds in humans.</p> <p>Whenever possible, pilot experiments will be performed using the same strain, sex and supplier of mice as those intended for later studies involving disease models to ensure consistency and better prediction. Tolerability of drug treatments may be confirmed in tumour-bearing animals so that we are aware of the combined adverse effects of treatment and tumour development, if any.</p> <p>Appropriate statistical methods will be used to design experiments and to confirm the finding so that the scientific data reported are reliable.</p> <p>We consult current and emerging guidelines on animal research and implement improvement in regulated procedure when applicable. These include attempts to reduce stress by sugar-dipping oral dosing needle to make it easier for the animals, and using coloured restrainers. Use of temporary tail vein cannulation may also replace surgical cannulation of animals.</p>
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Project	Pharmacokinetics, delivery and fate of dosed biopharmaceuticals and new chemical entities (NCE's)	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>When researching, and developing potential new medicines it is important to understand how potential new medicines will be affected by the body (pharmacokinetics) and whether the medicine is likely to have the desired effect (pharmacodynamics). New medicines have to be as safe and effective as possible before they are given to patients and this involves giving the medicines to animals before man.</p> <p>The work on this project will provide information or 'data' on what happens to a potential new medicine</p>	

	<p>after it has been given or ‘administered’; how long does it remain in the body, what effect it has, which by-products are produced and where do they go after the medicine is given. The results will inform further development of more effective medicines and support development of new and improved ways of taking them.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The data produced by this project will contribute to development of potential new medicines to treat patients with a variety of conditions (for example, cancer, HIV and heart failure). Data generated will allow them to be progressed from the laboratory into early studies in humans. Data from some of the studies will be used to determine the dose that should be used in other studies (for example animal toxicity or clinical trials). Some of the work carried out will be used to develop dosing systems that are more convenient, easier to use, less painful and/or reduce the frequency of taking medication for patients</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The majority of the studies will use rodents as they offer appropriate anatomy/physiology to investigate the pharmacokinetics and pharmacodynamics of new medicines with results likely to give an indication of what will happen in humans. However, as pig skin is very similar to human skin some studies, particularly those investigating delivery to the skin, will use mini pigs. In the five-year course of this project licence up to 2000 on mice, 500 rats and 150 pigs will be used to test substances. (This is based on a typical study requiring 3 animals per dose group to be tested to obtain a statistically significant effect) Up to 200 mice, 80 rats, and 80 pigs will be used to provide samples from untreated animals which will be used to set up and validate tests or to use as controls. All studies will go through a scientific review where consideration of numbers, methods and design is reviewed with strong consideration of the 3Rs</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the</p>	<p>Healthy animals will have potential new medicines administered by various routes but most often intravenously (directly into the vein), subcutaneously (just under the skin) or intraperitoneally (into the abdominal cavity). Blood samples will be taken either with a needle and syringe from a blood vessel near the surface of the body or a cannula which has</p>

end?	<p>been temporarily implanted into a vein. Adverse effects are not expected with these procedures with animals experiencing very little discomfort. Very rarely non-specific signs such as changes in behaviour or posture, weight loss, and reduction in food and water intake will be seen. If this happens action will be taken such as stopping study procedures and seeking veterinary advice. Where an animal cannot be treated, it will be humanely killed to prevent unnecessary suffering. The project has clear guidance on actions to be taken when animals experience side effects, including immediate euthanasia to prevent unnecessary suffering. Samples of organs and tissues are often taken after death to measure the concentration of a potential new medicine in them. During procedures, such as administration or blood sampling, animals are briefly restrained either by holding or placing animals in to devices such as slings or restraint tubes that briefly minimise movement to avoid injury to the animals and ensure success of the procedures. Most animals are housed in pairs or groups; for some studies, animals are on their own for short periods in special cages to collect urine and faeces for analysis to see how the body is removing the medicine from the animal. Animals are monitored for general signs (as mentioned above) that may be due to the potential new medicine being tested. However, the doses administered under this project are typically low so that no side effects would be expected following administration. At the end of the study most rodents will be humanely killed and terminal samples will be taken for further analysis. At the end of a study rodents and minipigs will have a health check and provided the criteria set by vets are met, the animals will be returned to stock to be used again on a new study.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Research programmes have evolved substantially in the past decade and scientific areas are now heavily dependent on non-animal experiments. However, how the potential new medicine is absorbed and, distributed around the body and excreted from it still needs to be tested during the research of the potential new medicines. The mechanisms by which medicines are handled in the body are complex and cannot be adequately evaluated with non-animal</p>

	<p>tests, therefore currently the properties of potential new medicines can only be fully understood by using a combination of non-animal models and animal approaches.</p> <p>Regulatory bodies that review and authorise new medicines to the market require data from this type of study before allowing potential new medicines to progress into human studies.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We are continually researching new technologies and processes that will aid reduction in animal use. Progress within these technical and scientific approaches required during animal experiments are constantly challenged by researchers across the world and published data is regularly reviewed and considered by our team. Our team have a history of developing and implementing methods to reduce animal usage and these will be used on our studies whenever possible. Such methods include: micro sampling methods, whereby multiple small volume samples are taken from an individual animal, thereby reducing the number of animals required for a range of studies.</p> <p>Discussions with a panel of internal experts will advise on optimisation of any study design to ensure the right species and procedures will be used to maximise the chance of success.</p> <p>We obtain statistical input into study design to ensure the appropriate and minimal animal numbers are used to meet the scientific objectives.</p> <p>Analysis of all samples will be via highly sensitive techniques that are more consistent with those used in later stages of research. This data will be reviewed by a panel of experts to determine if a substance can progress to the next stage of development or not.</p> <p>Animals suitable for reuse will undergo a thorough health check by the vet, where there should be no lasting effects as a consequence of the previous use on the health, behaviour or welfare of the animal including those caused by compound, procedures or housing. There should be no signs of infection and bodyweight should be stable. There should be no other health, behaviour or welfare issues unrelated to the study.</p>

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Early phase drug discovery uses rodents as a primary model before any further species are considered where it is clear other species, such as fish, fruit flies, or worms for example cannot be used. Rodents offer the most appropriate biological anatomy to reproduce a biological/drug interaction that can be measured.</p> <p>Pigs are considered to be one of the major animal species used in translational research, Pigs will be used for device delivery as the architecture of their skin is similar to humans and are increasingly being used in preference to the dog or monkey as the choice of nonrodent species in testing of pharmaceuticals.</p> <p>Minimising welfare issues will be addressed by understanding the requirements, collaborative decision making/planning and using staff with experience at recognising potential issues should they occur. Close working relationships will ensure the study is required and that the study design appropriately meets the objectives.</p> <p>Where blood samples are taken, micro sampling techniques will be used whenever possible, to make sure the minimum sample (e.g. microliters) volume is obtained using the least invasive techniques possible. For example, where possible the same needle stick wound will be used for more than one sample by wiping with a swab to remove the scab allowing the flow of blood for collection. This means fewer needle sticks are required. The frequency and volumes used when administering potential new medicines will also be minimised.</p>
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Project	Phenotyping Genetically Altered Mice Using Imaging	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The overall aim of this work, by using mice which express alterations in genes which are known to cause disease in humans, is to devise ways in which we can use imaging to follow the progression and severity of disease, and track the effects of candidate drugs on disease progression, in an effective, efficient, and minimally-invasive way.</p> <p>Neurological disorders such as neurodegenerative disease like Alzheimer's and Parkinson's are a national and global cause for concern, with estimated cost to the UK economy predicted to double, from £26 billion to £55 billion in the next 25 years if no cure is found.</p>	

Around 5% of diagnoses of such disorders are familial in origin, meaning that specific mutations and/or inherited causative genes have been described. As such, development of transgenic and knock-out/in mutant mouse models have enabled the in-depth and comprehensive study of disease processes in the rodent brain, and allowing us to translate our findings from the mouse, back to the human, with the aim of improving care and disease diagnosis in patients. As such, generation and study of mouse models in this way facilitates the vital development of new diagnostic protocols, and therapeutic strategies.

This project will use in-vivo imaging to help us understand the role and function of genes within the brain, that have been associated with disorders such as Alzheimer's and Parkinson's, which will enable us to remove major "bottlenecks" in the development of new diagnostic tests for diseases and human disease therapies. By using genetically altered mice, which harbour mutations in genes known to cause disease, we can study the effects of such genetic alterations on the whole body/organ structure and function. This is of paramount importance when considering neurodegenerative disease such as Alzheimer's and Parkinson's disease, in which gene mutations affect only the brain, leaving the rest of the body spared. Historically however, studies aiming to understand the effect of these genetic alterations are invasive, costly, labour intensive, and require large numbers of animals to be used for individual study. Imaging techniques (such as MRI and PET), being minimally-invasive, allow for high-throughput study of genetically altered animals, and permit repeated measurements to be taken during life and with age. In this project we therefore aim to:

- Use high resolution imaging to study genetically altered mice (and appropriate healthy control animals) to study in-depth, the effect of genetic alterations on the whole body/organ structure and function, and how such mutations lead to disease.
- Use repeated imaging of the same group

	<p>of animals as they develop and age, to assess the onset and progression of genetic disorders. This will allow us to use imaging biomarkers, such as quantifying blood flow in the brain, to assess and quantify the functional relevance of gene alteration during age.</p> <ul style="list-style-type: none"> • Where necessary, use additional subsidiary experiments to confirm, validate and extend the finding from our imaging experiments, acting as a confirmatory step in the design and use of new imaging techniques.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Developing new techniques to image the brain and more specifically brain affected by disease is likely to achieve a better understanding of the causes and mechanisms of neurological disease which, ultimately, could be crucial for the development of new therapeutic drugs. Additionally, as preclinical imaging protocols can be easily translated to clinic, this project could result in better tools to diagnose and follow-up neurology patients.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use mice and over the five years we will use a maximum of 1000 animals.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>As we are using animal models which harbour genetic alterations known to cause disease in humans, animals may, towards the later stages of their life, present with clinical symptoms synonymous with those observed in humans. The health of the animals however will be extensively monitored to ensure they are in as good health as possible, to minimise any pain or lasting harm. Imaging, being minimally-invasive, like in humans is expected to have very few adverse effects. But in order to image animals successfully, anaesthesia is required, often for hours at a time. General anaesthesia suppresses the heat regulating mechanisms of the body, and hence by closely monitoring the temperature of the animal while it is being scanned, we can overcome this by appropriately maintaining the body temperature by using heated air blowers. Similarly after animals are</p>

	<p>imaged they will be kept in a warming cabinet and will be kept under continuous observation until they have recovered from the procedure. In conjunction with imaging the animals, we may administer them with contrast agents in order to enhance imaging of specific organs or systems in the body. The majority of these contrast agents are already used in humans, and therefore we don't foresee any adverse effects. Yet animals will be closely monitored during, and after experiments to ensure they have not suffered from contrast agent administration. Lastly, we may administer animals with test drugs, in order to determine the success of these in treating the genetic disease being studied. These agents have all either already been given to humans and/or mice and are hence not anticipated to exert undesirable side-effects on the animals here. However, during experiments the health and welfare of animals will be closely monitored, and veterinary advice sought if any unforeseen adverse effects do arise. At the end of the experiments, animals will be culled using the most appropriate humane method.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Understanding how genes are regulated has important implications in both normal development and in progression of some diseases. The transgenic mouse allows the progression of genetic disease to be observed and quantified, in a whole living organism, allowing any compensatory effects secondary to the effect of mutation or deletion of the gene of interest to also be observed. Whilst, ideally, animals would be replaced by other approaches, no such alternative currently exists that provides an equivalent model of the complex behaviour of genetic interaction and impact upon disease. Furthermore, mice are straight-forward to maintain, cost-effective, and bespoke apparatus is available for imaging.</p>
<p>2. Reduction Explain how you will assure the use</p>	<p>It is possible to calculate the numbers of animals required for experimentation based on previous data. Imaging lets animals be used as their own control, allowing paired comparisons,</p>

<p>of minimum numbers of animals</p>	<p>and imaging is inherently sequential, using significantly fewer animals to achieve the same statistical power as conventional designs. In all cases we ensure that we have calculated the minimum number of animals required for the experiment to give us useful data. This approach also reduces the likelihood that the animal experiment would have to be repeated.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>For this project we will be working with laboratory mice that have been genetically modified, as we need to study the effects of disease genes on the mammalian whole body/organ level. The mice that have the most appropriate phenotypes for this program of technology development are animals that have been genetically manipulated to have gene deletions or mutations that largely have been defined <i>a priori</i> - there is already considerable data on the transgenic mouse models and several papers on the mouse. Modern accurate genetic techniques act as a refinement and allow us to manipulate genes in mice and express human disease genes in the right part of the body at the right time to lead to disease processes and symptoms highly reminiscent of the human disease being studied. The use of imaging technologies will refine animal experiments due to the minimally-invasive nature of many of the procedures planned. In addition the development and refinement of imaging technologies employed, i.e. reducing scan times, increasing sensitivity/selectivity will similarly aid refinement of animal procedures. In addition the very use of sensitive minimally-invasive imaging technologies will enable refinement via reduction of the number of animals needed, thus avoiding unnecessary suffering. Any adverse effects on animal welfare and wellbeing will be prevented by employing continuous monitoring throughout imaging procedures, and frequent animal observation during recovery. And only fully recovered, healthy animals will be used in subsequent imaging sessions. As such animals will be removed from study at the first sign of any adverse effects.</p>

Project	Physiological and molecular effects of glucocorticoid rhythm disruption.
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The adrenal hormone cortisol is important for the normal function of a healthy body and mind. It maintains our immune system, and controls energy levels, fat and sugar metabolism. It also controls our memory, mood and how we cope with stress. Cortisol is made in large amounts at the start of the day when we need energy, with large spikes in hormone production prior to waking. These pulses of hormone decrease during the late afternoon and early evening, as the inactive part of the day approaches. Unfortunately, this hormone pattern becomes disturbed when people are stressed, sleep</p>

	<p>deprived, or during shift work or jet lag, leading to problems with cortisol regulated processes in the body and brain. Weight gain and metabolic syndrome can arise, as can depression, anxiety and memory loss. Similar problems can also arise with patients prescribed synthetic steroid drugs. These synthetic 'corticosteroids' are widely used to treat many medical conditions, including asthma and arthritis, but they also have side effects on metabolism, memory, mood.</p> <p>The main focus of our work is to gain a better understanding about how changes in the normal daily cortisol rhythms can affect the function of different organs in the body, especially the liver because it is an important metabolic regulator, and the brain. Currently, it is still unknown how the early stages of metabolic and psychiatric disease develop as a result of stress related illness and with clinical steroid treatment. We plan to identify how the physiological processes in the cells within the liver and brain are affected, so that we can target these pathways in their treatment. Our research will also ultimately inform medical professionals about ways to develop better strategies to reduce the side effects of synthetic steroid treatment.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>To advance understanding of the consequences of disrupting the normal pattern of cortisol release and test strategies to minimise the consequences of synthetic steroid treatment. The primary and short-term beneficiaries of the work will be scientists working in the field of endocrine and circadian research. In this context the work is expected to greatly advance understanding of the significance of cortisol patterns in regulating physiological process. In the medium term the findings of the work will benefit clinicians in the design of treatment plans for patients suffering from conditions requiring corticosteroid treatment. In the long term the work is expected to improve the quality of life of patients receiving corticosteroid therapies by informing treatment strategies that maximise efficacy and minimise side effects.</p>
<p>What species and approximate numbers of animals do you expect</p>	<p>Over the 5 year duration of the licence the work is expected to use: Rats 2000 Rats (GAA) 4000</p>

to use over what period of time?	
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Our experimental treatments will induce very early changes in metabolism, along with very early stages of impaired memory and mild depression-like symptoms in the rats for a short while (up to 12 weeks maximum treatment times). The non surgical treatments with synthetic glucocorticoids provided in drinking water are mild in severity, and the surgical procedures (including adrenalectomy and cannulation) are moderate severity. The rats habituate well to the infusions that we perform, and apart from the early changes in metabolic and cognitive function, their health and well-being is not overtly impacted in our experiments. In the experiments where we induce chronic stress with constant light exposure for 6 weeks, the rats are checked by our qualified REDACTED technicians daily, and have never been found to exhibit overt signs of distress. We have the utmost commitment to animal welfare in our research. It is essential, both for the well-being of the animals and for the scientific outcomes of the research, that any potential distress to the experimental animals is minimised. Therefore, all animals will be habituated to handling before starting any experiments and all appropriate measures taken to ensure that normal physiology is maintained throughout the study. All surgeries will be performed using adequate anaesthesia and animals will be given post-operative pain control in line with best veterinary practice. At the end of the study the animals will be killed using a humane method and their brains and tissues will be dissected and analysed.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The use of animals in these studies is essential as the effects of hormones on metabolism and cognition can only be assessed in living animals. It is not possible to use non-protected species for these studies as they don't have the necessary hormone system.</p>
<p>2. Reduction</p>	<p>We will ensure that only the minimum number of animals are used by careful experimental design</p>

<p>Explain how you will assure the use of minimum numbers of animals</p>	<p>and the use of power calculation based on data obtained from our previous studies. Using this approach each experimental group will provide tissues for analysis to address multiple questions. Statistical support will be sought from bio-statisticians based within the faculty.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rats have been chosen for these studies because they have the necessary hormone systems, are large enough to enable the required serial blood sampling to be performed without compromising the animal's wellbeing and they are able to perform the behavioural tests needed to assess cognition. Before starting any studies, all animals will be habituated to handling. All appropriate measures will be taken to ensure that normal physiology is maintained throughout the study. All surgeries will be performed using anaesthesia and animals will be given post-operative pain relief.</p>

Project	Physiological regulation of innate immune responses	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	<p>White blood cells are critical for our defences against infections, but if they remain in the tissues they cause damage. In the lung disordered inflammation driven by the persistence of white blood cells is implicated in the pathogenesis of common and serious lung conditions including chronic obstructive pulmonary disease (COPD). Neutrophils are one of the first white blood cells to reach these areas. The processes that regulate their recruitment, persistence, activation and removal are critical in enabling an effective immune response whilst preventing tissue damage. The removal and activation of these innate immune cells is dependent in part upon their ability to die in a programmed manner, a process regulated in part by their physical environment including oxygen and nutrient availability. We now know that immune cells can sense changes in oxygen and nutrients by regulating a number of intra-cellular signals.</p>	

	<p>We also know that these pathways are tightly linked to the energy status of cells, and can reprogram subsequent immune responses ie generate immunological memory. Relatively little is known about the processes that enable these white blood cells to adapt to the physiological stresses to which they are exposed in the inflammatory niche or how changes in energy states and oxygen availability regulate subsequent white blood cell behaviour. We propose to investigate whether lack of oxygen (hypoxia) and access to extracellular nutrients can regulate the ability of white blood cells to generate energy, protect themselves from damaging stresses, kill bacteria, reprogram immune responses and enable an effective immune response. In the longer term we hope these insights will allow development of novel treatments for diseases of disordered inflammation currently lacking in clinical practice today.</p>
<p>Why is it important to undertake this work?</p>	<p>Respiratory disease kills one in five people in the UK, with almost 30,000 of these deaths a consequence of chronic obstructive pulmonary disease (COPD). To date there remains very little in the way of effective treatment strategies to target some of the most common inflammatory lung diseases typified by COPD. Consequently, they remain a significant disease burden to society. COPD causes breathing problems that get worse over time. In developed countries COPD mainly tends to present in middle-age or older adults who smoke as a consequence of long term inflammation in the airways. If we were able to shed light on some of the basic molecular pathways regulating the innate immune response and identify molecules that can selectively regulate neutrophil death and clearance, whilst preserving key anti-bacterial functions, this may be of help to the future development of effective anti-inflammatory strategies so desperately needed for the effective treatment for these common and disabling chronic lung diseases.</p>
<p>What outputs do you think you will see at the end of this project?</p>	<p>Academic success will be measured annually through presentation of research work within an international forum. Publication of work within high impact biomedical research journals will be used as a further bench mark of success, and the impact these manuscripts have on the broader research community. Economic impact will be determined indirectly through the number of institutions with whom I develop new or expand existing professional relationships, the development of partnerships with</p>

	<p>industry, and the knowledge transfer that occurs as a consequence of these interactions. Through the exposure of sixth form students to a week of work experience and REDACTED students to short 2 to 6 month research projects within our group I also hope to engage individuals who may not pursue an academic career, and increase their understanding of the importance of basic science research and what it entails. We further hope to expand our societal engagement with a regular contribution to the science in schools programme. Through the provision of biomedical research opportunities to postgraduate students, clinical lecturers, clinical fellows and academic trainees I also aim to develop the academic respiratory physicians of the future. Finally, if we were able to shed light on some of the basic molecular pathways regulating lung inflammation, this may be of help to the future development of effective anti-inflammatory strategies so desperately needed for the treatment of common and disabling chronic lung diseases.</p>
<p>Who or what will benefit from these outputs, and how?</p>	<p>Respiratory disease kills one in five people in the UK, with almost 30,000 of these deaths a consequence of chronic obstructive pulmonary disease. To date there remains very little in the way of effective treatment strategies to target some of the most common inflammatory lung diseases typified by COPD. Consequently, they remain a significant disease burden to society. COPD causes breathing problems that get worse over time. In developed countries COPD mainly tends to present in middle-age or older adults who smoke as a consequence of long term inflammation in the airways. If we were able to shed light on some of the basic molecular pathways regulating neutrophil persistence at sites of inflammation and identify molecules that can selectively regulate neutrophil death and clearance, whilst preserving key anti-bacterial functions, this may be of help to the future development of effective anti-inflammatory strategies so desperately needed for the effective treatment for these common and disabling chronic lung diseases.</p>
<p>Will this work be offered as a service to others?</p>	<p>No</p>
<p>How will you look to maximise the outputs of this work?</p>	<p>Attendance at international scientific meetings and presentation of research findings will provide free dissemination of research to other users with a common research interest. The extension of collaborative</p>

	<p>interests and the contribution to the publication of research articles in high quality biomedical journals will maximise the outputs from this work.</p>
<p>Explain why you are using these types of animals and your choice of life stages.</p>	<p>We study mice because their immune system and lung anatomy is similar to man. There are many resources available to use with mice and genetically modified mice are available to test key factors controlling the immune response. We test key hypotheses in human cells or in patient samples in vitro before studying mice to reduce numbers. We study mice from 4 weeks of age to enable access to sufficient numbers of myeloid cells for phenotypic and functional assessment.</p>
<p>Typically, what will be done to an animal used in your project?</p>	<p>Our principal models involve activation of the innate immune response using bacterial products and bacterial infections, changes in oxygen availability (hypoxia), changes in glucose availability, and drugs that directly activate the immune system. We will study physiological, biological and cellular responses. Animals with specific genetic changes to signalling pathways that are important in controlling the innate immune response will be studied in addition to animals that do not have specific genetic alterations. Typically, animals will be exposed to an infection or inflammatory challenge in the tissues including lung, skin and peritoneum, or systemically following the administration of intravenous agents. Acute responses will be studied over 24-48 hours whilst more sustained responses will be studied over 7-14 days. The majority of animals will be exposed either one or two concurrent interventions that activate the immune response eg. exposure to hypoxia and a bacterial product and one additional intervention to suppress this response eg. treatment with a drug to limit the immune response or the study of an animal with a genetic alteration. To explore the importance of oxygen and nutrient availability for inflammation outcomes, animals will be exposed to different levels of environmental oxygenation (down to 8% oxygen) and alterations in circulating glucose in a chemically induced model of diabetes. Agents will be administered by injection, inhalation, or oral administration. Outcome measures will include physiological assessments for example non invasive temperature and blood pressure measures, cellular and nutrient changes in blood parameters following blood sampling. These procedures will require the restraint of animals during the sampling process. In a minority of animals (less than 5%) organ function will be ascertained by whole animal imaging in</p>

	<p>the anaesthetised state, and invasive physiological monitoring undertaken with the use of surgically implanted telemetry devices.</p> <p>This work will provide fundamental answers as to how the immune system is regulated in a physiological setting and provide new insights into how we can therapeutically target a dysfunctional immune response to improve outcomes for inflammatory disease states for which no effective treatments currently exist.</p>
<p>What are the expected impacts and/or adverse effects for the animals during your project?</p>	<p>The breeding and maintenance of genetically modified mice will require offspring to be marked and tissue samples taken for genotyping for example by ear clipping and microchipping. Animals used in experimental models will, for the majority, experience immune cell activation, which can result in a diminished appetite, weight loss, roughening of the fur, inability to groom, increased rate of breathing, a drop in body temperature, and reduced mobility. Adverse effects will be minimised by accurate dosing of agents, by following appropriate anaesthetic protocols and by regular monitoring of mice for evidence of excessive sickness. Activation of the innate immune response within the tissues may result in impaired tissue function leading to increased respiratory effort, local skin inflammation with abscess formation and systemic illness responses as outlined above. Animals experiencing exaggerated sickness responses including sustained respiratory distress, weight loss of more than 20% or other signs of illness (rough fur, inability to groom, immobility, inactivity, pale feet) will be removed from the experiment and killed by a schedule one method. In general, acute responses will be studied over 24-48 hours whilst more sustained responses will be studied over 7-14 days.</p>
<p>What are the expected severities and the proportion of animals in each category (per animal type)?</p>	<p>We propose half to two thirds of all animals (experimental and breeding), and the majority of the experimental animals will fall within a moderate severity category (including induction of lung injury, localised skin inflammation, acute peritonitis, systemic inflammation, chronic lung injury, diabetes, and exposure to hypoxia). Animals used for breeding and maintenance will fall within a mild severity category, whilst animals exsanguinated under general anaesthesia will be non-recovery.</p>

<p>What will happen to animals at the end of this project?</p>	<ul style="list-style-type: none"> • Used in other projects
<p>Why do you need to use animals to achieve the aim of your project?</p>	<p>The ex vivo study of human peripheral blood cells cannot replicate the complexity of cell-cell interactions of human innate immune responses and their role in host pathogen responses. Whilst we maximise the use of initial in vitro screens, the need to study these interactions in a physiological context in in vivo models remains. Furthermore, neutrophil experimentation remains limited by the inability to genetically modify neutrophils in vitro, severely limiting the ability to verify in vitro novel therapeutic targets without the use of genetically modified mice.</p>
<p>Which non-animal alternatives did you consider for use in this project?</p>	<p>The proposed research project would run in parallel to a series of experiments in which we phenotype ex vivo human blood and tissue neutrophils in health and disease states under a range of physiological and pathological culture conditions.</p> <p>We have also considered the use of none-mammalian animal models.</p>
<p>Why were they not suitable?</p>	<p>The ex vivo study of human cells cannot replicate the complexity of cell-cell communication, or the inflammatory niche. Therefore, whilst we maximise the use of initial in vitro screens, the need to study these biological responses in a physiological context in in vivo models remains. Furthermore, neutrophil experimentation remains limited by the inability to genetically modify neutrophils in vitro, severely limiting the ability to verify the biological consequence of manipulation of the HIF/hydroxylase pathway members without the use of genetically modified mice.</p> <p>Non-mammalian models are not currently suitable models because of major differences in pulmonary anatomy and in immunological systems. Where advances allow we will incorporate their use to reduce mammalian studies.</p>
<p>Enter the estimated number of animals of each type used in this project.</p>	<p>mice: 33,000</p>

<p>How have you estimated the numbers of animals you will use?</p>	<p>We calculate numbers of mice to be studied by power calculations. Power calculations are based on previous published data from our group and others. We have employed standard statistical methodologies to arrive at our estimated sample sizes. With respect to breeding programmes, we aim to litter mate match cre- with cre+ offspring of the same sex wherever possible using the LysMcre lines to ensure that experiments are appropriately controlled. This results in the requirement for higher breeding numbers than a straightforward whole animal transgenic line and has been factored into the calculation of number of breeding and maintenance mice required.</p>
<p>What steps did you take during the experimental design phase to reduce the number of animals being used in this project?</p>	<p>We minimise numbers of mice by collecting the maximum samples amounts and types from individual mice, by the use of new assays, such as imaging based approaches, which would enable the kinetics of responses to be measured in single mice and by refining assays in terms of sample volume and variability.</p>
<p>What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?</p>	<p>Before each experiment is conducted, a detailed protocol will be written covering (i) a statement of the experimental objectives; (ii) a description of the experiment, covering such matters as the experimental treatments, the size of the experiment, and the experimental material; and (iii) an outline of the method of analysis of the results. Factorial designs are preferred, and power analysis is used where appropriate.</p>
<p>Which animal models and methods will you use during this project?</p>	<p>The mouse is the species of choice for two reasons: First, there is extensive experience in studying immune biology in murine models in our laboratory and others. Secondly, the transgenic models we propose to study are largely confined to the mouse and central to this proposal. In the context of inflammatory responses, outcomes are dependent upon the route of delivery, the dose of pathogen/pathogenic product or sterile agent, the strain of pathogen/pathogenic product and the genetic background of the mice. We have already extensively performed dose titrations on all the agents and so we do not anticipate significant morbidity or mortality. In particular to the viral models, we have titrated the doses of virus down to induce only very mild clinical symptoms and typically <10% weight loss when used as a single insult. Where multiple administrations of inflammatory insults occur, mice may theoretically be more susceptible to the effects of pulmonary</p>

	<p>inflammation/infection after sequential inflammatory mediators (e.g. repeated bacterial challenge). To minimise the potential for adverse effects these experiments will only proceed using doses of mediators which, on their own, would be expected to cause minimal clinical signs of slight hair piloerection, <10% weight loss, no reduced mobility and normal respiratory rate. This is specifically designed such that the combined stimuli are expected to produce a mild-moderate severity banding.</p>
Why can't you use animals that are less sentient?	This proposed studies require organisms with fully developed respiratory and innate immune systems.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	To stay informed about advances in the 3Rs, both myself and members of my research group attend local meetings targeted at reviewing the 3Rs and understanding animal research. We follow this up within my own research group with quarterly meetings every year to revisit the use of animals research within the group to enable effective implementation of the 3Rs.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	As a research group with in excess of 10 years experience in modelling the innate immune response, we have developed a general physiological scoring system that we use to monitor the animals on an experimental procedure. Prior to a new program of experiments a discussion with the NVS will take place in order to decide appropriate frequency of monitoring of animals based on the expected signs for each agent used. In some instances supportive measures such as supplemental oxygen and pre-emptive fluid boluses will be given to reduce the severity of any adverse effects. Animals displaying >20% weight loss, rough fur, inappetence, inability to groom, immobility or pale feet will be removed from the experiment and killed by a schedule 1 method. We have specifically included the use of rectal thermometer to measure core body temperature to allow our results to be directly compared with previous studies. I have also added the potential to measure temperature by less invasive infrared thermometer to give us the opportunity to directly compare both methods and in future refine our studies.
What published best practice guidance will you follow to ensure experiments are conducted	We have consulted the NC3Rs (National Centre for the Replacement, Refinement and Reduction of Animals in Research) research paper: Prescott MJ, Lidster K (2017) Improving quality of science through better animal

in the most refined way?	welfare: the NC3Rs strategy. Lab Animal 46(4):152-156. doi:10.1038/labam.1217 and the ARRIVE guidelines to ensure both that experiments are conducted in the most refined way and that our work is subsequently reported in a way which maximises published information and minimises unnecessary studies. Study of the PREPARE guidelines and review of the FRAME websites prior to planning new experimental programs enables us to consider factors that are not readily available in the scientific literature which can influence the validity and outcome of studies on animals, improving our overall experimental design.
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Project	Plasticity and function of the visual system	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We are aiming to achieve a better understanding of how the visual system works and develops at a cellular level, both in the young and the adult, in order to find new ways to tackle a range of disorders of vision as well as of brain development more generally.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	1. A detailed knowledge, at the single-cell level, of how one might enhance or restore the ability of the adult brain to adapt to change will help us in refining novel means of intervention in the treatment of amblyopia ('lazy eye') in humans. This is a condition affecting up to 4% of the population for which no established treatment works beyond the	

	<p>age of 7. 2. A better understanding of how genetic defects that affect the ability of the brain to learn and adapt can lead to conditions such as autism spectrum disorders will point the way to the development of drugs tailored to compensate for the molecular deficits, or to gene therapy targets. 3. An investigation of how input from higher brain areas to the visual areas affects vision will provide insights into the way higher brain functions such as attention and memory are interlinked with sensory processing. 4. A better understanding, in an animal model of glaucoma, of the sequence of events that leads to the death of cells in the retina and the role of nerve growth factors in maintaining a healthy retina may point the way to a future treatment option for the second most common cause of blindness in the UK (accounting for 18% of all cases of blindness).</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice, 2500 animals over a period of 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Animals will be surgically prepared, under general anaesthesia, for brain imaging studies. Post-operative pain of moderate severity is the most likely adverse effect. In rare cases, infections may occur. All animals will be humanely killed in the end of the studies.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The function of the visual system can at present only be studied in intact animals which can integrate sensory experiences over time and produce behavioural responses in return. While we can study certain questions in brain slices it is impossible to maintain a whole brain and eyes alive in a dish, and even if this were possible, we would be lacking a behavioural readout.</p> <p>Computer-based modelling can help us to interpret our results; however, real data collected in vivo are needed to feed into any</p>

	models to ensure they have a sound basis.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Chronic (longitudinal) studies will be used extensively, an approach that reduces the total number of animals both by obtaining more data from each animal and by increasing their statistical power. In addition, we will use cutting-edge imaging techniques which enable us to gather information on whole areas of cortex and many individual neurons at the same time, again ensuring that fewer animals are needed.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse is our species of choice for all experiments. Compared with animals models previously used in visual neuroscience such as cats and ferrets they have a shorter developmental time span and allow easier visual access to the brain for imaging purposes. But above all, they offer the advantage of ready genetic modifiability not available for any other mammalian species. This is critical for examining the function of specific genes/proteins in cortical plasticity e.g. using knock-out models.</p> <p>For all surgical procedures, animals will be under general anaesthesia. Analgesics will be given prior to surgery. Post-operative care will involve the use of analgesics and antibiotics where necessary. During post-operative recovery, animals will be closely watched until anaesthesia has worn off. Afterwards their health will be checked regularly.</p>

Project	Polyclonal Antibody Development	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project is to continue the production of antibodies in sheep. Antibodies are proteins that are made in response to a vaccine (although in the "real world" they are made in response to exposure to an infection like a cold or the 'flu).</p> <p>The antibodies will be used to help researchers look at how cells that are normal or diseased work. For example, so that we can find where particular "target" proteins are located in cells and tissues. The antibodies can help identify where processes are going wrong or how the cell reacts to differences in the outside environment by helping us to see whether the</p>	

	<p>amount of protein goes up or down.</p> <p>Antibodies can help us to “pick out” the molecules we are interested in, and so let us purify them or be able to see what these molecules do. They do this by acting a bit like a flag – they only attach to the particular molecules we want - so that we can separate out just the bits of the cell machinery that we need by taking only the molecules attached to a “flag”.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Without antibodies for diagnostic tests, doctors wouldn’t be able to find out whether or not people are ill with some types of disease. This project will make antibodies against parts of cells whose function we don’t yet understand or where we think that these components could be involved in disease. The antibodies made in this project will let us find out about new parts of the cell whose function we don’t understand or know yet, or let us work out how much of certain components are there, for example is there more of a particular molecule in the cells of a person with Parkinson’s Disease.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Approximately 800 sheep over 5 years (some animals have already been immunised under the authority of a previous licence) and we are continuing to take blood from them</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The sheep will receive a number of injections (usually up to 6) at the same time to make up the “vaccination”. They will be injected under the skin in their neck (where the skin is loose) and/or into a muscle in their rump or leg. These are all sites that a farmer would use to inject a vaccine into a sheep. The amount of liquid injected will be small (only about 0.5ml) for any injection. The sheep are held still for this and it shouldn’t hurt them any more than you getting an injection at the doctor’s. The sheep seem completely normal afterwards. They can be revaccinated. This would usually be up to 3 “booster” vaccinations, but would occasionally be up to 6 times. Animals then have blood samples taken from time to time (normally less than once a month). The first sample or two will be small to check that the sheep is producing</p>

	<p>the antibodies as expected – but then we take a bigger sample (for the sheep it's about the same amount to them as to a person who gives a blood donation). The animals are well handled and familiar with people and the technicians taking the samples trained and very experienced. This reduces the stress for the sheep of having blood taken and we expect them to suffer no more than the pain associated with a needle prick and a little potential discomfort at being held still.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Although alternative methods for using antibodies to help us work out how cells function are being developed we simply don't have ways of mimicking the way that the immune system works at the moment. This means that we still need an animal for the production of high-quality and specific antibodies. The reason that sheep are so good for this purpose is that they can give us a lot of blood compared to if we used a smaller species of animal like a mouse. In order to ensure that every alternative to the use of an animal is considered, each request for a new antibody will be reviewed locally by a Vet, the holder of this licence and an ethics committee. Where a requester has not shown that they have looked at all alternatives, or has not justified the work with clear benefits, the request will be rejected.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>For each project that looks at a specific part of how cells work, a small number of sheep (1-3) will be immunised. This is usually enough to produce the amount of antibodies we need.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Sheep are a very common choice for antibody production, as each animal can give a large volume of blood without being harmed and they can do this on several occasions. Sheep are able to be trained to be used to being handled and sampled so that they don't find the process stressful.</p>

Project	Polyclonal Antibody, Normal Serum and Antigen Production	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
	<input checked="" type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
	<input checked="" type="checkbox"/>	Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The production of antibodies and antigens using animals is required by UK based Companies that manufacture Diagnostic Test Kits for the detection of disease in man and Pharmaceutical Companies for the production of vaccines.</p> <p>Diagnostic test kits are used by Blood Banks and Hospitals throughout the world for the detection of common bacterial and viral diseases in man that include Meningitis, Hepatitis, MRSA, Syphilis, Influenza, Salmonella, Shigella and Streptococcus infections.</p>	

	<p>The key component of many diagnostic test kits are antibodies and antigens specific to the infecting agent, currently there are no methods available for the production of specific polyclonal antibodies using non animal alternatives, similarly the growth of certain bacterium such as the one causing Syphilis cannot be achieved with tissue culture techniques.</p> <p>The majority of diagnostic manufacturers require normal animal sera for the dilution of antibodies and control components.</p> <p>It is a legal requirement to test donated blood for disease.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The real value of diagnostic kits is the rapid diagnosis of infection so that appropriate treatment can be given immediately. The use of appropriate diagnostic tests is part of a progressive effort to minimise pain, stress and discomfort in man</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Rabbits are the most common animal used for the production of polyclonal antisera raised by bacterial antigens A maximum of 1800 rabbits per annum will be used</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Animals used for antibody production will be dosed with antigen over periods of 2 weeks to six months depending on the antigen, blood sampling will take place at intervals for the purpose of assessing antibodies, in all cases the final samples will be taken under general anesthesia. Animals are monitored at all stages of the processes to limit adverse affects, dosing is reduced or omitted if there is a concern that further inoculations may cause distress to the animal, distress would normally be mild, exhibited by reduced food and water intake. Each animal is weighed prior to each procedure to monitor animals for early signs of reaction to the antigen. On completion of each schedule of work blood will be harvested under terminal anesthesia.</p> <p>Animals used for the production of syphilis pathogens are dosed with antigen once and</p>

	<p>monitored closely for 2 weeks.</p> <p>Animals are weighed prior to the procedure; adverse effects are controlled by pain relief and husbandry refinements.</p> <p>On completion of the schedule, tissue is harvested under terminal anesthesia.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Currently there are no methods available for the production of specific polyclonal antibodies using non animal alternatives, similarly the growth of certain bacterium such as the one causing Syphilis cannot be achieved with tissue culture techniques.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The current plan of work uses animals that produce larger volumes of serum and bacteria antigens due to their size and weight (purpose bred strains). Large Reductions of animal use have been achieved in the last 5 years using this approach.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rabbits have been historically used for antibody production, they were originally chosen for their ease of use (blood sampling & antigen dosing), ease of housing, plentiful supply and ability to produce high quality antibodies.</p> <p>Refinement is achieved in many ways including; use of disease free stock, an ongoing training / coaching system of staff to ensure good welfare, environmental enrichment, objective health monitoring and maximization of yields in the Laboratory.</p>

Project	Population genetics and radio tracking of an invasive reptile species
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research <input type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Aim: to assess whether an introduced, non-native reptile is likely to become a destructive invasive species, and to formulate a control and eradication strategy should the need arise.</p> <p>Objectives: to understand the distribution, population dynamics, genetic variability and viability, and key habitats of the species, to allow the formulation of a control plan exploiting this knowledge should the need arise.</p>
What are the potential benefits likely to derive from this project (how	Our results will allow us to assess the likelihood of a reptile becoming an invasive

science could be advanced or humans or animals could benefit from the project)?	species. Invasive species are the second biggest threat to biodiversity worldwide and by studying this population we hope to avert future losses. Second, our data would inform any future eradication efforts for this species if this becomes necessary, and be used for the formulation of a control and eradication plan. Finally the development of refinements to the use of implanted radiotelemetry and modelling would inform future practices with native species.
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 50 over the course of three years for genetic samples (blood/tissue), up to 50 over five years for radiotransmitter insertion.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We anticipate moderate severity through transmitter insertion procedure. However, other studies have demonstrated little to no long term ill effects of these procedures. Blood sampling should cause no more than transient distress, and we will use aseptic technique and monitor for possible adverse affects of infection or inflammation. At the end of the study all animals will be released to the wild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The population we hope to study is free-ranging and wild. We are interested in the ecology of this species and so we have to observe natural behaviours. There is no suitable alternative. Surgically implanted radiotransmitters are essential, as previous pilot projects have shown external attachment to be inviable in this species. Radiotagging is essential to allow animals to be located even when hidden from sight, and thus identify key habitats and habitat features, which is essential for control efforts.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Multiple transmitter insertions into a single animal will be minimised, but they are essential for females to elucidate breeding and hibernation behaviours. Males will not undergo multiple insertion procedures. We will also reduce the number of multiple insertions required for females, and do not expect to

	<p>carry out this procedure for more than 2-3 animals per season.</p> <p>No animals would undergo protocols 1 (blood sampling) and 2 (tracker insertion). We propose to collect the blood sample required for genomic analysis during surgery using a capillary tube to negate the need to take blood prior to surgery.</p> <p>We will monitor the benefit each animal brings to the study and stop adding new individuals once we have sufficient data.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>All animals will be handled for minimal times, and transported in dark, cloth bags inside boxes to minimise stress. Proper painkillers and anaesthetics will be used during surgery, and relevant protocols followed. The capture, tagging and handling methods have all been refined over many years and are intended to cause minimal harm. However we acknowledge that there is a non-zero risk of adverse effects, and we will closely monitor individuals and intervene humanely if necessary. We are very experienced with this and similar species and wholeheartedly believe ourselves able to judge situations we are likely to encounter as well as anyone. Because animals are tracked with radiotelemetry, we are able to monitor well-being during the course of the study very effectively and intervene to provide veterinary care or euthanasia if needed. The transmitters we will use are being refined through contact with the manufacturer. They are having modifications to increase battery life to reduce the need for multiple insertions, so each snake can be tracked for longer to obtain more data per insertion.</p>

Project	Potent monoclonal antibodies for biologics discovery	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project licence will be used to immunize rodents to generate antibodies that can be used as therapeutic medicines, as reagents to support clinical programs, as reagents for early stage research into new therapeutic target discovery, and to help improve the methods currently used to produce these medicines and reagents.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will generate high quality medicines which will be used to treat patients who have diseases of high unmet medical need (i.e. diseases affecting millions of patients worldwide) such as cancer, asthma, metabolic disease, pain and neurodegenerative diseases, and a number	

	of other diseases across different therapy areas.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years we would expect to use 1000 mice 50 rats
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The aim of this licence is two-fold. (1) To support the generation of biologic drugs for treatment of diseases such as cancer, diabetes, asthma where there are currently inadequate treatments available to very large patient populations. This will be achieved either through generation of antibodies that can serve as therapeutic drugs in their own right, or to generate antibodies which can help identify and validate new targets for therapy, or to generate antibodies the help monitor the therapeutic drugs themselves in studies before and during clinical trials. (2) To develop optimized protocols for the generation of highly effective antibodies to difficult antigen classes in wildtype and genetically modified mice. For both aims of the licence, this will involve injecting mice with substances including protein/peptides in solution, expressed on the surface of recombinant cells/inactivated bacteria or inserted into membrane mimics, expressed as RNA/DNA or via non-replicating virus with or without adjuvant such as oil-based adjuvants like Freund's complete/incomplete or aluminium-based adjuvant. Immunization will include the following routes: subcutaneous, intraperitoneal, intravenous, intradermal, intramuscular. Blood samples will be taken throughout the study. In order to obtain a large blood sample, the animals will be anaesthetised and then animal killed. The protocol used is a standard procedure for the generation of monoclonal antibodies. The protocol has been categorised as mild because the procedures undertaken are not expected to result in the animals developing any clinical signs of disease or ill health. On occasion, some mice might experience a transient piloerection (ruffled fur), and/or reduced activity. The administration of substances and blood sampling volumes and frequencies will adhere to published best practice guidelines (LASA) which will keep adverse effects to a minimum. Monitoring and procedures are in place to ensure that animal welfare will be

	considered at all times. At the end all animals will be killed.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The use of animals in this project is required to generate effective antibody reagents and antibody medicines for different types of disease with high unmet medical need (e.g. cancer, diabetes, asthma). While there are in vitro technologies for antibody generation available, these methods are not suitable for all disease targets. For example, it is extremely challenging to generate medicines to disease targets which are found on the surface of cells using in vitro technologies.</p> <p>Antibodies to simple soluble protein targets can be generated using in vitro technologies, such as phage display. However, in order to generate high affinity antibodies from in vitro phage display libraries, each antibody derived in vitro will need to undergo further significant in vitro manipulation to achieve the desired biological affect in man. Such affinity maturation projects are often very long, very labour intensive, and typically performed on one antibody at a time. Where large panels of high affinity antibodies are required as anti-idiotypes, parallel reagents or tool antibodies to support the pre-clinical and clinical development of a biologic drug, the exclusive use of in vitro antibody generation methods would result in significant delays in the progression of therapeutic candidates into key proof of concept clinical trials by years which, for the major diseases which we aim to tackle such as cancer, diabetes, asthma, etc., could potentially result in many more patients dying from such diseases than is necessary.</p> <p>Additionally, in vitro technologies can produce inferior quality antibodies that require significant in vitro engineering to get to medicine standard. This engineering can often have deleterious effects on their manufacturability and efficacy as medicines.</p> <p>A review of the most appropriate approach for generating our medicines will be carried out at the beginning of each programme. We will also</p>

	<p>apply the learnings from successful projects carried out on this licence to help build our knowledge of how to make <i>in vitro</i> antibody generation technologies more reliable in future.</p> <p>Furthermore, we aim to introduce new technologies to allow us to deep mine <i>in vivo</i> generated antibody repertoires by screening B-cells directly from immunized mice for antigen specificity. This new technology has the power to generate very large antibody data sets which will be used to help train machine learning algorithms. The aim of this initiative is to determine if we can train machine learning algorithms to design high affinity antibodies <i>in silico</i> to ultimately replace both <i>in vivo</i> and <i>in vitro</i> antibody discovery technologies. The datasets generated from <i>in vivo</i> studies are especially valuable as these antibodies have been generated naturally to the target antigen, and then affinity matured by natural <i>in vivo</i> sequence diversification processes, whereas <i>in vitro</i> generated antibodies are generated by selection of mis-matched antibody gene pairings, followed by non-natural molecular biology techniques to achieve affinity matured antibodies. This can give rise to undesirable traits identified later on in their development as biologic therapeutic drugs. Currently, there is no <i>in vitro</i> system that can be used to model the complex antibody generation process that the <i>in vivo</i> antibody generation and affinity maturation provides.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use the experience gained from the previous licences to guide the design of our studies.</p> <p>We will always plan to use the minimal number of animals for each experiment and will constantly analyse the data that we generate to see if further animal reductions can be made for the future. To minimise animal numbers, the choice of the immunization strategy and adjuvants will be partly based on previous experience, literature search and in consultation with other experts within the company. Where appropriate, small pilot studies will be performed to test a strategy or technology before deciding on whether to conduct experiments involving</p>

	<p>larger numbers of animals. If deemed relevant, statistical tools (e.g. power analysis) will be used to design the studies. We have access to in house statisticians who we will work with as necessary when planning such <i>in vivo</i> studies. We will use randomisation, blinding etc. where appropriate to avoid biases.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice and rats are small and easily handled species with a highly characterised immune system and well-defined biology. Mice and rats are also short lived, have rapid generation times, and are easier to look after than other larger animals. The immunization protocol used has minimal impact on animal welfare (mild severity) while giving each project the highest likelihood of achieving the desired scientific outcome, i.e. a large panel of high affinity antibodies to a given target to support biologic drug discovery and development for serious clinical diseases such as cancer, asthma, diabetes, etc.</p> <p>All animals are purpose bred for scientific use and kept in state of the art high quality pathogen free facilities to keep them healthy and clean until use, with access to food, water and environmental enrichments to enable the animals to live normal, good quality lives. Animals will typically be group housed and monitored by trained and competent animal technicians. Experimental procedures may involve a limited number of injections and/or small blood samples over a period of several weeks. These will be conducted according to best practice guidelines. Dedicated Technical staff perform all procedures detailed on this licence, and have been appropriately trained and supervised.</p> <p>The nature of the antibody response in mice and rats is very well characterised. The routes of injection used in this project have all been shown to cause no adverse effects whilst inducing effective antibody responses in most cases.</p> <p>Where appropriate, small pilot studies will be conducted to ensure that the methods used provide for the maximum animal welfare in relation to the study objectives.</p>

Project	Poultry Respiratory Infection, Pathology and Immunobiology	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The objectives of this programme of work are:</p> <ol style="list-style-type: none"> 1. To improve understanding on the disease and immunity in poultry infected with respiratory viruses of infectious bronchitis and metapneumoviruses 2. To examine the impact of vaccination in improving respiratory protection against virulent viruses 3. To understand the underlying mechanism which confers the protection when on or more respiratory vaccine viruses are given 	

	<p>either alone or simultaneously in chickens</p> <p>4. To strengthen the virulence of respiratory viruses by passaging in poultry.</p> <p>Our research group has continuously used non-animal models to understand the pathogenesis of poultry viruses. However, to appreciate the virus behaviour in the host (in birds), such as how the virus infects the birds, which tissue it multiplies and what immune responses induced, how the birds response to vaccination, and does it provides the protection needed. The only option is to use the most susceptible host; chickens for infectious bronchitis virus (IBV), and both turkey and chicken for avian metapneumovirus (AMPV). IBV is the most significant disease causing massive economic losses (poor body weight gain, increased feed consumption, condemnation at abattoir, drops in egg production and quality) and welfare concerns (respiratory disease, wet litter – lameness). AMPV causes respiratory disease in younger birds but prominently causes drops in egg production and quality. Though vaccination has been practiced for decades, due to the emergence of new strains of these viruses, it is essential that continuous scientific work are in-progress to examine the pathogenesis, immune responses, effective vaccination strategies, and potential development of innovative vaccines.</p> <p>The proposed study will complement our field epidemiological and diagnostic work, which is being carried out alongside the stakeholders in the poultry industry. Conventional and advanced molecular tools will be used for the characterization the virus and host responses. Findings from studies proposed here will have a short- and long-term impact on the control and prevention of IBV and AMPV in chicken and turkey flocks worldwide. The objectives of this programme of work are:</p> <p>To improve understanding on the disease and immunity in poultry infected with respiratory viruses of infectious bronchitis and metapneumoviruses</p> <p>To examine the impact of vaccination in improving respiratory protection against virulent viruses</p> <p>1. To understand the underlying mechanism</p>
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	<p>which confers the protection when on or more respiratory vaccine viruses are given either alone or simultaneously in chickens</p> <p>2. To strengthen the virulence of respiratory viruses by passaging in poultry.</p> <p>Our research group has continuously used non-animal models to understand the pathogenesis of poultry viruses. However, to appreciate the virus behaviour in the host (in birds), such as how the virus infects the birds, which tissue it multiplies and what immune responses induced, how the birds response to vaccination, and does it provides the protection needed. The only option is to use the most susceptible host; chickens for infectious bronchitis virus (IBV), and both turkey and chicken for avian metapneumovirus (AMPV). IBV is the most significant disease causing massive economic losses (poor body weight gain, increased feed consumption, condemnation at abattoir, drops in egg production and quality) and welfare concerns (respiratory disease, wet litter – lameness). AMPV causes respiratory disease in younger birds but prominently causes drops in egg production and quality. Though vaccination has been practiced for decades, due to the emergence of new strains of these viruses, it is essential that continuous scientific work are in-progress to examine the pathogenesis, immune responses,</p> <p>The work will provide scientific information, which will be shared through publications, presentations at meetings, and may lead to identification of new vaccines or vaccination strategies through a better understanding of the virus-host interactions up to the molecular levels. Following are the direct and indirect benefits expected:-</p> <p>1. <i>Increasing preparedness of the poultry industry to face arrival of new strains of IBV and AMPV.</i> By proactively identifying and characterizing newly emerged IBV and/or AMPV, including virulence studies, appropriate intervention strategies could be implemented early. This will avoid the dead of millions of birds due to diseases and production losses (e.g. poor body weight gain or eggs).</p>
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	<p>2. <i>Increasing preparedness of the poultry industry to face arrival of new strains of IBV and AMPV.</i> By proactively identifying and characterizing newly emerged IBV and/or AMPV, including virulence studies, appropriate intervention strategies could be implemented early. This will avoid the death of millions of birds due to diseases and production losses (e.g. poor body weight gain or eggs).</p> <p>3. <i>Increasing preparedness of the poultry industry to face arrival of new strains of IBV and AMPV.</i> By proactively identifying and characterizing newly emerged IBV and/or AMPV, including virulence studies, appropriate intervention strategies could be implemented early. This will avoid the death of millions of birds due to diseases and production losses (e.g. poor body weight gain or eggs).</p> <p>4. <i>Decreasing the adverse impact of newly emerged IBV and AMPV through the use of scientifically-tested vaccination strategies.</i> Normally, with the arrival of new strains of IBV/AMPV, introducing a new vaccine will take 5-10 years. Meanwhile, currently available vaccines could be used in a strategic vaccination programme to induce higher and wider immunity against the new IBV/AMPV. This can be quickly organised and executed at our place for better poultry welfare, health and protection in UK and worldwide.</p> <p>5. <i>Decreasing the adverse impact of newly emerged IBV and AMPV through the use of scientifically-tested vaccination strategies.</i> Normally, with the arrival of new strains of IBV/AMPV, introducing a new vaccine will take 5-10 years. Meanwhile, currently available vaccines could be used in a strategic vaccination programme to induce higher and wider immunity against the new IBV/AMPV. This can be quickly organised and executed at our place for better poultry welfare, health and protection in UK and worldwide.</p> <p>6. <i>Generation of future vaccines targeting the</i></p>
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	<p><i>induction of correct and higher immune development. With new strains for viruses, alongside better understanding on the disease process and immune-responses, new vaccines will be targeted to replicate and induce immunity at the respiratory lining, which have primary importance in protection against IBV and AMPV.</i></p> <p>7. <i>Generation of future vaccines targeting the induction of correct and higher immune development. With new strains for viruses, alongside better understanding on the disease process and immune-responses, new vaccines will be targeted to replicate and induce immunity at the respiratory lining, which have primary importance in protection against IBV and AMPV.</i></p> <p>8. <i>Reduction in use of antibiotics as an indirect benefit. An important reason for antibiotics use is due to respiratory disease complex induced by IBV and/or AMPV. Better diseases understanding and better intervention is likely to reduce complication with <i>E coli</i> and avian mycoplasmas. Thus, antibiotics use can be reduced, which will contribute in avoiding antimicrobial resistance.</i></p> <p>9. <i>Reduction in use of antibiotics as an indirect benefit. An important reason for antibiotics use is due to respiratory disease complex induced by IBV and/or AMPV. Better diseases understanding and better intervention is likely to reduce complication with <i>E coli</i> and avian mycoplasmas. Thus, antibiotics use can be reduced, which will contribute in avoiding antimicrobial resistance.</i></p> <p>10. <i>Reduction in use of antibiotics as an indirect benefit. An important reason for antibiotics use is due to respiratory disease complex induced by IBV and/or AMPV. Better diseases understanding and better intervention is likely to reduce complication with <i>E coli</i> and avian mycoplasmas. Thus, antibiotics use can be reduced, which will contribute in avoiding antimicrobial</i></p>
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	<p>resistance.</p> <p>11. <i>Reduction in use of antibiotics as an indirect benefit.</i> An important reason for antibiotics use is due to respiratory disease complex induced by IBV and/or AMPV. Better diseases understanding and better intervention is likely to reduce complication with <i>E coli</i> and avian mycoplasmas. Thus, antibiotics use can be reduced, which will contribute in avoiding antimicrobial resistance.</p> <p>12. <i>Global contribution on scientific knowledge on pathogenesis, immune responses and vaccine development to wider scientific community.</i> All our findings will be published in peer-reviewed journals for the benefit of other scientists and workers in the field of IBV and AMPV specifically, and for those working in the field of coronaviruses and metapneumoviruses.</p> <p>13. <i>Global contribution on scientific knowledge on pathogenesis, immune responses and vaccine development to wider scientific community.</i> All our findings will be published in peer-reviewed journals for the benefit of other scientists and workers in the field of IBV and AMPV specifically, and for those working in the field of coronaviruses and metapneumoviruses.</p> <p>effective vaccination strategies, and potential development of innovative vaccines. The proposed study will complement our field epidemiological and diagnostic work, which is being carried out alongside the stakeholders in the poultry industry. Conventional and advanced molecular tools will be used for the characterization the virus and host responses. Findings from studies proposed here will have a short- and long-term impact on the control and prevention of IBV and AMPV in chicken and turkey flocks worldwide.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The work will provide scientific information, which will be shared through publications, presentations at meetings, and may lead to identification of new vaccines or vaccination strategies through a better understanding of the virus-host interactions up to the molecular levels. Following are the</p>

	<p>direct and indirect benefits expected:- 1. Increasing preparedness of the poultry industry to face arrival of new strains of IBV and AMPV. By proactively identifying and characterizing newly emerged IBV and/or AMPV, including virulence studies, appropriate intervention strategies could be implemented early. This will avoid the death of millions of birds due to diseases and production losses (e.g. poor body weight gain or eggs). 2. Decreasing the adverse impact of newly emerged IBV and AMPV through the use of scientifically-tested vaccination strategies. Normally, with the arrival of new strains of IBV/AMPV, introducing a new vaccine will take 5-10 years. Meanwhile, currently available vaccines could be used in a strategic vaccination programme to induce higher and wider immunity against the new IBV/AMPV. This can be quickly organised and executed at our place for better poultry welfare, health and protection in UK and worldwide. 3. Generation of future vaccines targeting the induction of correct and higher immune development. With new strains for viruses, alongside better understanding on the disease process and immune-responses, new vaccines will be targeted to replicate and induce immunity at the respiratory lining, which have primary importance in protection against IBV and AMPV. 4. Reduction in use of antibiotics as an indirect benefit. An important reason for antibiotics use is due to respiratory disease complex induced by IBV and/or AMPV. Better disease understanding and better intervention is likely to reduce complication with E coli and avian mycoplasmas. Thus, antibiotics use can be reduced, which will contribute in avoiding antimicrobial resistance. 5. Global contribution on scientific knowledge on pathogenesis, immune responses and vaccine development to wider scientific community. All our findings will be published in peer-reviewed journals for the benefit of other scientists and workers in the field of IBV and AMPV specifically, and for those working in the field of coronaviruses and metapneumoviruses.</p>
<p>What species and approximate numbers of animals do you expect</p>	<p>The proposed work in this project will use specific-pathogen-free and commercial chicken and turkey breeds that are available in the UK.</p>

to use over what period of time?	Approximately 1600 birds might be used over the 5 years period. The scientific findings produced from our studies using less than 2000 birds, will likely benefit the welfare and health of poultry worldwide (producing more than 800 billion chicken eggs per year, and 50 billion broilers are slaughtered in a year).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The reasons for doing the work are to optimise the use of poultry vaccines in the following ways: (i) by knowing what new types of IBV are prevalent in a country/region and adjusting vaccine protocols accordingly, (ii) by understanding the broad protection offered when two different IBV vaccines are given (iii) by developing a novel AMPV vaccine based on a recent isolate and (iv) by studying the interaction between different live vaccines given simultaneously in the short life of the chicken, so that temporal adjustments can be made to the programme. These diseases are specific to domestic poultry and in order to test the vaccines, it is essential to use the host birds. Molecular or antigenic interrelationships between vaccine and field viruses are not in themselves helpful in predicting the outcome of vaccination challenge trials. The respiratory viruses to be used cause relatively mild infections, which normally resolve in about 7-10 days. The commercial vaccines cause no distress. All animals will be monitored 1-4 times daily pending protocol used. Numbers in experimental groups will be kept to a minimum consistent with producing meaningful results and allowing for individual variation. At the end of the experiments, all birds are humanely killed using Home Office Schedule 1 Methods.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	The reasons for doing the work are to optimise the use of poultry vaccines in the following ways: (i) by knowing what new types of infectious bronchitis virus (IBV) are prevalent in a country/region and adjusting vaccine protocols accordingly, (ii) by understanding the broad protection offered when two different IBV vaccines are given (iii) by developing a novel avian metapneumovirus (AMPV) vaccine based on a recent isolate and (iv) by studying the interaction

between different live vaccines given simultaneously in the short life of the chicken, so that temporal adjustments can be made to the programme.

These diseases are specific to domestic poultry and in order to test the vaccines, it is essential to use the host birds. Also, use of birds were unavoidable as this was a precondition to demonstrate efficacy of IBV or AMPV vaccines. Other non-animal models and molecular interrelationships between vaccine and field viruses are not in themselves helpful in predicting the outcome of poultry vaccination challenge studies.

The strains of the viruses being used in the proposed studies are known to cause mild to moderate disease, and birds are closely monitored with two to three, or more visits daily. If birds become ill, the bird will be humanely killed. Birds can be stressed due to handling, collecting swabs or blood. For this reason, only trained members allowed to handle and undertake these procedures. Same bird will not be subjected to repeated sampling on the same day. All personal are well trained to recognise disease and stress signs.

Poultry will be killed humanely at the end of each study.

As outline above, *in ovo* and *in vitro* systems are always the first line of investigation, however, it would not be possible to study the spontaneous living poultry responses.

When it is essential, we will move to use of live poultry. Both IBV and AMPV are diseases specific to domestic poultry and in order to assess a virulent strain of virus or vaccines, it is highly advisable to use living whole birds. This is as host-antigen interactions, particularly pathogenesis and immune responses, could be thoroughly studied. As such, it is essential to use host poultry when absolutely needed. For vaccine assessment, protection studies in the respective host provide undisputable results and are accepted by authorities (e.g. European pharmacopeia), scientist, veterinarians and producers worldwide.

<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of birds used will be kept minimal and will not affect the scientific output of the studies. For this, we consult statisticians and refer to past publications, protocols set by national and international regulatory bodies (e.g. European Pharmacopoeia). All work and preparations are strictly controlled including biosecurity to avoid repeating of any experiments, to minimise use of birds.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Chickens and turkeys will be used because they are the most susceptible host to the diseases under study.</p> <p>The respiratory viruses to be used cause relatively mild infections, which normally resolve in about 7-10 days. The commercial vaccines cause no distress. All animals will be monitored daily. Numbers in experimental groups will be kept to a minimum, consistent with producing meaningful results and allowing for individual variation.</p> <p>At all times, the birds are kept in the best environment, where floor-space, ventilation, light and lightings, feed and feeding, water and watering, behavioural needs (e.g. perching) and others requirements are provided to optimal standards. Any birds of welfare concerns, either due to health or not, would be put-to-sleep humanely.</p>

Project	Preclinical cardiovascular evaluation of drugs	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The main objectives of this study are to look at the effects of drugs on blood flow to different parts of the body and to determine whether these effects could explain some of the clinical vascular events seen in humans. Where possible, this project will also try to understand the ways in which these drugs may affect the cardiovascular system. The project will look at both drugs that are currently being developed as possible treatments to make sure they are safe in the cardiovascular system, and also drugs that have 'failed' in clinical trials, to see whether any adverse cardiovascular side effects could have been detected much earlier using our model.	

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The overall purpose of this project is to examine whether the adverse cardiovascular effects of compounds identified in the late stages of clinical development, could have been predicted preclinically, thereby providing a possible novel preclinical model for safety testing. The benefits of this project include obtaining a clear understanding of the cardiovascular effects of compounds that failed in clinical trials. The outcomes could lead to this methodology forming an important part of future projects. The potential benefits would ensure protection from cardiovascular adverse events occurring in man in the future. Moreover, finding out that drugs potentially increase cardiovascular risk during early preclinical development could reduce the use of not only rats and mice, but also dogs and monkeys, which are often used in later stages of drug development.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The rat is the animal of choice, since there is an extensive background literature on cardiovascular regulation in this species, the cardiovascular system resembles that of man in many ways, and implantation of the measuring devices is not possible in mice because they are too small. Across the lifetime of this project, it is estimated that a maximum of 704 rats will be used. For a typical rat in the full experimental schedule, surgery to implant flow probes (maximum of 3) around blood vessels, such as the renal and mesenteric arteries and the descending aorta, and 10-14 days later, to implant catheters in blood vessels will be carried out under general anaesthesia with operative and post-operative analgesia. On the day following catheterization, rats are dosed with the drug of interest. Experimental recordings are made for a maximum of 4 days.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In order to minimise animal use, short-term experiments run over a maximum of 4 days, with animals acting as their own controls, and being exposed to more than 1 compound, if appropriate. Possible adverse events include reactions to anaesthesia, issues with wound healing, damage to catheters, or reactions to the drugs. The expected severity level of the</p>

	<p>procedures is moderate. The health and well-being of animals will be closely monitored, particularly post-surgery, and any concerns raised with the NVS or deputy. As a consequence of discussions with the NVS, or following completion of the experimental protocol, animals will be killed by a Schedule 1 method.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The use of animal models is essential to enhancing our understanding of cardiovascular responses to drug administration and provides a means of replicating elements of the treatable phases of disease to drive forward new therapeutic options. The 3Rs will be implemented where possible to improve the scientific models used in this project, as demonstrated by our use of the EDA design tool. Although <i>in vitro</i> work can often replace some aspects of whole animal studies, the aim of the current project is to evaluate the complex cardiovascular effects of compounds in a robust model of integrated, intact systems.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We have implemented strategies that allow examination of the effects of different pharmacological interventions, in the same surgically prepared animal, on different experimental days. This achieves a reduction in the use of animals requiring surgical preparation without unduly increasing the burden on each animal (other than by extending the time for which the animal is held in the experimental condition), because the effects of the experimental interventions are minor and transient, relative to the burden of surgery. Our rigorous data interpretation helps to inform the design of further experiments, with support from the EDA tool, ensuring robust experimental design and forward planning which contributes to reduction in overall animal use.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having</p>	<p>The rat offers a model system that shares a level of commonality in its cardiovascular physiology that is considered a close parallel to that of man. The choice is therefore a rodent model system at the lowest possible neurophysiological sensitivity</p>

<p>regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>that is able to produce data of significant physiological significance that may be, as much as realistically possible, considered valid for extrapolation to the living human situation.</p> <p>The methodology used in this study is continually refined. The probe size is considerably smaller than other commercially available systems, surgeries are performed by highly trained personal licence holders, and new refinements to techniques are implemented on an on-going basis. Animals are very closely monitored during the post-surgical phase (every 15 min) and any concerns discussed with the NVS or deputy. Rigorous checks ensure animals do not experience any additional harm or suffering.</p>
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Project	Preclinical evaluation of a new chemical cross-linker for the treatment of keratoconus	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Keratoconus is a progressive condition, affecting young and working age people, in which the cornea becomes misshapen significantly disrupting the path of light into the eye. It is a lifelong condition and a significant health burden in work-age adults; and is a leading cause of corneal transplantation in the UK. One of the key features of keratoconus is a loss of physical stability in the cornea, with a 40% reduction in the stiffness of the cornea compared to a normal cornea. Corneal collagen cross-linking increases the stiffness of the cornea and can reduce the progression of this	

	<p>disease by preserving corneal integrity due to strong bonds formed within the collagen. Over the past decade, collagen cross-linking using ultraviolet A (UVA) radiation combined with riboflavin has been used but it has several limitations. In particular, the exposure to UVA radiation risks toxicity to cells in two layers of the cornea called the stroma and the corneal endothelium. Also for effective stiffening it is necessary to remove another layer called the corneal epithelium to allow penetration of the riboflavin, which is painful for the patient and increases the risk of infection. We have developed a cross-linking solution and demonstrated that it stiffens the cornea in a laboratory model of the cornea using porcine and human tissue to a similar extent to the existing treatment but with added benefits such as reduced patient discomfort and ease of application. This project aims to test how effective the solution is at stiffening the cornea in a suitable animal model, determine its long term effectiveness and if it is safe.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The current treatment for keratoconus involves strengthening of the cornea by administration of riboflavin and activation by UVA irradiation, which is toxic to corneal cells and may result in long term damage. There is also a need to remove the most superficial layer of the cornea to allow the riboflavin to reach the correct part of the cornea. This process causes significant discomfort for the patient and a risk of infection. We have developed a safer corneal cross-linking procedure which will be easier to use by the clinician and more comfortable for the patient as there is no need to remove the corneal epithelium. Patient discomfort will be reduced and risk of infection reduced.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will be using rabbits and rats. We expect to use approximately 30 rats and 50 rabbits. The minimum experimental period of time will be 24hrs and the maximum will be 180 days.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity?</p>	<p>The rat and rabbits will be undergoing eye surgery under general anaesthesia. Pain killing drugs will be given to minimise post-operative discomfort, which should resolve within</p>

<p>What will happen to the animals at the end?</p>	<p>approximately 72 hours. We expect a moderate severity. Animals will be euthanised using schedule 1 procedures and then whole globes and other tissue removed to study any structural changes in the cornea.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>It is not possible to completely replace the use of animals in this study as we require a physiologically relevant model to test our chemical cross linker before moving to clinical trials in humans, the next step in the translational pipeline. The rat and rabbit are suitable models due to their large eye size relative to its body and its anatomical similarity to the human cornea.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>In terms of reduction, initial experiments will use tissue from pigs and humans to refine the formulations of the chemical cross linker. This will allow us to limit the number of experimental groups, and so animal numbers, that we require for the animal experiments. Statistical design has been conducted to determine the lowest number of animals that will be required to provide meaningful results.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We have chosen the rat /rabbit as our model due to its similarity to the human cornea in terms of corneal layers and dimensions. In order to obtain Medicines and Healthcare products Regulatory Agency (MHRA) approval for a 'First in Man' clinical trial toxicological evaluation must be performed in a rodent (rat) and non-rodent species (rabbit).</p> <p>All surgical procedures will be optimized using rabbit eyes in the laboratory and cadaveric rabbits prior to the surgery in live animals, which will reduce the risk of complications occurring in the animals. All surgery will be performed by a consultant ophthalmologist who is highly trained in the procedure, performing similar procedures on a weekly basis in patients. This means that the animals will receive similar care and attention that a patient would expect.</p>

Project	Pre-clinical evaluation of animal trypanosomiasis vaccine candidates
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input type="checkbox"/> Basic research <input checked="" type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
What's the aim of this project?	The purpose of this research is to develop Animal African Trypanosomiasis (AAT) vaccine candidates that can protect animals against trypanosome infection by preventing the parasites from moving into the bloodstream from the site of infection in the skin. Our previous research has identified multiple parasite proteins that could elicit a protective immune response, which we now need to test in an animal model.
Why is it important to undertake this work?	Animal African Trypanosomiasis (AAT) is a vector-borne livestock disease that is endemic throughout sub-Saharan Africa. It is a leading cause of livestock mortality in the developing world and is a principal limitation on agricultural productivity. No vaccine has ever been

	<p>produced for animal trypanosomiasis, yet millions of animals are infected each year. Identification of protective antigens will allow us to formulate an experimental vaccine for use with livestock in clinical trials. There is an urgent need for sustainable solutions to animal trypanosomiasis across the developing world due to high disease transmission and drug failure.</p>
<p>What outputs do you think you will see at the end of this project?</p>	<p>Our work is expected to have the following key benefits: –</p> <ul style="list-style-type: none"> - We will evaluate novel parasite antigens for their ability to raise protective antibodies against trypanosome parasites. Ultimately, identification of protective antigens will allow us to formulate an experimental vaccine for use in clinical trials. There is an urgent need for sustainable solutions to animal trypanosomiasis across the developing world due to high disease transmission and drug failure. Developing successful vaccines will depend on identifying immunogenic antigens and optimal adjuvants. - For antigens that have protective effects after challenge, we will determine correlates of protection through comparison of immune transcriptomes from protected and control animals. These studies will reveal those host genes that are involved in a protective immune response, helping us to understand the optimal conditions for sterile immunity. <p>These findings will be published in peer reviewed journals, and may form the basis for the development of a novel vaccine formulation for animal trypanosomiasis that can be used in clinical trials.</p>
<p>Who or what will benefit from these outputs, and how?</p>	<ol style="list-style-type: none"> 1. Academic scientists (knowledge): we will create new knowledge about how novel parasite antigens elicit immune responses in hosts, about the contribution of fly bite to immunological response, and about the molecular host-parasite interaction during early infection (within 3 years). 3. Parasitology discipline: by challenging the consensus that vaccination against trypanosomes is impossible, we will facilitate progress towards this important aim (within 5 years). 4. Animal health NGOs: protective antigens that we identify will facilitate the work of NGOs (REDACTED), seeking to develop novel interventions from new basic knowledge (5-10 years).

	<p>5.Reducing AAT (animals): protective antigens would provide a vaccine that would reduce animal mortality and morbidity. These antigens may be cross-reactive with homologs in human trypanosome species, and so protect against both human and animal trypanosomiasis (10+ years).</p> <p>6.Reducing AAT (people): reduction in AAT will increase the wealth and prosperity of farmers in the developing world, from small-scale farms using animals for draught to large-scale livestock production systems (10+ years).</p> <p>7.Reducing AAT (societies): reduction in AAT will reduce economic loss and government costs in disease control, leading to greater socio-economic development and food security. This will contribute to UK Government commitments towards international development (10+ years).</p> <p>8.UK economy: our protective antigens may be patented for commercial application, and exploitation of a successful vaccine may contribute to the wider UK economy (5+ years).</p>
<p>Will this work be offered as a service to others?</p>	<p>No</p>
<p>How will you look to maximise the outputs of this work?</p>	<p>To maximise scientific impact, we have a strategy to connect with a range of target audiences. Results (both successful and unsuccessful approaches) will be disseminated to the research community through presentations at international conferences (e.g. WAAVP), UK meetings (e.g. British Society for Parasitology) and through primary publications. Significant developments in application that arise from this work will be accompanied by appropriate press releases administered through Institutional Press Offices, which have vast experience of the local, national and international print, online and broadcast media and will co-ordinate press releases, and help ensure the research is presented in an accurate and balanced way. Our institutions have strong international, one-health programmes and maintain formal partnerships with animal health institutes across Africa and South America. We will use these institutional links to advocate AAT vaccine development to African/ S.American animal health agencies. We will recruit an industry partner to optimise vaccine formulation and test its properties in a disease setting.</p>

<p>Explain why you are using these types of animals and your choice of life stages.</p>	<p>We are studying the complex, global immune response of host to trypanosome, trying to identify an authentic response that protects a host from infection. Immune responses are highly multi-factorial and, in the context of trypanosomes, largely uncharacterised. Therefore, at present, it is not possible to fully recapitulate the complex processes that follow immunisation or the innate and acquired immune response that follows infection using an in vitro model. A mammalian species is required for the study of immune responses to trypanosomes, and to accurately model bovine infections. Many of the immunological pathways involved in resistance to infection and the development of protective adaptive immunity are shared between mice and livestock. The mouse is also an excellent model system for <i>Trypanosoma</i> infection, since it is a natural host for these parasites, and can be infected through physiologically relevant routes, recapitulating livestock disease phenotypes.</p>
<p>Typically, what will be done to an animal used in your project?</p>	<p>Typically, an animal will receive a vaccination. This will be delivered as three injections, spaced around two weeks apart. We will take a small drop of blood from the tail of the animal in order to test for production of antibodies. After a rest period of 2-4 weeks, the animal will be infected with trypanosomes. These parasites are spread by the bite of the tsetse fly, and we will mimic this route of transmission here. We will then follow the spread of the parasite in the infected animal by periodically sampling drops of blood from the tail, and by looking for bioluminescent signals from the parasite. The latter will involve the animal being anaesthetised and placed in an imaging chamber. Infections will last between one and three weeks, before the experiment ends.</p>
<p>What are the expected impacts and/or adverse effects for the animals during your project?</p>	<p>As a result of infection with the parasite, animals may develop a chancre at the bite site, which will self-cure within 3-10 days, when parasites move to blood (or are cleared). Blood stage infection may result in periodic lethargy, piloerection, and loss of appetite, coincident with peaks in parasitaemia (approximately every 3 days). These systemic symptoms are generally not observed until after the first peak of parasitaemia. Many experiments will be terminated after the first peak of parasitaemia, minimising clinical symptoms. Some will last for up to 3 weeks, with stringent monitoring of welfare throughout.</p>

<p>What are the expected severities and the proportion of animals in each category (per animal type)?</p>	<p>We expect around 50% of mice to be classified as mild, and 50% as moderate. This will depend on whether the experiment is terminated before the appearance of clinical signs, which we will do wherever compatible with scientific aims.</p>
<p>What will happen to animals at the end of this project?</p>	<p>killed</p>
<p>Why do you need to use animals to achieve the aim of your project?</p>	<p>The aim of this project is to evaluate the ability of specific parasite antigens to induce protective antibodies in the host after immunisation, and following fly bite challenge with trypanosomes. We are studying the complex, global response of the host immune system to trypanosome infection, trying to identify an authentic response that protects a host from infection. Since the ultimate goal is to design a vaccine that protects livestock, we must study a mammalian immune system. Mice are the least sentient model for studying this and are an amenable species with which to test the hypotheses.</p>
<p>Which non-animal alternatives did you consider for use in this project?</p>	<p>In reaching this point, we have adopted sophisticated <i>in silico</i> models to replace as much <i>in vivo</i> work as is compatible with achieving our aims. We have used a reverse vaccinology approach to identify plausible antigens for vaccination, screening pathogen genome sequences for elements of protein structure that might imply cell surface-related roles. We have thereafter used <i>in vitro</i> assays to screen serum from naturally infected animals to identify strongly immunogenic antigens among our list of target antigens, removing the need for experimental infection. However, we still require the use of <i>in vivo</i> models to confirm the most significant findings arising from our <i>in silico</i> analyses and <i>in vitro</i> assays, and most importantly, to test the ability of antigens to elicit protective immune responses at a systemic level. This will be crucial if we are to translate our findings to the development of novel therapeutic strategies.</p>
<p>Why were they not suitable?</p>	<p>Immune responses are highly multi-factorial and, in the context of trypanosomes, largely uncharacterised. Therefore, at present, it is not possible to fully recapitulate the complex processes that follow immunization or the innate and acquired immune response that follows infection using an <i>in vitro</i> model. Furthermore, one of our</p>

	<p>chosen parasites, <i>Trypanosoma vivax</i>, cannot be cultured in vitro and must be maintained in a vertebrate host. Animal models are therefore required to culture the pathogen and to accurately determine whether vaccination produces a protective immune response.</p>
<p>Enter the estimated number of animals of each type used in this project.</p>	<p>mice: 700</p>
<p>How have you estimated the numbers of animals you will use?</p>	<p>Our aim is to identify antigens that provide protective effects against trypanosome infection, when used to immunise mice. We are looking for antigens that completely prevent bloodstream infection, and restrict the parasite to the bite site. Therefore, we do not require large sample sizes because we are not interested in detecting slight reductions in parasite burden.</p> <p>The key issue when calculating sample size then becomes the variability in infection levels in the control (unvaccinated) group after fly-bite challenge. We have conducted an extensive survey of the literature in order to determine how many mice we will require per group to ensure statistical significance. In the almost all examples of <i>T. congolense</i> and <i>T. vivax</i> fly-bite infections of livestock, and <i>T. congolense</i> and <i>T. brucei</i> fly-bite infections of mice, ALL of the experimental animals developed parasitaemia, though levels were quite variable. We used this data to perform sample size calculations using G*Power software. We found that 5-9 animals per group would be required to achieve the appropriate statistical power. We will also perform our own pilot studies to complement this analysis.</p>
<p>What steps did you take during the experimental design phase to reduce the number of animals being used in this project?</p>	<p>We used G*power to compute statistical power, and perform sample size calculations. We conducted an extensive review of the literature to determine variation in blood parasitaemia.</p>
<p>What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?</p>	<p>Age and sex-matching of mice will be used to reduce intra- and inter-group variation. Inbred mice will be purchased from an external vendor to provide experimental groups of uniform age and weight. The maximum amount of information will be extracted from each experimental animal by collecting tissue for flow cytometry, cytokine</p>

	<p>bead array, histology, and transcriptomic analysis of both host and pathogen gene expression. Where possible, tissue will also be archived for future investigations.</p> <p>We will perform pilot experiments to determine the proportion of mice becoming infected, time to patency, and variation in peak parasitaemia. These will be used to refine our sample size calculations, and where possible, reduce group sizes.</p>
<p>Which animal models and methods will you use during this project?</p>	<p>We will use a mouse model of trypanosome infection. Mice will be immunised with candidate peptide antigens, and then challenged with <i>T. congolense</i> or <i>T. vivax</i> via Tsetse fly bite. This mimics natural infection.</p>
<p>Why can't you use animals that are less sentient?</p>	<p>Ultimately, we hope to develop a vaccination that protects against <i>T. congolense</i> and <i>T. vivax</i> in livestock. To do this, we need to know that immunisation with our selected antigens generates a protective immune response in adult mammals (immature or non-mammalian host immune systems would respond differently, and would not accurately predict chance of vaccine success in the field). Mice are the least sentient model species with which to achieve this. Many of the immunological pathways involved in resistance to infection and the development of protective adaptive immunity are shared between mice and livestock. The mouse is also an excellent model system for <i>Trypanosoma</i> infection, since it is a natural host for these parasites, and can be infected through physiologically relevant routes, recapitulating livestock disease phenotypes. Since it takes several weeks for an effective immune response to develop, and several days for signs of infection to become apparent, we cannot conduct our studies on terminally anaesthetised animals.</p>
<p>How will you stay informed about advances in the 3Rs, and implement these advances effectively,</p>	<p>We will take advantage of regular training opportunities offered by our animal facility in conjunction with the NC3Rs, in addition to online training resources and bulletins published by the NC3Rs. We will attend subject-specific conferences and liaise with collaborators in the</p>

<p>during the project?</p>	<p>field to keep informed of any developments in our particular model. We will continuously seek to refine our experimental procedures to reflect new advice.</p>
<p>How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?</p>	<p>We have extensive experience of monitoring and scoring schemes for detecting adverse effects in experimental animals. This scheme has clearly defined humane end points to minimise suffering. Any animal that exhibits symptoms consistent with these defined end points will be humanely euthanized by a Schedule 1 method.</p> <p>Where possible, we will perform analysis at time-points that allow for adequate induction of an immune response, but that precede development of clinical signs of disease. All of the infection models we intend to use have been used extensively, and are well defined, allowing us to confidently predict these time-points. For example, in our collaborators' experience, mice remain overtly in good health for 5 days following subcutaneous infection with <i>T. vivax</i>, at which point antibodies can be detected in the lymph nodes. Much of our analysis to determine correlates of protection could therefore be performed at this time-point, for antigens that have been shown to be protective in earlier experiments. In some cases, it will be necessary to proceed beyond these time-points to confirm that no bloodstream infection develops. Following infection, animals will be assessed on a daily basis using clinical scoring systems that have well-defined endpoints to ensure that there is no unnecessary suffering. Any animals showing signs of approaching these endpoints will be euthanised immediately.</p>
<p>What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?</p>	<p>We will refer to the PREPARE guidelines (https://norecopa.no/prepare) and to guidance documents published by the NC3Rs (Responsibility in the use of animals in bioscience research, ARRIVE, Experimental Design Assistant) and UKRIO.</p>

Project	Preclinical Imaging in Biomedical Research
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input checked="" type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of this work is to provide unique data in rodents, (i) to support the development of tool radiolabelled compounds for the medical imaging technology, positron emission tomography (PET), (ii) to characterise novel drugs and therapies using imaging; and (iii) to use imaging to provide key knowledge of healthy and disease states within the living body.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit)	This project is expected to optimise clinical imaging studies in several ways but particularly by the development of tool compounds for imaging. The development of drugs by our

<p>from the project)?</p>	<p>collaborators and customers including both Pharma and biotech companies, will be facilitated, thereby reducing attrition. In addition, using imaging and related techniques as an important tool, the work will contribute new fundamental scientific knowledge on normal and disease processes within the body.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Approximately 700 rodents (rats, mice, Guinea pigs & gerbils) may be used per year.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Where possible, normal free-living rodents will be used under this project. Animal models of disease such as Parkinson's Disease, respiratory disease, rheumatoid arthritis, liver disease and cancer may be generated and used, where the experimental question may not be answered using normal animals. Aseptic techniques will be used for surgical preparation of recovery animals, together with antibiotic/analgesic cover, where appropriate. Possible toxic effects of compound administration are expected to be rare and mild as the lowest possible dose possible to give scientific valid data will be used. The body temperature and other physiological parameters of the animals will be monitored throughout the procedures where possible. If adverse effects were to occur then suitable action based upon the best care practice and/or veterinary advice will be taken with immediate effect. Typically, studies will involve an imaging technique to provide quantitative in vivo data on a compound or biological process. Alternatively, a simple dosing and sampling regimen may be employed. Blood samples and/or tissue samples will be collected when required. By analysing the data, the concentration of radioactivity in the tissues will be determined and biological parameters generated. The majority (>95%) of the animals are not expected to show any signs of adverse effects that impact materially on their general wellbeing. No more than 5% of animals are expected to show clinical signs of a moderate severity. The animals will be humanely killed at the end of the procedure.</p>

Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>New drugs and PET tool compounds are required. Studies using tissues and cells or computer simulation are typically used to investigate the characteristics of compounds and/or their targets of interest. However, as these drugs and PET tool compounds are being developed for administration in humans, their effects on the body as a whole and the effect of the body on the drugs themselves are important to know. Therefore they need to be investigated in live animals. In addition, some biological processes may only be investigated in live animals</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>For each study, the minimum number of animals required to obtain scientifically valid data will be used. Imaging enables the characteristics of a compound or a process within the body to be measured over time, negating the use of a larger number of animals killed at discrete time points and will thus reduce the number of animals that would otherwise be used.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rodents, commonly rats and mice, will be used under this project as the lowest sentient species that may be used to produce satisfactory results. There is a considerable amount of historical data using these species for imaging studies. Opportunities for experimental refinement will be sought throughout the project. Good, sympathetic handling techniques will be used to minimise discomfort to the animals. To reduce distress, single housing will be reduced to a minimum and additional enrichment will be provided wherever possible.</p>

Project	Preclinical research of novel pain therapeutics	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our principal objective is to develop new therapeutic strategies to treat chronic pain. To achieve this we will identify nervous system mechanisms contributing to chronic pain and test novel therapeutics targeting these mechanisms. The sites and mechanisms of action of substances and medical devices known to provide pain relief in humans, whose mechanism of action is currently unknown, will also be identified and this knowledge used to develop new, improved therapeutic approaches to treat chronic pain.	
What are the potential benefits likely to derive from this project	Our studies will provide new insights into the treatment of pain and will provide further stimulus in	

<p>(how science could be advanced or humans or animals could benefit from the project)?</p>	<p>the scientific community opening new lines of investigation for the development of new and improved analgesic therapies. The availability of new classes of compounds, targeted towards novel mechanisms involved in the generation and maintenance of pain will provide relief from suffering and a better quality of life for millions of sufferers worldwide.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>In total, we expect to use approximately 6000 adult Rats, 2500 adult Mice and 250 adult Guinea-Pigs over a period of 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Under a typical experimental setup, for example, nerve ligation followed by behavioural testing, animals will only experience mild-moderate discomfort, associated with exposure to the pain models. This discomfort is typically transient in nature, with animals returning to baseline behavioural status on return to their home cages, as indicated by return of social interactions and normal physiological function. This suggests that the majority of animals (98%) will not experience any adverse effects that significantly impact on quality of life. A minority (no more than 1%) may show moderate discomfort, for example a slight limp or lack of use of affected limb for a few days following surgery. Should more severe effects be observed, and at the end of the experiment, animals will be immediately killed using approved procedures.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Pain is complex, arising from integration within a fully functioning nervous system that can only be investigated in models with intact circuitry comparable to that involved in human pain perception. Thus no suitable alternatives exist to address underlying mechanisms contributing to pathophysiology.</p> <p>Once molecular targets/pathways are identified, detailed mechanistic studies will use <i>in vitro</i> systems.</p> <p>Data generated is subject to mathematical/systems biology approaches to develop predictive,</p>

	<p>computer-based models of gene and neural networks for future replacement and refinement of experiments.</p> <p>Wherever possible <i>in vitro</i> techniques will be employed including: cultured and/or dissociated neurons, isolated brain and spinal cord slice preparations.</p> <p><i>Ex-vivo</i> tissues from animal models will also be utilised wherever possible.</p> <p>Wherever possible we will supply tissues to other researchers and collaborators to maximise model use</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We have vast experience of performing these experiments over approximately 25 years and using this experience coupled with mathematical calculations, we have accurately predicted the number of animals that will be required for each experiment to provide confidence in results generated. Biostatisticians are consulted as and when deemed necessary to confirm the use of minimum numbers of animals.</p> <p>Animal number is also minimised by ensuring tissue is analysed in multiple ways (e.g. tissue used in one specific experiment will later be used for other studies)</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>All models utilised in these experiments are the 'gold-standard' models for neuropathic pain. Since no model encompasses all symptoms/elements of the disorder the use of multiple models is a requirement for a complete understanding of the biological mechanism that lead to the diseased state of the disorder.</p> <p>The use of post-operative analgesia is incompatible with the procedures as the aim of the project is to investigate pain. A mini-project performed under our previous project licence performed upon recommendation of the home office inspector showed that analgesic use prior to surgery in the animals significantly altered the output of the experiments, with animals showing reduced central pain sensitisation and increased variability post-surgery. Thus, to refine and reduce usage in these models, analgesic use post-operatively is</p>

	<p>contraindicated.</p> <p>Rats and mice provide the basic model and are the lowest vertebrate groups on which well characterised, minimal severity models of pain have been developed and characterised anatomically, neurophysiologically and behaviourally. Guinea pigs have certain attributes more comparable to man where the involvement of these faculties is known, guinea-pig would be the first-choice model.</p> <p>To minimise suffering and maximise output we will adhere to our existing (and where appropriate, develop) standard operating procedures for all surgical and behavioural protocols. In all models, animal health and welfare is carefully monitored and documented using standard criteria based on adverse effects. Wherever possible cultured tissue and donated human post-mortem tissue is used. Animal husbandry procedures are constantly reviewed and improved to maintain and enhance quality of life through-out the project.</p> <p>Furthermore, regular contact with collaborators, pharmaceutical/biotechnology partners, statisticians, the establishment named veterinary surgeon and animal welfare officer will ensure refinement of existing and novel approaches.</p>
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Project	Preclinical screening in drug discovery	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The aim of this project is to accelerate the discovery of new drugs to treat people suffering with distressing and disabling psychiatric and age-related brain disorders, such as Parkinson's disease, stroke or schizophrenia. This will be achieved using our panel of screening techniques in living animals, which include high-resolution magnetic resonance imaging (MRI), electroencephalography, autoradiography, behavioural and histological measurements. This allows us to comprehensively measure the effectiveness of drugs in the brain. These screening techniques are incorporated within our imaging research facility and will be offered either as a service to pharmaceutical industry, or utilized through academic collaborations.</p> <p>The project has two main objectives:</p>	

	<p>1) To measure structural and functional brain changes in rodent models representative of age-related and psychiatric disorders to develop biomarkers of pathology that can be measured in both humans and animals</p> <p>2) To provide a service for assessment of new drug treatments for age-related and psychiatric disorders using our preclinical screening techniques</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The value of this approach is three-fold. Firstly, rodent models allow one to assess the precise effects of genetic, environmental or drug-induced manipulations on brain structure and function in the absence of confounding factors usually present in human studies. Secondly, the use of MRI (clinically comparable technology) allows the collection of parallel assessments in living rodents and humans, maximising the possibility for translation of experimental findings to the clinic. Thirdly, and most importantly, in rodents one can measure brain structure and function in ways that are technically and ethically impossible in living human subjects, bridging the gap between imaging and neuropathology to identify the underlying mechanisms. This will accelerate the discovery and translation of new drugs for the treatment of disabling and distressing psychiatric and age-related brain disorders such as e.g. schizophrenia, stroke or Parkinson's disease. Currently there are only limited treatment options for these disorders and these are only partially effective at relieving patient symptoms. Furthermore, no treatments exist to slow the progression of these disorders and thus cure patients of their illness. Together this represents a serious unmet medical need our research will attempt to address.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use approximately 2680 rats and 2060 of mice over the length of project that will be 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In all cases we endeavour to minimise any suffering of experimental animals. More than half of all protocols will be performed under anaesthesia and therefore animals will not be conscious or feel any pain. However, for purposes of translating the results of our experiments into clinical findings and to understand how the living systems respond to pharmaceuticals, we will sometimes perform recordings in conscious animals. This may involve taking blood samples, injecting substances into the body and taking metabolic, behavioural and/or electrophysiological measurements from the brains of these animals through injection of radioactively</p>

	<p>labelled tracers or previously implanted devices. These will always be conducted in a manner that causes minimum amount of suffering. Any animal that shows unexpected adverse effects will be immediately humanely killed. All surgical and brain imaging procedures will always be conducted under anaesthesia, animals will receive pain killers, and local analgesics will be applied to wound sites. To aid recovery, animals will receive additional hydration, electrolyte replacement and wet-mash food for few days following surgical interventions. During such time they will be inspected daily and euthanised in case of any unexpected adverse effects. Most animals will be killed humanely within 12 weeks of starting any procedure; in a few cases we will keep the animals for up to a year. In such cases they will be housed in social groups, under standard housing conditions.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The animals have to be used because it is not yet possible to model complex brain diseases adequately in cells or by using a computer model. This is because our knowledge about the structure and function of the nervous system, and the pathological events in these diseases is not yet sufficiently advanced. Therefore, one of the main aims of this work is to help advance our knowledge of complex psychiatric and age-related brain diseases that will eventually lead to improved <i>non-animal</i> modelling of such conditions.</p> <p>Also, as this project is largely concerned with the effects of drugs on the brains of living organisms, little replacement is possible. For example, one of our aims is to investigate how experimental drugs affect the brain, by measuring changes before and after drug treatment on brain blood flow, which is linked to changes in brain activity. This cannot be replicated in cells in a dish for example, due to a lack of adequate models of brain blood circulation.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>By using neuro-imaging techniques in living animals to measure brain structure or function we can substantially reduce the number of animals needed. This is because these techniques allow continuous (repeated) monitoring and are non-invasive. This means we can follow the same animal over time. This is ideal because we can see what happens to the same animals before and after treatment and control for variation in response between animals. These methods can also be integrated with other approaches such as measurement of brain blood flow, metabolism or the behaviour of an animal. In this way we can acquire different, but complimentary measurements from the same animal.</p>

	<p>Combined, this approach greatly reduces the number of animals needed per experiment.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives.</p> <p>Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The genetics of the rat and the mouse and the anatomy and physiology of their brains are well understood and validated. Our laboratory has extensive experience (>10 years) in the use of rodents in neuroimaging and behavioural experiments. Rodent models of brain diseases, including psychiatric and age-related disorders, which we intend to study, have already been optimised and characterised by multiple laboratories; therefore we will only use valid and proven models for testing new drugs to treat these disorders. For example, we will prioritise the use of rodents that carry identical or similar gene mutations that cause human psychiatric and age-related brain disorders as an ideal model system.</p> <p>Despite the use of multiple measurements we will not cause unnecessary suffering to the animals. The majority of our experiments are performed under anaesthesia, thus it is possible to measure several aspects of brain function simultaneously without causing additional discomfort and suffering. We will only use behavioural tests that are relevant and appropriate to the drug or the model under study. The minimum number of sessions required to produce sufficient data will be conducted, so that each animal experiences only a minimum of required testing.</p> <p>These behavioural assessments will only be undertaken when there is no existing data to demonstrate that a drug may be potentially useful to treat a given brain disorder. The behavioural tests we propose to use are well established and are not of substantial severity.</p> <p>Drugs will only be tested in an appropriate disease model, at an appropriate dose and route of administration, and only when it is predicted that the compound might be have a positive effect.</p>

Project	Preclinical testing of snakebite therapies
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>To protect the health of human patients, this project will use a mouse model to preclinically test the effectiveness and safety of antibody based anti-toxins and other toxin-neutralising therapies.</p> <p>This will help ensure that ineffective or toxic therapeutics are identified before their use in human patients</p>
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit)	The benefits of this project will be the approval for human use of treatments and other toxin-neutralising therapies demonstrated to be effective and safe in a proven animal model.

<p>from the project)?</p>	<p>The project will also establish the initial dose of therapies for human trials and assist product manufacturers meet regulatory requirements. The relevant pharmacopoeias stipulate that the effectiveness of each batch of manufactured product should be preclinically tested in mice before it is used in human patients. Thus, in line with regulatory and public health policy, outputs from our previous project licenses resulted in:</p> <p>1) Provision 37,000 vials of treatments to help save the lives in West Africa. 2) Cessation of manufacture of a poorly efficacious product destined for sSA - before it reached market. 3) Validation of efficacy and stability of products to be used by the NHS and European countries for human treatment. It is important that human victims of toxins receive appropriate treatment: the inappropriate distribution in Ghana, Chad and Central African Republic of untested products resulted in an increase in mortality of treated patients from 0.5% to 12%. This example demonstrates that while the cost to mice of these preclinical tests are high, that benefit to humans is greater. The cost/benefit ratio of preclinical testing is therefore greatly skewed in favour of human health benefit.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We anticipate the need for 1245 mice for 35 assays to test the toxicities of these natural toxins (7/year), and 1945 mice for 55 assays to test the toxin-neutralising efficacies of antibody-based treatments or inhibitory molecules (11/year) over 5 years. We anticipate requiring 120 mice for dose range-finding studies and 180 mice to determine the potential toxicity of the antibodies or inhibitory molecules.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Toxins cause severe cardiovascular and neurological effects in mice. Humane end points will be used throughout for rapid implementation of schedule 1 killing. At the end of the experiment all animals will be killed using a schedule 1 method</p>
<p>Application of the 3Rs</p>	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The mouse model of toxicity has proved a satisfactorily accurate representation of the multiple effects of toxin-induced pathology in humans. There is no <i>in vitro</i> alternative assay yet devised that can accurately predict the (i) toxicity of a venom and (ii) the efficacy of an antivenom or small molecule inhibitor.</p> <p>We continue to actively investigate methods which may provide accurate assessment of therapeutic efficacy <i>in vitro</i>.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The literature and results of previous experiments is closely examined to reduce the range of toxin doses, and therefore the numbers of mice, needed to establish the statistical validity of the assays. To further minimise the numbers of mice required to achieve the objective, we use (i) preliminary range finding studies and (i) dose-staging methods to significantly reduce the numbers of mice required to accurately determine the lethality of the toxin/s and/or potency of the antibody or inhibitor therapy.</p> <p>Statistical analysis is performed on all the results, and the minimum number of mice required for statistical validity is used throughout.</p> <p>During this project we will continue our vigilance to identify methods that show promise in reducing the numbers of mice required for these assays.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the physiologically least advanced rodent species that could be used for the preclinical assays.</p> <p>All the previous preclinical tests on antibody potency have been performed on mice. It would therefore be illogical to change the animal model species. The physiology of mice has been well characterised and the effects of toxin/s can therefore be accurately determined. The consistent use of mouse genetic strains (eg CD1) reduces independent variability and therefore (i) reduces the number of animals required for statistical validity and (ii) increases</p>

the validity of comparing results from different experiments.

To refine protocols, we will:

- Undertake *in vitro* tests to reduce the number of candidate therapies requiring *in vivo* testing
- Undertake *in vitro* tests to reduce the number of candidate therapies requiring *in vivo* testing
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- Undertake *in vitro* tests to reduce the number of candidate therapies requiring *in vivo* testing
- Undertake *in vitro* tests to reduce the number of candidate therapies requiring *in vivo* testing
- Use tests of the shortest possible duration
- Employ human endpoint observations at frequent intervals throughout the experiments
- Maximally implement analgesia
- Use range-finding and dose-staging protocols to reduce the numbers of use required
- Use existing and develop new less-severe humane end points to reduce pain, harm and distress

Project	Predictive models of human neurological disorders	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>We aim to better understand how the brain and other parts of the nervous system are dysregulated in common neurological disorders such as migraine, autism and dementia, in order to develop effective treatments. We will investigate how disease predisposing genes alter the way particular part of the nervous system respond and contribute to disease from a molecular level to the whole organism level.</p> <p>In order to address these current scientific unknowns, we will generate human disease in a dish models using stem cells. Stem cell have already been made from patients with neurological disorders and will be turned into</p>	

	<p>various brain cell types to understand how disease genes affect the functioning of these cells. In parallel we will investigate animal models which have been genetically altered to express neurological disease gene variants comparable with the patients. We will then compare the disease in a dish model with the animal model to validate them as a non-animal alternative for therapeutic drug screening.</p> <p>The disease in a dish models and animals will then be used to better understand disease mechanism (for example which cells and which nerve circuits are critical for disease development) and to develop new therapies that are effective and safe. The anonymised clinical data from patients with autism, dementia and migraine we also have access to, will be correlated with the outcome of experiments on the animal models and stem cell in vitro models.</p> <p>Human conditions are multifactorial and likely involve many different mechanisms. We are investigating genetic alterations that we believe are exemplars of the conditions under study - migraine, autism and dementia. We thereby aim to learn the key principle of disease causation, demonstrate the validity of human cellular models and develop therapies for these conditions that are likely to translate into safe and effective medicines.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>A key primary benefit is the development of better disease models for neurological disorders, the models will lead to a better understanding disease mechanism, provide better models for preclinical drug discovery and eventually lead to newer and more effective treatments. We will work with biotech and Pharma to bring our preclinical discoveries to clinic through licensing the intellectual property arising from this project. Ultimately this will then benefit the millions of people suffering these conditions worldwide. It will also demonstrate the value of stem cell models derived from human patients for investigating disease mechanism and drug discovery and could therefore lead to reduced animal experimentation. Through publication of findings in academic journals, the information is likely to be of interest other researchers with an</p>

	interest in the function of the nervous system in health and disease
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use mice and over 5 years we expect to use 15000 mice
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We expect the level of severity to be mild to moderate. We do not expect genetically altered for these experiments will have severely detrimental phenotypes. If harmful effects are seen, the animals will be killed by an approved method to prevent ongoing suffering. Typically animals will have a genetic alteration that is associated with migraine, autism or dementia. Animals may undergo invasive procedures such as administration of drugs or surgical procedures in order to modify the expression of the gene of interest and understand how this affects disease development. Disease will be assessed by examining the tissue of animals using molecular techniques, assessing nerve circuit function by electrophysiology techniques in brain sections or in the case of migraine, investigating changes in sensory responses. Animals will have analgesia and anaesthetic as required. Complications from such procedures such as infection, swelling or bleeding will be carefully monitored for and appropriate action taken early to minimise suffering. Animals will be routinely examined for their appearance (including weight loss, breathing patterns, coat condition and discharges), posture and behaviour (including abnormal movement, aggression and vocalisation). This will ensure animals are healthy before experiments and suffering is minimised. Animals will be killed at the end of experiments in order to analyse tissue.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The demonstration that abnormalities identified in cultured cells are relevant to a given disease requires investigation in whole organisms. Animals are being replaced in certain aspects such as in a number of areas of drug discovery. This includes work on stem cells cultured from patients with a disease of interest. An aspect of

	<p>this project is to demonstrate that stem cells are a suitable means of replacement and the cellular abnormality is relevant to a given disease. Nevertheless, the full assessment of a disease process and a putative new drug requires animal studies, until projects such ours, can demonstrate that cellular models can have equivalent or superior predictivity to animal models.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>To minimize animal numbers our experiments are always preceded by preliminary studies using cells in culture followed by pilot studies in animals. We will use optimized techniques to reduce experimental error. We will ensure our experiments have sufficient animals to detect an effect and will use the appropriate statistical tests. Where possible we will try to undertake control experiments in the same animal, or test multiple hypotheses in the same experiment using factorial design. We will ensure however that animal suffering is minimized and does not exceed the moderate severity limit. We will also follow best practice standards such as the ARRIVE guidelines which include good experimental design for sufficient statistical power, randomisation and blinding.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are sufficiently close to humans to be of relevance for human neurobiology and neurological disease and genetic alteration is well established. The evaluation of mice through anatomical, behavioural and neurophysiological studies has been refined over several decades.</p> <p>In this project we will undertake a continuous process of refinement of procedures allows the use of more specific and minimal invasive methodologies, use of minimally invasive and least stressful behavioural and physiological assessments. Suffering will also be minimized through appropriate use of analgesics (dosing based upon our prior experience of achieving effective analgesia) when undertaking surgeries which will employ aseptic surgical technique. We will use the most refined route and mode of anaesthesia for the developmental stage of the animal. Analgesics will be applied as a jelly rather than injection where appropriate. Animal</p>

	<p>models of migraine will be refined to minimize stress and suffering whilst ensuring robust outcome measures with changes in sensory thresholds. Refined procedures include: collection of faeces to measure CORT levels instead of typical measurement using invasive blood collection methods; Orofacial sensory assessment device will be carried out to with apparatus using no restraint to the animal. Animals are routinely group housed in ventilated and sound-attenuated light tight chambers (LTCs) in which the light environment can be carefully manipulated. This enables us to collect various physiological and behavioural data without disturbing the animals. Where the light environment is experimentally manipulated within LTCs, light intensity is closely monitored below the levels which would result in retinal damage. Additionally, mouse strains have been considered when exposed to a light pulse as albino mice can only tolerate a maximum of 1400 lux before retinal damage compared to non-albino mice which allow illuminance of up to 2000 lux.</p>
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Project	Prevention of bacterial infection	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The overall aim of work in the project proposal is to address the problem of bacterial infection, which is becoming an increasingly serious threat due to the rise in antibiotic resistance and the emergence of superbugs. We study how bacteria cause disease in humans, and then develop interventions to prevent or treat bacterial infection, usually through designing and testing vaccines. Work under this project addresses several pathogens, including major causes of meningitis, sexually transmitted disease, and dysentery.</p> <p>We will achieve this by undertaking work to identify critical molecules produced by bacteria that allow them to cause disease in humans. These molecules, and other structures on the surface of</p>	

	<p>bacteria can be targets of the immune system during natural infection, and might be successful vaccines. Therefore once we identify potential candidates, we will test them for their ability to induce protective immune responses.</p> <p>Also we do not know why certain people become infected with microbes while others remain perfectly healthy. Our studies will shed light on our genetic make-up which makes us susceptible to infections, in particular the deadly condition, bacterial meningitis.</p> <p>Furthermore there are no vaccines to prevent diarrhoeal disease caused by <i>Shigella</i>. Shigellosis affects over 150 million children per year in impoverished countries where water and good sanitation are limited. Part of the problem is that we have no reliable small animal model that can be used to test vaccines. Therefore we will work on developing novel models of <i>Shigella</i> based on the natural route of infection.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Benefits to humans - Knowledge of what makes bacteria such a threat to human health is important as it can be exploited for making vaccines and drugs. Bacterial vaccines are all based on molecules that are involved in the disease process, such as bacterial toxins and capsules. These vaccines continue to save millions of lives across the world every year. - Vaccines are a highly cost effective medical intervention and have been used improve human health across the world. Vaccines have led to the global elimination of smallpox and virtual eradication of polio; these diseases have been scourges for mankind over many centuries. The implementation of national strategies for immunisation have been highly effective in protecting populations from the threat of infectious diseases. However, as the threat of superbugs rises, we need to make vaccines against more pathogens that threaten human health, such as <i>Neisseria gonorrhoeae</i> and <i>Shigella</i>, which we will study in this project licence. - New treatments and diagnostics are urgently needed to combat the threat of antibiotic resistant pathogens. We live in an era when bacteria are becoming increasingly resistant to antibiotics. <i>Neisseria gonorrhoeae</i> and <i>Shigella</i> spp.. have developed resistance to multiple antibiotics, and we are now faced with</p>

	<p>strains of the gonococcus which are untreatable with conventional antibiotics. Therefore developing new ways of preventing and treating these infections are required, and will be addressed under work in this project. For example, antibodies are a key component of the human immune system that wards off challenges by exposure to microbes. In recent years, antibodies have been exploited for the treatment of human disease, especially cancer and inflammatory conditions. However, antibodies have not been extensively evaluated as part of novel therapeutics against bacterial infection. Antibodies can recognise and specifically bind to the surface of bacteria, making them attractive tools for diagnostics and therapeutics. We will generate a series of antibodies that target the surface of bacteria, and see if we can engineer them to kill the microbes.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Over the 5 year course of the licence, we will use up to 9,000 mice.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The level of severity for the overwhelming majority of animals (>90%) is under this project is expected to be mild. In Protocol 1, most of our work will involve the assessment of immune responses to bacterial antigens. Animals might develop local reactions to the vaccines at the site of injection such as skin sores or a local infection (less than 5%). Very rarely (far less than 1%) mice might have an allergic reaction. These will be monitored and treated appropriately. At the end of experiments, animals will be killed by a schedule 1 method. Serum samples will usually be taken at this time to reduce any distress of taking specimens when the animals are alive. When mice have been given live bacteria (Protocols 2 and 3), they might experience the effects of infection so the severity limit for these Protocols is moderate. We have developed a series of humane end points to minimise any discomfort experienced by animals during these experiments. For infection with <i>N. meningitidis</i> (Protocol 2), mice might develop reduced movement, hunched posture, and reduced feeding. However for most mice (>90% in Protocol 2), we obtain blood samples before they become unwell and use the quantity of bacteria in the bloodstream as our experimental endpoint, rather</p>

	<p>than the development of signs and symptoms. For infection with <i>Shigella</i> (Protocol 3), animals might experience general signs of infection (as for Protocol 2) as well as loose or possibly bloody stools, which would reflect intestinal infection, consistent with human disease. At the end of experiments, animals will be killed by a schedule 1 method. For breeding and maintaining transgenic animals, the severity limits will be mild and moderate for Protocols 4 and 5, respectively. Our current GA mice show no adverse effects and we will not be generating new lines under this licence. We plan to obtain mice from our collaborators who will be able to provide information about the health and welfare of animals.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The mammalian immune system is a complex component of host defence, made up of different cells types in several tissues and organs (lymph nodes, bone marrow, liver, epithelial cells etc.). Therefore we need to use animals to assess immune responses to antigens, and to examine the disease process in infection models.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>Ahead of any experiments, we reduce numbers by discussing experimental design in the laboratory and with technicians trained in animal welfare and care. We have training in medical statistics, and always ask what it the lowest number of animals that we can use and still obtain biologically relevant results.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We have developed humane endpoints for all our protocols, and during the last licence, all animal handling was performed by highly trained technicians employed at our user establishment.</p> <p>For the protocol that involves immunisations of mice (Protocol 1), we have included enpoints which carefully consider and monitor animals for local and general reactions to vaccines. Additionally, we will prepare the immunogens with adjuvants (substances that can help immune responses) which are well tolerated by mice.</p> <p>For the protocol that involves mice receiving live <i>N. meningitidis</i> (a cause of meningitis, Protocol 2), we</p>

will monitor mice to see if they develop signs of infection, and end experiments based on humane endpoints. Our extensive experience in monitoring mice means that we know when infection develops, so we can check animals very closely over this time.

For the protocol that involves developing models of *Shigella* infection (Protocol 3), we will check mice for signs of infection of the intestinal tract (the natural site of shigellosis, e.g. for diarrhoea, bloody faeces), and systemic illness (e.g. significant weight loss, ruffled fur). If mice develop defined criteria we will end experiments to reduce any distress.

For our breeding protocols (Protocols 4 and 5), we will transfer any new transgenic mice from collaborators who will inform us of any adverse effects of genetic modification. This will allow us to take steps to ensure the welfare of mice or decide whether or not proceed with experiments.

We will consult with highly trained NACWOs and Vets to refine our monitoring of mice in all protocols.

Project	Prevention of Post-surgical Adhesions	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Post-surgical adhesions (PSAs) consist of fibrous tissue which sometimes grows excessively and can lead to constriction of the bowel and other internal structures, cause significant pain and even result in female sterility.</p> <p>We will investigate the ability of new procedures, materials and/or devices to affect the formation of PSAs by applying them to pre-clinical models we have used and developed in house.</p> <p>In a systematic review of 87 studies including 110 076 patients the incidence of small-bowel obstruction due to postsurgical adhesions was 9% which is equal to 9906 patients over a period of</p>	

	<p>five years. If these figures are extrapolated to include adhesions at other sites (which have not yet been exposed to systematic review) it is likely that an excess of 10,000 patients per year could benefit from an effective postsurgical adhesion prevention strategy.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Reduction or prevention of post surgical adhesions in both humans and animals will reduce postoperative complications, enable efficient recovery to normal movement, reduce or remove the need to carry out subsequent surgery to remove adhesions and thus improve patient welfare, reduce hospital inpatient time and reduce the financial implications.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>400 mice, 400 rats, 120 rabbits, 180 sheep and 180 pigs.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The models we use create adhesions but we do not let these adhesions become painful to the animals as we treat just after creation to assess reduction or prevention and we know from our culture studies that the treatments we use have good potential to reduce or prevent PSAs so we would regard this licence as moderate in its severity. Some animals will be recovered from surgery and will be monitored for up to 12 months after the initial surgery. This may include repeated anaesthetics for the purposes of biopsy and/or non-invasive imaging. Any animals who show excessive signs of distress will be put down and examined in an attempt to determine the cause and also to assess the affect of the treatment applied to them. At the end of each study the animals will be put down and the tissue taken and examined to assess the efficacy of the treatment, also, where possible, tissue will be taken for other studies and/or educational purposes in an effort to maximise the usage and reduce overall number of animals used.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p>	<p>The formation of adhesions is a complex process involving many different components within the</p>

<p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>body (blood, lymph, enzymes, etc) all interacting and as such a complete live animal is needed to form adhesions for evaluation and subsequent treatment. Prior to live animal studies, procedures, materials or devices to be assessed will, where possible, be tested on cells or tissues in order to keep animal use to a minimum.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The ability to remove organs and tissues under terminal anaesthesia from animals in one study to be used for in vitro or ex vivo studies or transplantation/implantation or to be used for training reduces the need to retrieve these organs or tissues from dedicated donors thus reducing the number of animals required overall.</p> <p>All potential treatments, procedures or devices transitioning from the laboratory into live animal testing will go via pilot studies involving small numbers (typically 3) of animals - this is to be sure that the laboratory prediction is borne out in live tissues.</p> <p>For many of the studies carried out under these protocols, several sites of injury can be induced in the same animal without materially increasing suffering which allows us to reduce the number of animals required to produce scientifically relevant data. Also, the ability to use adjacent or remote tissues from the same animal as internal or autologous controls again allows a reduction in the number of animals required overall.</p> <p>For those studies carried out under Good Laboratory Practice (GLP) compliance, a regulatory process required by the MHRA and the FDA for all pre-clinical studies leading to requests for use in man, statistically robust appropriate information must be derived and this typically requires between 6 and 10 animals per experimental group to satisfy these parameters.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs</p>	<p>The models we use have been evolved over the last 20 years and are as refined as we can currently achieve. We use the smaller species (mice and rats) for initial studies to confirm that the laboratory prediction is borne out in live tissues but often need to use more appropriately sized animals (i.e. a similar size to humans) for many studies. Using a range of assessments including</p>

<p>(harms) to the animals.</p>	<p>non-invasive imaging (e.g. X-ray or Ultrasound) has further refined our techniques allowing us to obtain more information whilst minimising the impact on the animals' welfare.</p> <p>For some direct application treatments the rabbit can be used to assess efficacy however, to establish representative sized defects and relevant treatment doses, large animals are required. Also, for the new procedures, instrumentation is designed for humans and a representatively sized animal will therefore have to be used. There are some areas of anatomy which are specifically recognised within different species as best models – e.g. for meniscal cartilage the sheep is deemed more anatomically similar to humans than is the pig, while for bowel and vasculature the pig is deemed more representative of the human than the sheep. Choices of species will be dependent on the anatomic site under investigation.</p> <p>Appropriate monitoring of animals post-surgery and intervention if necessary with pain relief medication will ensure animal comfort. Our experience is that the animals are not in any pain during these studies probably because most are treated and those that are not are not allowed to progress to the level of adhesion formation where humans would present with symptoms.</p>
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Project	Production and Maintenance of GA Rodents	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>To produce unique strains of genetically altered Laboratory mice as required by various research programmes within the establishment.</p> <p>To remove potentially harmful diseases and organisms through a process called rederivation thus improving their health status</p> <p>To freeze tissues from genetically altered mice for future use.</p> <p>On a temporary short-term basis, to breed and maintain strains of genetically altered Laboratory mice for use within other research programmes within the establishment.</p>	

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This licence improves efficiency by centralising a service and allows a core of highly skilled individuals to provide a service allowing production of new lines/strains of mice without the need for continuous training of new individuals. By utilising a small team of highly trained technicians the procedures involved will be carried out to the highest possible standards. It will allow unique genetically altered strains of mice to be produced and go on to be used in applied human medicine research programmes. It will allow strains to be cryopreserved (deeply frozen) for future use or transportation reducing the number of animals or the need to ship live animals. It will allow new researchers to start work at the establishment without interruption whilst research licence applications are being processed.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Approximately 7100 mice over 5 years. However this is based on the previous experience and assumes that this production licence will be used regularly throughout its lifespan</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Animals under this project will be subjected to the following: Hormone injections Vasectomy surgery Embryo transfer surgery Additionally, embryos (eggs) from Mice will be used to create new genetically altered Mice by either adding DNA or taking it away. To create a new genetically altered type of mouse scientists will either add or take away a specific part of a gene or specific DNA by inserting it into a growing mouse egg either by injecting directly into the egg or using a machine to temporarily dissolve the eggs protective layer to allow the DNA to enter. This egg will then be transferred to a new "mother" mouse and allowed to grow. When the mouse is born it is hoped to have taken the modification and once old enough can be bred so that it passes on the same modification to its offspring allowing researchers to study it and the effects of the missing or added DNA. Regarding the genetic alterations themselves the vast majority of animals are normal and will show no changes in behaviour, have no health implications and show no noticeable detrimental</p>

effects. Much like in human IVF, the mice are given a series of small hormone injections (normally this involves 2 separate injections) designed to supercharge the mouse ovary into producing lots of eggs and ensure they are in the right place to be collected. The mice will suffer only very mild discomfort and return to normal quickly. This will be carried out by experienced staff trained in the procedure. 24hrs after the second injection the mice are humanely killed and any eggs (embryos) carefully collected. Vasectomy surgery (preventing a male from fathering offspring by surgically cutting relevant tubes) is carried out under general anaesthesia using strict sterile techniques. It is a short surgical procedure taking approximately 30-40 minutes. The animals are given medicine to relieve any pain throughout and monitored closely for the duration of the surgery and should return to normal behaviour quickly. They can be mated to produce "pseudo-pregnant" female mice (this convinces the female mice that they are pregnant, and they begin to go through the process as if they were allowing any implanted eggs to hopefully develop normally.) Post-surgical infection is extremely rare but any mouse showing any post-surgical complications or infection will be checked by a vet and either treated or humanely killed. Embryo transfer surgery (transferring a modified egg into a new mouse) is also carried out under general anaesthesia using strict sterile techniques by highly trained staff. It is also a short surgical procedure taking 30-40 minutes. Following any modifications to the eggs as described above, the fertilised embryos (originally from donor female mice) are transplanted through a small cut into the oviducts of pseudo-pregnant female mice (recipients). The animals are given medicine to relieve any pain and monitored very closely throughout the surgery and expected to return to normal behaviour quickly. Post-surgical infection is extremely rare but any mouse showing post-surgical complications or infection will be checked by a vet and either treated or humanely killed. If successful, the mice are allowed to give birth and kept with the offspring until they are old enough to fend for themselves. Unless required for health screening (to check if

	<p>there are any unwanted diseases or pathogens) the recipient mothers are then humanely killed. This Licence also allows mice to be held temporarily for other researchers while they await their own licence to be granted. This allows them to save a little time as their mice can be ready to go and in sufficient numbers when needed.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Breeding and the genetic alterations affect all systems and as such live animals are required. Mice are the most appropriate species for this as 1) the entire mouse genome has been mapped 2) they are relatively easy to manipulate on a genetic level and 3) they can produce in a short space of time, a large number of genetically identical animals.</p> <p>nevertheless, we are constantly reviewing procedures and current literature to look for alternatives.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Minimum numbers will be used by ensuring that any mouse colonies are kept at the lowest possible size to keep them going for as long as required. Careful management of the colony will ensure no excess animals are produced. Where possible the most up to date techniques will be employed to ensure that the maximum number of embryos are gained from the smallest number of mice.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice used in this licence are essentially dictated by needs and requirements of scientific researchers who will request use of the services offered by this licence. Under the previous licence we were able to implement several key refinements (better hormone, refined surgical practice, individual information sheets for each genetically altered mouse line) and hope to continue to look for more during lifetime of this licence. We are also continually gathering information on each line to monitor them for any unexpected effects and take any required action.</p> <p>Impact on the animals' welfare will be minimised</p>

	<p>by the use of experienced and skilled staff, appropriate anaesthesia and pain relief used in all surgical procedures (and other procedures where possible), and a culture of care promoted across the facility. Surgery will be carried out aseptically.</p> <p>The facility is a modern, purpose-built animal unit operated within the guidelines provided by the UK government. All equipment is regularly serviced and maintained. There is a veterinary surgeon on site and available for advice.</p> <p>We are in regular communication with similar animal units to discuss better ways of doing things and pass on what we have learned</p>
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Project	Production and Maintenance of Genetically Altered Mice	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The production of genetically altered (GA) animals to unravel the functional role of genes employs a variety of model organisms amenable to gene manipulation. Incredible advances in technologies over the last 20 years have enabled many subtle and controllable genetic manipulations of the mouse genome. The mouse itself is essential to our research because, as a mammal, it shares many of the developmental milestones and disease states that we, as humans, will experience in our lifetimes. Changes in the genes of	

	<p>the mouse can closely mimic the changes seen in human disease as the mouse has the same basic tissues and organs, and shares much of its physiology with humans. Through this work we will better understand the role of genes in human disease, and allow us to examine gene activity during development in the context of other genes, other cells, other tissues.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>All models that are created are aligned to the goals and aspirations of the other projects this licence will support thus minimising animal usage at this stage of their own projects. The expertise available to us and the application of well-developed strategies ensures high quality mouse models with targeted and, where possible, standardised mutations. We actively share our knowledge and resources with the scientific community to allow them to take advantage of the production processes we have optimised. Through the use of GA mice, the effects of an altered gene can be studied in great detail, and provides us with a window into its biological role and in turn an insight in to its role in disease.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mouse 450,000 animals predominantly for breeding and production of GA mice. The vast majority of these mice (>90%) are expected to remain below the mild severity limit. 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>This is a service licence for our Institute that will generate GA mice. We alter the DNA of the mice by microinjection of genetically modified stem cells or nucleases such as CRISPR that alter the DNA sequence in a targeted manner. This is done in early stage embryos. These embryos are generated via superovulation by intraperitoneal injection of hormones into donor mice. After microinjection embryos are replaced surgically into a pseudopregnant recipient to develop to term. They are made pseudopregnant by mating to a male that has previously been surgically vasectomised. All surgeries use a</p>

	<p>general anaesthesia and analgesics followed by post-operative monitoring from trained and skilled technicians. We breed selected mice born from these embryo transfers, including GA mice to generate cohorts for phenotypic analysis to help understand the effect of the genetic alteration or for use on other project licences at the institute for further in depth analysis. The vast majority of mice will show no adverse effects with less than 10% showing some harmful effects caused by the genetic alteration. The harmful effects caused will be addressed where possible with husbandry and veterinary support. Where this will not help, the mice will be humanely killed. Sperm from each novel genetically altered line will be cryopreserved and shared with other researchers around the world for more detailed study. We may be required to generate a small number of animals, typically one or two litters, for specific tissue analysis. This will determine if the expression of the gene of interest is as expected prior to generating a full-scale colony and potentially wasting animals. We may also recover live mice from cryopreserved material to share with other researchers where they do not have the technical ability to recover the lines themselves from frozen sperm. An in vitro test of each frozen line is carried out to ensure that it is still capable of fertilisation post freezing (IVF). During the course of the project, if at any stage an animal experiences adverse effects that cannot be ameliorated, it will be killed humanely and in a timely manner. All animals that have reached the end of their study will be killed using a humane method of killing.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Extensive <i>ex vivo</i> analysis is integral to all at our Establishment and is always the first option considered when new biological areas of interest are identified. However to study the full effect of a gene mutation it is</p>

	<p>essential to study it in context with all molecular, developmental and physiological interactions provided within a living mammalian system. Due to its similarity and availability of extensive genetic manipulation techniques, the mouse is ideally suited to allow us to study these interactions.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We continuously look at ways to minimise the number of animals used to propagate mutant models. Embryo numbers and recipients are carefully aligned to the number of clones required, while efficiency rates are routinely monitored ensuring the fewest number of animals are required to obtain the required number of new mutant models. Archiving has moved to using sperm as the predominant method of cryopreservation, again reducing the number of animals required to secure a line. These cryopreserved lines are deposited for distribution to the scientific community allowing for a global offset of our production rates by reducing the need to reproduce a mutation at other research institutes. We have calculated that each colony we produce requires 300 mice to reach a stable colony. By distributing and archiving in sustainable archive we have been able to potentially reduce the global production by around 750,000 animals should all colonies be recovered and progressed. The development of new gene editing technology (CRISPR/Cas9) should bring benefit both to the reduction of the numbers of animals used and refinement in the ability to create more bespoke mutations</p> <p>Initial characterisation of tissue may be carried out on some of the lines to ensure the gene of interest is expressing as expected. This would save larger colony cohorts being generated with incorrect gene expression thus wasting animals.</p>
<p>3. Refinement Explain the choice of species</p>	<p>Comparative anatomical, embryological and physiological studies have shown that</p>

<p>and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>mice and humans have the same basic organ systems, skeleton and reproductive cycles. These similarities, coupled with the rapid advances in technologies available to manipulate the mouse genome, make the mouse the most suitable model to mimic human disease condition.</p> <p>Mice are only created if they are required for experimental analysis in line with the programs of the establishment. Careful monitoring and adaptation of our processes has led to refinement of the stages of the processes required for the maintenance and provision for experimental purposes. This has seen us reduce the average cage holding within the facility from 19 to 13 per colony showing elements of reduction driven by refinement. This has been underpinned by the development of software that allows us to have greater oversight on the operation and requirements of large scale production. This is now being made available to other establishments to allow them to also gain such benefits.</p> <p>On-going review of breeding and production data coupled with standardised welfare observations, allow us to further refine procedural, production and breeding protocols both within this licence and in the provision of optimal breeding strategies for other project licences at the establishment.</p> <p>Husbandry and health monitoring of all animals under this licence is performed by a team of highly competent Animal and Scientific Technicians that are assessed under the Institutes competency assessment program. Cleaning regimes are minimised to ensure stress and disturbance to breeding and stock animals is reduced. Environmental enrichment is provided to account for the individual needs of the animals e.g. nestlets for nest making by pregnant or lactating females.. All animals will be group housed where possible.</p> <p>All surgical techniques will look to adopt the principles of aseptic techniques as</p>
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	described in the LASA Guiding Principles for Preparing and Undertaking Aseptic Surgery http://www.lasa.co.uk/pdf/lasa_guiding_principles_aseptic_surgery_2010.2.pdf
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Project	Production of antibodies, antisera and blood products	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	This Project provides for the production of antisera, antibodies and related materials to support the delivery, diagnostics and development of animal and human healthcare nationally and internationally.	
Why is it important to undertake this work?	<p>This project provides a continuing supply of in-vitro diagnostic reagents to clinical laboratories, nationally and internationally and underpins fundamental and applied research for the development and application of new materials in support of human and animal healthcare.</p> <p>Alternative methods for antibody production are in use and employed whenever possible however, there are currently no methods available for the production of</p>	

	<p>specific polyclonal antibodies using non animal alternatives.</p> <p>The production of antibodies and normal sera using animals is required by Companies that manufacture Diagnostic Test Kits for the rapid detection of disease in animals and humans. The key component of these kits being antibodies and antigens specific to the infecting agent, normal sera for the dilution of antibodies and normal sera for the negative control component.</p> <p>Much of the research is aimed at developing techniques to reduce the future need for animals.</p>
What outputs do you think you will see at the end of this project?	This Project provides for the production of antisera, antibodies and related materials to support the delivery, diagnostics and development of animal and human healthcare nationally and internationally.
Who or what will benefit from these outputs, and how?	<p>This project provides a continuing supply of in-vitro diagnostic reagents to clinical laboratories, nationally and internationally and underpins fundamental and applied research for the development and application of new materials in support of human and animal healthcare.</p> <p>Alternative methods for antibody production are in use and employed whenever possible however, there are currently no methods available for the production of specific polyclonal antibodies using non animal alternatives.</p>
Will this work be offered as a service to others?	Yes
How will you look to maximise the outputs of this work?	High quality products provided to clients for further research or analysis projects.
Explain why you are using these types of animals and your choice of life stages.	This project uses domestic livestock species, which for the purpose of this project are defined as those species generally regarded as farm livestock, i.e. cattle, pigs,

	<p>sheep, goats, camelids and poultry. The species of animal selected will be based on antibody specificity, quality, quantity and species specificity for the end product required. These factors are all different according to species of animal and the life stage that they are at.</p>
<p>Typically, what will be done to an animal used in your project?</p>	<p>Animals are immunised by injection with the material to which a response is sought and subsequently blood samples are withdrawn.</p> <p>IgY Polyclonal Antibodies are produced by immunising chickens and collecting the eggs to obtain and purify the IgY.</p>
<p>What are the expected impacts and/or adverse effects for the animals during your project?</p>	<p>The severity limit for this work is mild and no adverse effects are expected. Occasionally a local reaction at the injection site may be seen, which may result in a small swelling and possible temporary increase in body temperature.</p>
<p>What are the expected severities and the proportion of animals in each category (per animal type)?</p>	<p>The severity limit for this work is mild and no adverse effects are expected.</p> <p>This project uses domestic livestock species, which for the purpose of this project are defined as those species generally regarded as farm livestock, i.e. cattle, pigs, sheep, goats, camelids and poultry.</p> <p>Cattle – 200</p> <p>Sheep – 75</p> <p>Pigs – 50</p> <p>Poultry – 100</p> <p>Goats – 30</p> <p>Camelids – 50</p>
<p>What will happen to animals at the end of this project?</p>	<p>kept-alive, rehomed, used-in-other-projects</p>
<p>Why do you need to use</p>	<p>Alternative methods for antibody production are used</p>

animals to achieve the aim of your project?	<p>where available however, there are currently no methods available for the production of specific polyclonal and monoclonal antibodies using non animal alternatives.</p> <p>Much of the research is aimed at developing techniques to reduce the future need for animals.</p>
Which non-animal alternatives did you consider for use in this project?	There are currently no methods available for the specific types of polyclonal antibodies required by our clients.
Why were they not suitable?	There are currently no methods available.
Enter the estimated number of animals of each type used in this project.	<p>fowl: 100</p> <p>pigs: 50</p> <p>goats: 30</p> <p>sheep: 75</p> <p>cattle: 200</p> <p>camelids: 50</p>
How have you estimated the numbers of animals you will use?	This is an established procedure and the minimum number of animals will be used in each experiment to produce the amount of antibody or blood required for use in the tests.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	Effective management and the use of established Standard Operating Procedures minimises the number of animals required to produce antiserum or blood, and the time that any individual animal is on Procedure.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	Tissue and products will be shared where possible to maximise the use of the products.

<p>Which animal models and methods will you use during this project?</p>	<p>All animals used for production of antibodies or blood products are housed in a low stress naturalised environment and animals are maintained as members of a social group. During the study the animals will be closely monitored by well trained, experienced animal staff. Few adverse effects occur and any signs of ill health are immediately investigated and treated.</p>
<p>Why can't you use animals that are less sentient?</p>	<p>Fish and amphibia or less sentient life-stages cannot be used for this project as they do not produce mammalian antibodies. Non-vertebrates also cannot be used as they have a lack of an adaptive immune system.</p> <p>The animals that are to be used in this project need to have</p> <p>For blood collection the products needed are species specific.</p>
<p>How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?</p>	<p>Through regular contact with advisory bodies and effective staff training.</p>
<p>How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?</p>	<p>Periods of observation will be designed to ensure that any effects resulting from any procedure will be detected early. All animals used are under regular veterinary supervision by the Named Veterinary Surgeon. Animals will be trained so that any handling will be less stressful.</p>
<p>What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?</p>	<p>Work will follow published monographs, LASA Guidelines on administration of substances and NC3Rs Guidelines on blood sampling.</p>

Project	Production of Biological Samples	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The success of any regulatory or non-regulatory study is dependant on the use of equipment that can generate datasets accurately; And this is very much reliant on the equipment used being validated and calibrated appropriately. Indeed, this is a fundamental requirement of working to Good Laboratory Practice. The work undertaken within this project licence will allow us to validate the suitability of equipment before it is used on important, scientific studies, calibrate it before use thereby, ensuring it is capable of generating data that is accurate and can be trusted.</p> <p>Another aspect of work that will be served by</p>	

	<p>the performance of studies under this licence is the development of methodologies that can be utilised on in-vitro studies e.g. in-vitro assays.</p> <p>The primary objective of this licence is therefore, to obtain blood and/or urine samples from laboratory animals for the purpose of establishing and/or validating methodologies for use on <i>in-vitro</i> studies, or to validate/calibrate laboratory equipment which in turn will allow future regulatory studies to be performed under Good Laboratory Practice standards.</p> <p>The animals used on these studies will have been acquired from other regulatory safety or efficacy studies, which for a variety of reasons, including studies being terminated early and animals were not used or, the animals were used, but euthanasia was not part of the objectives. On these occasions, providing that the animals are in good clinical health, have been checked by a Named Veterinary Surgeon and the project licence has adequate authority for re-use, these animals may be considered for transfer to this licence.</p> <p>Transferring these animals to this licence has the benefit of reducing the number of animals used. Indeed, it would not be ethical to euthanise animals unnecessarily, only to obtain more animals at a later date for use on studies performed on this licence.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The potential benefits associated with the attainment of the objectives are as follows: 1. The biological samples collected will allow in-vitro studies to be performed and the data generated used to make an assessment of the bio-availability of a drug and an indication of its pharmacological effects before going into animals. 2. The validation and calibration of equipment and the establishment of methodology prior to use on regulatory and non-regulatory studies will allow data to be collected in a Good Laboratory Practice (GLP) compliant manner which is acceptable to international regulators.</p>
<p>What species and approximate</p>	<p>In the majority of cases biological samples will</p>

<p>numbers of animals do you expect to use over what period of time?</p>	<p>be taken from rats and mice. However, there will be occasions when samples will be taken from rabbits and the beagle dog. Numbers will be relatively low and will not exceed 400 mice, 800 rats, 50 rabbits and 100 dogs in a five year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Taking blood from animals using established methods causes transient pain or discomfort only. No adverse effects are expected and the severity is Mild. The volumes taken will be within Internationally accepted Good Practice guidelines for each species and should not interfere with the welfare of the animals. Effectively, no adverse effects should be observed and the expected severity is Mild. On the occasions where large volumes are required i.e. may exceed Good Practice Guidelines then samples will be taken under General Anaesthesia and the animal will not be allowed to recover. Urine samples will be collected from rats and mice by putting them into a purpose built cage (metabolism cage) which allows urine to collect in a glass vessel. Urine samples will be collected from dogs by catheterisation. Both methods will be of Mild severity and will cause transient discomfort only. Once samples have been collected, the animals may be considered for future sampling, or for re-use on future regulatory studies. If however, there is no justification to re-use them or further sampling is not required then they will be humanely euthanised.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The primary purpose of this licence is to obtain biological products i.e. blood and urine to support <i>in-vitro</i> studies which require these products as part of validation, calibration or method development. At this time there are no suitable substitutes for blood or urine that are internationally accepted for these purposes and it is therefore, still a necessity to acquire biological fluids directly from animals.</p>
<p>2. Reduction</p>	<p>In the majority of cases, the quantity of biological product necessary to achieve the</p>

<p>Explain how you will assure the use of minimum numbers of animals</p>	<p>objectives of the study will be known. By using readily available literature on recommended blood sampling volumes and circulatory blood volumes for the selected species it will be a relatively straightforward process in determining how many animals will be required in order to acquire the volumes required.</p> <p>Where necessary, animals will be considered for re-use, thus reducing the numbers of animals used on this licence even further.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Regulatory and non-regulatory studies performed at the establishment typically involve the use of rats, mice, rabbits and where justified, beagle dogs. Where <i>in-vitro</i> studies are needed to support in-vivo studies then it is necessary to use samples from the same species. Samples will be taken using established techniques that have been refined to the extent of causing the animals transient discomfort only. Where objectives allow, samples will be taken under terminal anaesthesia thereby, minimising pain, suffering and distress.</p> <p>The procedures required on this licence are of Mild severity only and will cause only transient levels of pain, suffering and distress to the animals.</p>

Project	Production of Immune Cells and Sera	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Monoclonal antibodies derive from a natural immune defence mechanism whereby foreign proteins, for example on invading bacteria, are specifically identified and neutralised. These properties have led to a wealth of applications in the pharmaceutical industry. The aim of this project is to produce monoclonal antibodies to support drug discovery activities with applications ranging from novel therapeutics, tools for drug target discovery and validation and development of assays.	
What are the potential benefits likely to derive from this project (how science could be advanced or	This project supports a number of drug discovery programmes that are directed at patients with severe disease and high unmet	

humans or animals could benefit from the project)?	medical need.
What species and approximate numbers of animals do you expect to use over what period of time?	The project utilises 3 species, rabbits, rats and mice that have all been shown to elicit good antibody responses in the past. Per annum we expect to use approximately 60 rabbits, 50 rats and 150 mice.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The expected level of severity is mild with transient pain and discomfort associated with the injections used to immunise the animals. Anaesthesia will be used to reduce discomfort where appropriate. At the end of the immunisations animals will be killed and immune cells harvested to screen for cells producing antibodies of interest.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	Where appropriate we will use an in vitro method of antibody discovery. However this method is often poor in finding rare antibodies with the desired functional characteristics. In these circumstances the quality of a natural immune response directed at the target protein is more likely to succeed.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	Where appropriate proteins will be combined to allow immunisation against more than one protein in a single animal. We will instigate any immunisation with low numbers of animals and if unsuccessful try a different approach again in low numbers. This should reduce numbers from a single immunisation strategy directed at a large number of animals.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	All animals have environmental enrichment and our rabbits are socially housed unless there is a specific need not to.

Project	PRODUCTION, BREEDING & CRYOPRESERVATION OF GM MICE	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input checked="" type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The purpose of this licence is to provide research scientists a full transgenic service facility to include the production of new Genetically modified mouse lines, a cryopreservation and rederivation service.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our team has a vast amount of experience in the production, breeding and cryopreservation of new mouse models. A new bioengineering facility was recently set up within the Institute. This facility will offer advice and expertise in the design, testing and assessment of the quality of new Embryonic stem cell lines and constructs prior to their use in the generation of new mouse models. This facility, in combination with our experience and expertise, will benefit many	

	<p>scientists across several disciplines. The areas of research we will produce mice for include stem cell biology, haematopoiesis and haematology, cancer biology infection and inflammation. A central service facility for the production of, and cryopreservation of, genetically modified mice ensures that a minimum number of mice are used by avoiding unnecessary duplication of breeding colonies, stud and vasectomised males and keeps wastage to a minimum.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We estimate that we use we will use in the region of 8000 mice over the 5 year lifespan of this PPL based on our previous experience.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Harmful adverse effects are rare and unpredictable in the production of new GM lines but any animal showing an unwanted harmful phenotype will be killed by a schedule 1 method. Approximately 50% of the total mice used in this project will be superovulated and should not experience more than transient discomfort from 2 injections 48 hours apart. Occasional over aggressive males may cause injury to females. If this occurs those males will be killed by a schedule 1 method. Good aseptic surgical technique will minimise rare complications that arise following surgery. Mice having undergone surgery (vasectomy or embryo transfer) will be monitored closely post operatively and will be administered analgesia. Any mouse failing to fully recover within 24 hours of surgery will be killed by a schedule 1 method. The majority of animals will be transferred to the end users PPL where HO permission has been granted for their use. Those that are not transferred may be bred under the authority of this PPL and will be killed by a schedule 1 method.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The analysis of the effect of a gene at early developmental stages in different tissues and the study of interactions between factors requires a whole animal model. Although Zebrafish and lower vertebrates may be appropriate model systems for studying many developmental processes, a mammalian model still remains necessary in order to fully understand the effects of many human genes and</p>

	<p>their disease-associated mutants and other complex physiological systems that mammals share.</p> <p>The justification for individual experiments will be covered in the end-user's licences. Mouse is the model of choice for genetic modifications modelling human diseases because of the availability and ease of manipulation of mouse ES cells. Where possible, modified embryonic stem cells will be analysed <i>in vitro</i> to determine which constructs require analysis in intact animals.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Breeding colonies to supply the service will maintained at the lowest possible levels and any excess mice will be made available to other users. We will advise users to allow us to cryopreserve lines at the earliest time possible to avoid tick over breeding. Sperm freezing wherever possible will be encouraged over embryo freezing. This will reduce the number of mice required to cryopreserve a line by eliminating the need to superovulate large numbers of female donors and maintain stud males to produce fertilised embryos for freezing. All steps in every process will be carefully monitored to minimise numbers.</p> <p>Experimental procedures will be updated as appropriate and new technologies will be introduced as they develop to minimise mouse numbers.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mouse is the species of choice for genetic modifications modelling human disease because of the availability and ease of manipulation of mouse ES cells.</p> <p>Best practice will be used for all procedures and staff will keep up to date with new methodologies and implement new procedures as they arise.</p> <p>Wherever possible constructs and/or manipulated ES cells will be produced and tested in the bioengineering facility in an <i>in vitro</i> system before going on to produce new GM lines.</p> <p>Analgesia will be used wherever appropriate. Vasectomies will be via the scrotal sac rather than abdomen. Most embryo transfers to date have been carried out using a surgical method. This requires a general anaesthetic and a potential welfare issues which would apply to any animal undergoing a surgical procedure. We will evaluate a non surgical embryo</p>

	<p>transfer method which would only cause a mild momentary discomfort and has the potential to eliminate the need for anaesthetic and any post surgical complications. The recent availability of a transgenic line in which males are sterile in the homozygous state will eliminate the need for future surgical vasectomies.</p>
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Project	Production, maintenance and analysis of genetically altered livestock	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The aims of this project relate to the creation of specific changes to the genes of pigs or sheep. They include experiments to make pigs that are resistant to diseases that blight the commercial pig industry, the creation and analysis of sheep models of human diseases, and refinements to the way we make genetic changes to animals.</p> <p>Diseases of livestock are a huge economic burden on the farming sector, and can result in considerable uncertainty in production systems. One of the diseases we are working on is caused by Porcine Reproductive and</p>	

Respiratory Syndrome Virus (PRRSV). This global disease causes abortion in pregnant pigs, death of newborn piglets and a failure to thrive in older animals. There are currently no effective vaccines or treatments for this disease. It is estimated that in the EU alone PRRSV is responsible for approximately €1.5 billion of production loss every year. A second disease that will be addressed in this application is caused by African Swine Fever Virus (ASFV). This virus causes a disease in pigs that is similar to Ebola in humans, and currently there is no good vaccine or treatment. Until recently this virus was restricted to Africa, but in 2012 there was a case of ASFV in Ukraine, which has since spread throughout the Balkan states and Russia and this year has spread as far west as the Czech Republic. For both of these viruses one must not only consider the financial losses of farmers but also the significant suffering experienced by infected animals.

Animals are often used by scientists as models of human diseases, allowing us to better understand disease processes or to test new treatments. Mice are commonly used for this purpose, but either the biology of mice or their small size can sometimes make them inadequate models. By generating a pig with a genome-editing enzyme in all of its cells, we will enable potential therapeutic drug targets to be validated before the drugs have been made. This could potentially prevent extensive experimentation in other animal models investigating drug targets that do not work. The Cas9 pig will enable us to determine the effect of treatments that target a particular gene, without the expense, and delay, of creating a drug to target that gene or protein.

We are currently investigating the use of sheep as models of two different human diseases. Cystic fibrosis (CF) is a disease associated with repeated chest infections and a significantly shortened lifespan. Mouse models of CF do not develop the lung disorder experienced by humans. A pig model of CF has proven useful for better understanding the human lung disorder, but also develops an intestinal blockage requiring corrective surgery as soon as piglets are born. We think that the genetic

change we are making in sheep will result in a model with lung but not intestinal disease, allowing us to test new therapeutic approaches without the welfare concerns associated with additional surgery. The second model is of Batten disease. This is actually a group of closely related genetic disorders that result in the death of cells in the brain resulting in death of affected patients. Mutation in a gene called PPT1 cause the most severe form of this disease, with affected children dying before puberty. There are good mouse models that have greatly increased our understanding of this disease. However, a larger model with brain structure more similar to humans is now required to investigate the application of therapeutic approaches.

Most biological traits (e.g. height or intelligence) are influenced by many interacting genes. Small differences in the sequence of the genes inherited from each parent contribute to the nature of the offspring. Standard livestock husbandry involves breeding the “best” animals together, but it can often take many generations and therefore many years to reach a desired goal. We previously used genome editors to make precise changes to single genes of pigs and sheep. We now want to try to change several genes at the same time to test how efficiently this process could be used for agricultural improvement.

Our work on disease resistant animals should benefit agriculture (both farmers and animals). Discoveries made under our last project license are already being implemented by an international animal breeding company, and we are progressing those same projects under this license. The main uncertainty at present is not the value of disease resistant animals in agriculture (everybody wants healthy animals) but the way the technology we use to manipulate livestock genomes will be regulated by authorities. As such, we are also working on ways to minimise the amount of genetic change while at the same time maintaining the observed benefits. Our models of human diseases aim to allow pharmaceutical companies or charitable organisations to better evaluate therapies that they have developed in other systems. Both of

	<p>the proposed models of human disease have the potential to overcome limitations of existing animal models and provide a tool to both evaluate therapies and further improve our understanding of these diseases. The ability to manipulate several genes at the same time will initially benefit the scientific community through a better understanding of applications (and limitations) of the molecular tools we use. In the longer term we anticipate that such approaches will become increasingly common as part of commercial livestock husbandry practices.</p> <p>The work within our centre requires delivery of a low technology pipeline for production of gene edited cattle, that can be applied in sub-Saharan Africa. Our current work with gene editors in pigs and sheep involves microinjection of reagents into the single-cell embryo. This is a highly skilled technique and the associated equipment is big, fragile and expensive. We have developed an electroporation protocol using abattoir-derived bovine zygotes which is technically less difficult and utilises cheaper and smaller equipment. The blastocysts that we generate using this procedure appear good. The gene that we have been editing encodes the prolactin receptor, and cattle breeds with natural variants of this gene demonstrate increased thermal tolerance in the event of heat stress. We will now transfer edited blastocysts to recipients to produce calves, thereby validating the pipeline. We will then assess thermal tolerance (also relevant to sub-Saharan Africa) in appropriate individuals by measuring rectal temperature at different times of day and at different times of year.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Our work on disease resistant animals should benefit agriculture (both farmers and animals). Discoveries made under our last project license are already being implemented by an international animal breeding company, and we are progressing those same projects under this license. The main uncertainty at present is not the value of disease resistant animals or environmentally resilient animals in agriculture (everybody wants healthy animals) but the way the technology we use to manipulate livestock genomes will be regulated by authorities. As</p>

	<p>such, we are also working on ways to minimise the amount of genetic change while at the same time maintaining the observed benefits. Our models of human diseases aim to allow pharmaceutical companies or charitable organisations to better evaluate therapies that they have developed in other systems. Both of the proposed models of human disease have the potential to overcome limitations of existing animal models and provide a tool to both evaluate therapies and further improve our understanding of these diseases. The ability to manipulate several genes at the same time will initially benefit the scientific community through a better understanding of applications (and limitations) of the molecular tools we use. In the longer term we anticipate that such approaches will become increasingly common as part of commercial livestock husbandry practices.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The work set out in this project will involve approximately 30 cattle, 330 sheep and 650 pigs over the next 5 years. Our experiments involving PPT1 require approximately 250 sheep. Our CF experiments will involve approximately 80 sheep. For experiments involving disease resistant pigs we will use approximately 350 pigs to supply animals for pathogen challenge studies under other licenses. In order to refine our methods of genetic alteration we will use a further 300 pigs.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Pigs, cattle and sheep are housed on our research farm. Pigs and cattle are housed indoors all year, but most sheep in these studies (with the exception of those involved in disease progression monitoring) will have access to pasture for most of the year. The majority of animals used in this project will be involved only in breeding, with no ill effect anticipated as a result of either the procedure or their genetic alterations. Some of the animals involved in human disease modelling (those carrying 2 copies of the altered gene) are likely to become sick. Because these are new models we do not know how fast disease progression will occur, so will monitor animals closely as they age. Once the first few animals (3-5) reach a pre-defined point (e.g. the first sign of significant</p>

	<p>disease) they will be killed and samples taken for analysis. Subsequent animals will be killed before they reach this point. Sheep will be anaesthetised by single injection of a suitable drug into a vein in the neck. Pigs will first receive an intramuscular injection of a sedative then anaesthetic via an ear vein. Both species cope well with anaesthesia. Sheep recover to standing within 10-15 minutes after cessation of anaesthesia, while pigs return to performing normal behaviours within several hours. Bronchoalveolar lavage of both sheep and pigs will be used to take samples from the lung. Animals will be anaesthetised throughout and it is our experience that they experience no apparent adverse effects from this procedure. For MRI and PET-CT imaging (to look at the brain or lungs) animals will be anaesthetised and remain unconscious throughout the scanning process. A sample of spinal fluid may also be taken at this time; there is a small risk of infection as a result of this procedure. This is avoided by careful use of hygienic methods. In creating new lines of animals some will undergo surgery. For pig embryo recovery donors are sedated then given an anaesthetic overdose to kill them prior to surgery. Recipients are anaesthetised and ovaries exposed through a small surgical incision in the abdomen. Genetically altered embryos are inserted into the uterus, and then the abdominal wall is sutured closed. One risk that all of the above procedures have in common is infection. However, our good practice means that this is very rare. At the end of these procedures some animals will be retained for breeding and some may be moved to other projects, but most will be killed at the end of their use, for example to provide tissues for analysis.</p>
Application of the 3Rs	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Where possible we do use alternatives to animals. For example, we can use computer analysis of the large amounts of genetic information that is now available to identify which genes are involved in eg disease resistance in farm animals. Also, there is a lot of work in cells to refine methods of genetic</p>

	<p>manipulation before they are used to create genetically altered animals. However, to fully understand how an animal responds to an infection there is no alternative to using whole animals.</p> <p>In modelling human diseases, much earlier work has been done in cell culture and in mouse models. However, for both of the diseases outlined in this project the mouse model either fails to replicate the human condition or is not appropriate, due to its size and physiology, for adequate testing of novel therapeutic approaches.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Every new experiment is carefully evaluated by experts in statistics, ethics and animal care and requires official approval before it can proceed. As part of this process we must set out clearly the goals and the experimental design we will apply to answer our questions. This is an ongoing process throughout the project that subjects every experiment to rigorous expert evaluation and ensures the minimum number of animals is used to meet our objectives.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The pig diseases we work on only infect pigs, so “lower” species cannot be substituted. This license is restricted to production, maintenance and breeding of these animals; any infection studies will be carried out on separate license authority.</p> <p>For development of a bovine genome editing pipeline suitable for application in sub-Saharan Africa we have carried out a large amount of work in first cultured cells and subsequently abattoir-derived zygotes. We now need to progress the project by producing live cattle to both validate the novel methodological approach and characterise phenotypic alterations associated with the genetic changes to the prolactin receptor gene. The questions we are asking are specifically related to cattle, and as such must ultimately be answered using cattle.</p> <p>Sheep have been chosen as a model for CF because they are large animals whose lungs are anatomically and physiologically similar to humans. They are amenable to bronchoscopy,</p>

and tolerate repeated interventions in this manner with no discernible clinical effect. Measures of toxicity relating to bronchoscopic interventions show consistency with related work involving human CF patients. Sheep are frequently used animal models and are widely accepted as a key element in the process of developing drugs to combat respiratory disease.

Similarly, sheep have been chosen as a model for Batten disease because they are large animals whose brain architecture is similar to that of humans (while mice are not). Sheep are amenable to handling for regular behavioural and neurological assessment and have good tolerance for the repeated anaesthetic required for consecutive MRI and/or PET-CT imaging over time.

Pigs have been chosen to test methodological improvements in the application of genome editors to livestock because they have multiple offspring in a single litter (typically more than 10). This means that fewer animals are required to undergo surgical procedures to generate statistically significant datasets.

Initial observations are usually of a small number of animals, enabling us to refine subsequent studies with larger numbers. Such a progressive approach allows us to better understand the outcomes of our genetic alterations and identify experimental and humane end points that minimise any suffering experienced by the animals

Project	Promoting tissue regeneration by targeting endogenous stem and progenitor cells	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim of this project is to elucidate the key events in tissue healing to identify novel clinical therapeutics for improving regeneration of skeletal, cardiac, bone and liver by targeting stem and progenitor cells already present in the body. Adult stem cells are an essential component of tissue homeostasis and have been shown to play indispensable tissue renewal and repair following injury. The greatest success in harnessing the regenerative potential of stem cells thus far has been for haematological disorders, although further improvements in engraftment would lead significant clinical benefit. A similar approach	

	<p>based on administration of exogenous stem and progenitor cells has not yielded meaningful improvements for solid organs. Even if strategies that rely on ex vivo expansion of the own individual patient stem cells were to be shown to be effective, this approach would be prohibitively expensive and unlikely to become widely available in the near future. Targeting the body's own stem cells overcomes the many hurdles associated with therapies reliant on the administration of cells grown up outside the body and should be more cost effective.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>By better understand how the body's own stem cells repair damaged tissues and organs, we will alleviate the suffering of patients affected by a myriad of conditions, including trauma, skeletal muscle disorders, liver disorders such as cirrhosis and myocardial infarction. At the moment, there is no approved therapeutics to promote regeneration of any of these.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use mice, no more than 26000 over a 5 year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The protocols in this PPL cover tissue regeneration studies in general but the purpose of each protocol is different since each protocol covers one organ (bone, muscle, cardiac, liver) and the likely/expected severity is different depending on the protocol. The majority of our protocols are of moderate severity. The liver and cardiac protocols are severe and we will ensure the welfare of all animals by monitoring them closely and addressing any issues promptly. Following cardiac surgery, we will monitor regularly in the 2-3 hours post-surgery, then at least three times daily for the first week and as twice daily thereafter. For the liver models the animals are expected to show weight loss but not any other visible adverse effects, as confirmed by experience in other centres that are using these models. The mice will be monitored closely and we will use a scoring system to record potential adverse effects. We minimise the suffering by adhering to LASA guidelines throughout, surgery is performed under aseptic conditions, analgesia is administered before painful procedures and</p>

	<p>they are checked regularly afterwards, with further analgesia administered as necessary. We liaise closely with the NAWCO/vets when necessary, and work with our collaborators, who are experts in the field, to obtain training in procedures that are not yet full-established in our lab. Welfare costs are minimised by using power calculations (statistical methodology) to limit the number of animals in each group. Animals will be killed at the end of each experiment by appropriate methods and further analysis will be done to achieve the highest level of information possible from each animal.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p><i>In vitro</i> work will be used to test hypothesis prior to <i>in vivo</i> and therefore reduce the number of animals required in subsequent <i>in vivo</i> experiments. <i>Ex vivo</i> assays will further analyse the <i>in vivo</i> findings. We will rely on extensive <i>in silico</i> modelling as well as <i>in vitro</i> assays using murine and human cell lines before proceeding to <i>in vivo</i> mouse models.</p> <p>Bone repair, haematopoiesis, skeletal muscle, cardiac and liver regeneration can only be fully followed in animal models. The healing of these tissues involves complex interactions between numerous cell types and cytokines, many of which have not been fully identified yet, so studying the regeneration of these tissues is not reproducible <i>in vitro</i>.</p> <p>The mouse genome is well-studied and more reagents e.g. antibodies and genetic knockouts are available for biological investigation than for any other laboratory animal, including rats.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Efficient colony management ensures that only colonies that are actively being used are allowed to mate and produce offspring. Those that are no longer required are cryopreserved and breeding and maintenance discontinued at the earliest opportunity. We manage our active colonies efficiently. Before commencing a new study, we draw up an experimental protocol, covering our objectives/hypothesis, an experimental outline, detailing experimental treatments, group sizes,</p>

	<p>the experimental material to be collected and a detailed method of analysis. We routinely use good laboratory practice aimed at reducing bias, such as randomisation of treatment and blinded assessment of outcomes. The number of animals will be minimised by performing multiple assessments such as non-invasive <i>in vivo</i> scanning on the same animal whenever possible and by using the maximum relevant tissues from each animal. We derive maximal information from any experimental sample.</p> <p>All group members have received training in statistical methods and we have consulted statisticians on aspects of our experimental design. Where appropriate, power calculations are performed prior to the start of an experiment to establish the required group size. In most cases, a significance level of 5% and 80% power will be used. In a typical study, consisting of more than 2 experimental groups, multiple comparisons will be made using ANOVA and eventually multiple t-test comparison.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse genome is well-studied and more reagents e.g. antibodies and genetic knockouts are available for biological investigation than for any other laboratory animal, including rats. The mouse is a relevant species for tissue regeneration/translational research. The main healing events are similar to those seen in humans.</p> <p>Our different regeneration models are well established, and they have been refined to the highest standard. We continuously look for further potential options of refinement. Furthermore, either our group is already proficient on them or we have established the necessary collaborations for the expertise knowledge and training required for the best refinement models.</p> <p>We refine the procedures by adhering to LASA guidelines throughout. Surgery will be performed under aseptic conditions, analgesia administered before painful procedures and animals will be checked regularly afterwards, with further analgesia administered as required. We liaise closely with the NAWCO/vets when necessary, and work with our collaborators, who are experts</p>

	<p>in the field, to obtain training in procedures that are not yet full-established in our lab. Animal numbers are minimised by using power calculations (statistical methodology) to limit the number of animals in each group. Animals will be killed at the end of each experiment by appropriate methods and further analysis will be done to obtain the highest level of information possible from each animal. We have already established a fracture model using a plastic bridging bar that allows serial CT scanning of each animal over time, thereby dramatically reducing animal numbers. The animals are able to ambulate freely after surgery. Our skeletal muscle injury model selectively causes the death of the muscle fibres, with minimal associated inflammation. The animals are able to weight bear immediately following recovery from anaesthesia and do not exhibit any signs of pain after 12 hours.</p>
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Project	Promoting tissue regeneration by targeting endogenous stem and progenitor cells	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim of this project is to elucidate the key events in tissue healing to identify novel clinical therapeutics for improving regeneration of skeletal, cardiac, bone and liver by targeting stem and progenitor cells already present in the body. Adult stem cells are an essential component of tissue homeostasis and have been shown to play indispensable tissue renewal and repair following injury. The greatest success in harnessing the regenerative potential of stem cells thus far has been for haematological disorders, although further improvements in engraftment would lead significant clinical benefit. A similar approach	

	<p>based on administration of exogenous stem and progenitor cells has not yielded meaningful improvements for solid organs. Even if strategies that rely on ex vivo expansion of the own individual patient stem cells were to be shown to be effective, this approach would be prohibitively expensive and unlikely to become widely available in the near future. Targeting the body's own stem cells overcomes the many hurdles associated with therapies reliant on the administration of cells grown up outside the body and should be more cost effective.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>By better understand how the body's own stem cells repair damaged tissues and organs, we will alleviate the suffering of patients affected by a myriad of conditions, including trauma, skeletal muscle disorders, liver disorders such as cirrhosis and myocardial infarction. At the moment, there is no approved therapeutics to promote regeneration of any of these.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use mice, no more than 26000 over a 5 year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The protocols in this PPL cover tissue regeneration studies in general but the purpose of each protocol is different since each protocol covers one organ (bone, muscle, cardiac, liver) and the likely/expected severity is different depending on the protocol. The majority of our protocols are of moderate severity. The liver and cardiac protocols are severe and we will ensure the welfare of all animals by monitoring them closely and addressing any issues promptly. Following cardiac surgery, we will monitor regularly in the 2-3 hours post-surgery, then at least three times daily for the first week and as twice daily thereafter. For the liver models the animals are expected to show weight loss but not any other visible adverse effects, as confirmed by experience in other centres that are using these models. The mice will be monitored closely and we will use a scoring system to record potential adverse effects. We minimise the suffering by adhering to LASA guidelines throughout, surgery is performed under aseptic conditions, analgesia is administered before painful procedures and</p>

	<p>they are checked regularly afterwards, with further analgesia administered as necessary. We liaise closely with the NAWCO/vets when necessary, and work with our collaborators, who are experts in the field, to obtain training in procedures that are not yet full-established in our lab. Welfare costs are minimised by using power calculations (statistical methodology) to limit the number of animals in each group. Animals will be killed at the end of each experiment by appropriate methods and further analysis will be done to achieve the highest level of information possible from each animal.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p><i>In vitro</i> work will be used to test hypothesis prior to <i>in vivo</i> and therefore reduce the number of animals required in subsequent <i>in vivo</i> experiments. <i>Ex vivo</i> assays will further analyse the <i>in vivo</i> findings. We will rely on extensive <i>in silico</i> modelling as well as <i>in vitro</i> assays using murine and human cell lines before proceeding to <i>in vivo</i> mouse models.</p> <p>Bone repair, haematopoiesis, skeletal muscle, cardiac and liver regeneration can only be fully followed in animal models. The healing of these tissues involves complex interactions between numerous cell types and cytokines, many of which have not been fully identified yet, so studying the regeneration of these tissues is not reproducible <i>in vitro</i>.</p> <p>The mouse genome is well-studied and more reagents e.g. antibodies and genetic knockouts are available for biological investigation than for any other laboratory animal, including rats.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Efficient colony management ensures that only colonies that are actively being used are allowed to mate and produce offspring. Those that are no longer required are cryopreserved and breeding and maintenance discontinued at the earliest opportunity. We manage our active colonies efficiently. Before commencing a new study, we draw up an experimental protocol, covering our objectives/hypothesis, an experimental outline, detailing experimental treatments, group sizes,</p>

	<p>the experimental material to be collected and a detailed method of analysis. We routinely use good laboratory practice aimed at reducing bias, such as randomisation of treatment and blinded assessment of outcomes. The number of animals will be minimised by performing multiple assessments such as non-invasive <i>in vivo</i> scanning on the same animal whenever possible and by using the maximum relevant tissues from each animal. We derive maximal information from any experimental sample.</p> <p>All group members have received training in statistical methods and we have consulted statisticians on aspects of our experimental design. Where appropriate, power calculations are performed prior to the start of an experiment to establish the required group size. In most cases, a significance level of 5% and 80% power will be used. In a typical study, consisting of more than 2 experimental groups, multiple comparisons will be made using ANOVA and eventually multiple t-test comparison.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse genome is well-studied and more reagents e.g. antibodies and genetic knockouts are available for biological investigation than for any other laboratory animal, including rats. The mouse is a relevant species for tissue regeneration/translational research. The main healing events are similar to those seen in humans.</p> <p>Our different regeneration models are well established, and they have been refined to the highest standard. We continuously look for further potential options of refinement. Furthermore, either our group is already proficient on them or we have established the necessary collaborations for the expertise knowledge and training required for the best refinement models.</p> <p>We refine the procedures by adhering to LASA guidelines throughout. Surgery will be performed under aseptic conditions, analgesia administered before painful procedures and animals will be checked regularly afterwards, with further analgesia administered as required. We liaise closely with the NAWCO/vets when necessary, and work with our collaborators, who are experts</p>

	<p>in the field, to obtain training in procedures that are not yet full-established in our lab. Animal numbers are minimised by using power calculations (statistical methodology) to limit the number of animals in each group. Animals will be killed at the end of each experiment by appropriate methods and further analysis will be done to obtain the highest level of information possible from each animal. We have already established a fracture model using a plastic bridging bar that allows serial CT scanning of each animal over time, thereby dramatically reducing animal numbers. The animals are able to ambulate freely after surgery. Our skeletal muscle injury model selectively causes the death of the muscle fibres, with minimal associated inflammation. The animals are able to weight bear immediately following recovery from anaesthesia and do not exhibit any signs of pain after 12 hours.</p>
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Project	Pro-oncogenic tissue repair and regeneration mechanisms driven by tumour cell death	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	(1) To understand how tumour cells (which carry the genetic mutations of cancer), especially dying tumour cells, interact with the normal host cells that are always found in cancer tissue; (2) to determine how these interactions lead to cancer progression; (3) to identify potential diagnostic or therapeutic targets.	
What are the potential benefits likely to derive from this project (how science could be advanced or	The research will provide much-needed information about our body's internal environmental conditions which are critical for	

humans or animals could benefit from the project)?	the growth of aggressive malignant tumours. This knowledge will help improve prospects for early diagnosis, cause, outcome and treatment of cancer. This will be of importance both to human and to animal healthcare.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 3000 mice, 4000 zebrafish and 20 rats over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Normal and genetically altered animals will be bred to study the mechanisms of tumour formation through transplantation of tumour cells from the laboratory to the animal or through spontaneous tumour formation. Because tumours will not be allowed to grow to a large size, adverse effects are expected to be absent or minimal (some weight loss, unkempt or greasy coat, pale or cold extremities). Animals will be sacrificed humanely at the end. Harms to the animal will be mainly through injections which will only cause transient and mild discomfort. Rarely there may be ulceration of tumour or swelling or irritation of an injection site.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The tissue environment of tumours is complex and it is not possible to study it without using animals. It cannot be recapitulated in the laboratory by cell or organ culture, although certain aspects of the tumour environment can be studied using simplified cell culture models, such as co-culture of tumour cells with white blood cells <i>in vitro</i> . We will also use fruit fly models where possible to avoid vertebrate animal use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This research group is highly experienced in the experimental models that will be used. Sample sizes will be minimized through rigorous experimental design and statistical principles. Reduction in animal numbers will also be achieved where possible via 'pre-screening' protocols – such as exposure of tumour cells to a specific reagent or cell – so that candidate

	<p>mechanisms can be identified in the laboratory through simplified cell culture and fruit fly approaches.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the mammals of choice for studies of cancer biology because of the number of tools available (such as important genetic variants of laboratory strains) and because of the established tumour models. These models are highly relevant to our work because they show the cellularity and tissue architecture of their human counterparts and so are very relevant for improving healthcare, ultimately. Rats will only be used as appropriate to produce antibodies against mouse molecules where raising antibodies in mice is not possible (antibodies will be used to test the targeting of specific molecules which may constitute future therapeutic targets). Because of their small size and optical transparency, zebrafish provide excellent genetic models which are particularly suitable for real-time cell imaging studies, especially of the early stages of cancer development. Fruit flies are similarly useful. However, mammals such as mice also need to be used since flies and fish are too far removed from humans to provide a comprehensive animal model for mechanisms relevant to human cancer. Furthermore, many tools (such as reagents for phenotyping of cells) are immediately and readily available for use in mouse models and these are not available for zebrafish systems. All protocols are well-established and known to produce mild or moderate adverse effects and personnel involved with the animal work have substantial expertise in carrying out the specified protocols and in observing (and responding appropriately to) adverse effects. Strict humane endpoints will be applied throughout.</p>

Project	Protein kinases and phosphatases in health and disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Parkinson's disease and Huntingdon's disease are serious conditions, causing progressive loss of brain function and muscle control. The underlying causes of the most common form of Parkinson's disease are unknown, but we are beginning to understand the genetic basis of the rarer inherited forms. About 20 clear-cut genes have been identified in which mutations are associated with the death of defined sets of nerve cells (neurons) and the symptoms associated with Parkinson's disease. This suggests that the products of these genes are involved in similar or the same molecular	

	<p>mechanisms as operate in the more common sporadic form of the disease. Our research involves making defined changes in the equivalent genes in mice, investigating what the molecular consequences are and determining how they may cooperate in causing signs of disease. We are already studying two genes, called LRRK2 and alpha-synuclein, each of which has been implicated in Parkinson's disease. Specific inhibitors of LRRK2 may prove to be new medicines for the disease, but it is still unclear how LRRK2 interacts with alpha-synuclein or with the products of any of the other genes implicated in the disease.</p> <p>The genetic basis of Huntington's disease is known, but the molecular events that lead to symptoms are not well-characterised and there are as yet no effective medicines. We have recently identified a family of enzymes called MINDY, whose activities might be beneficial in the treatment of Huntington's disease (and, indeed, Parkinson's disease). We wish to characterise these enzymes in detail in mice, to determine whether they would indeed be useful targets for new medicines.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Our basic research is aimed at understanding the molecular mechanisms that underlie the initiation and development of serious neurological disorders such as Parkinson's disease and Huntington's disease. We anticipate that this work may lead to the identification of new targets or new medicines for these devastating disorders.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to breed and maintain a number of mouse lines in which specific genes have been altered. We expect to use up to 18,000 mice over five years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In our experience, mouse models of these human diseases exhibit very few signs of adverse welfare, though tissues analysed after the animals have been humanely killed demonstrate informative molecular changes. We do not require animals, if they do show any signs, to progress beyond early signs of disease. Animals may be administered chemicals that are believed</p>

	<p>to be potential new medicines, in order to measure their effects on the molecular mechanisms and on the development of any outward signs of disease. These chemicals are not, in themselves, expected to cause any harm. We will conduct some tests of animals' memory and motor skills, but these too are not expected to cause harm.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>In order to define the role Parkinson's and Huntington's genes have in the biology of the different types of regions and neuronal cells brain tissues, a mouse model is needed. Also, to establish whether treatment with LRRK2 inhibitors will delay early deficits seen in mouse models of Parkinson's disease is also best addressed in a mouse model. The genes suspected of involvement in the development and progression of Parkinson's disease and Huntington's disease may have important activities in several distinct areas of the brain (and, in fact, in other organs of the body too). Their actions therefore cannot yet be modelled sufficiently well without using intact animals. We will also culture primary neurons from mice and make use of brain tissue to undertake in vitro studies of the signalling pathways. In parallel we are devoting significant effort into in vitro investigation of the genes we are studying in mice. The knowledge gained from both the in vitro and in vivo work is expected to provide new fundamental novel information on how genetic manipulations that mimic disease causing mutations influence signalling systems and biology in neurons and the brain. This would be expected to lead to follow up studies that could be undertaken in an in vitro system rather than in animal models.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will manage our breeding colonies so as to produce the right amount of tissues for laboratory analysis. When we are testing novel chemicals for their potential as medicines, we shall use statistical power calculations in order to design experiments that will give robust answers while not wasting animals. We will undertake power calculations to ensure that we use the lowest</p>

	<p>number of mice to get scientifically rigorous results. We have recently developed novel very sensitive and specific monoclonal antibodies that allow us to assess LRRK2 signalling pathway activity with unprecedented accuracy (manuscript in submission). These new reagents and assays will significantly increase the robustness of our assay and accuracy that we can monitor in vivo LRRK2 activity. This should help reduce the number of mice needed assess impact of mutations on LRRK2 pathway activity.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The ease with which specific genetic alterations can be introduced into mice, and the molecular similarities between their nervous systems and our own make them the ideal species in which to investigate the basic mechanisms of neurological disease. Our studies will not require animals to exhibit signs of advanced disease, indeed the majority of the animals will live apparently normal lives and will be killed humanely before tissues are harvested for detailed laboratory studies. All mice will be very carefully monitored to minimise welfare costs including monitoring signs of reduced weight loss, neglect of grooming, reduced ambulation, early signs of movement impairment and resistance to passive movements.</p>

Project	Protein misfolding diseases: pathogenesis and intervention	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The aim of this work is identification of strategies to ameliorate human diseases that arise when protein quality control fails, such as neurodegenerative diseases, diabetes and cancer.</p> <p>We have identified strategies that boost natural cellular defence mechanisms to fight protein misfolding, and demonstrated their efficacy in mouse models. We have evidence that our approaches may be beneficial in a broad range of diseases. We will test this exciting possibility in diverse models of degenerative diseases. The</p>	

	therapeutic potential is very high.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefit from the work described here is to provide further evidence that modifying the natural cellular defence systems against misfolded proteins can slow down human diseases in order to identify pathways that can be exploited therapeutically.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice are required as they provide good models of human diseases. I would expect that ~ 25 000 mice may be required.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mice will be maintained in social environment and environmental enrichments will be provided to improve the wellbeing of mice. Mice for which the disease progression will be monitored will be kept until they develop the disease symptoms like weight loss or hindlimb paralysis but will be culled the latest at this stage. Some mice will be administered with pharmacological substances which might cause a intermittent distress but no lasting adverse effects are expected. In most cases substances will be administered by oral route. In most cases behavioural tests that will be applied to mice are non-invasive and are not expected to cause any lasting distress or harm. On rare occasions mice might be subjected to a more invasive test like 'Morris water maze' in which mice will need to swim in water for one minute or 'hot plate' in which mice will be placed on a plate at the max. temp. 55C until they lick their paws or escape. Including these tests is essential to determine the efficacy of treatment in the disease prevention and is required to validate a tested compound as a potential future therapeutic. At the end of the procedure mice will be humanely killed and pathology as well as drug efficacy will be monitored by postmortem tissue analysis. The genetic modification of some mice might led to sudden unexpected deaths in a limited number (5%) of mice that are not preceded by any prior disease symptoms, therefore not possible to predict. In the past years we developed refined protocols and monitoring procedures that enabled us to decrease the incidence of mice found dead suddenly and unexpectedly. We continue to search for further refinements. Nevertheless, we

	are unable to predict and prevent unexpected deaths completely.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We have discovered novel, powerful and straightforward approaches to rescue from failure of protein quality control. We have a group of pharmacological modifiers with high potential to ameliorate protein misfolding diseases.</p> <p>Prior to being used in humans, there is a legal requirement for virtually all potential disease modifiers to be tested in animal models for the disease(s) in question. The mouse is best suited for this work since, of all existing models, mouse models are highly relevant to the human diseases.</p> <p>We have done as much as possible, and will continue in the future to carry out pilot experiments in cell lines or in ex vivo cultures. Whilst they will provide some useful information, cultured cells cannot fully replace mouse models. They do not provide physiological conditions nor the complex interactions amongst different cell types or different tissues. They also don't recapitulate the drug metabolism.</p> <p>The work in mice we propose to carry out is essential to validate our discoveries and may have a big impact on human health.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Mouse breeding will be carefully monitored to ensure that surplus animals are not generated.</p> <p>We will use the minimum number of animals necessary to give a statistically significant result. The number of mice needed will be evaluated based on the previous studies or power analysis. Statistician will be consulted.</p> <p>To reduce the sources of variability and bias mice will be randomly assigned to experimental groups. Experiments will be run in a blind fashion.</p> <p>Cryopreservation will be used to preserve important lines and remove the necessity to hold</p>

	stock for extended periods.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Existing animal models of common neurodegenerative diseases exhibit the essential features of the human diseases, a necessary prerequisite for the identification and evaluation of disease modifiers. As such, they are true models of the molecular and cellular features of the human diseases. At present, no valid alternative model exist.</p> <p>Signs of disease and the adverse effects will be limited to the minimum required for a valid scientific outcome and in all cases the general health and condition of an animal will remain the overriding determinant. Only those mice needed for the experiments will be kept until they develop the disease symptoms. Otherwise only young mice will be kept.</p> <p>All animal experimentation will comply with the local establishment guidelines. This document has been adopted by the local Ethical Review Process in order to inform researchers of the bounds within which their animal work should be conducted as well as to provide practical recommendations on various aspects of animal experimentation.</p>

Project	Provision of an outsourced drug development platform for the treatment of bleeding disorders	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	When a blood vessel is damaged twenty specialist proteins in the blood (clotting factors) combine with cells in the blood (platelets), which makes the blood sticky and bleeding eventually stops (haemostasis). This process is complex and tightly regulated, but if this regulation is abnormal then unwanted blood clots (thrombosis) can occur inside arteries and veins, leading to a clinical illness, such as stroke or deep vein thrombosis, or unwanted bleeding can occur, as in patients with haemophilia. The development of drugs to treat thrombosis and	

haemophilia forms the basis of this project licence.

The most common medicines prescribed for unwanted clotting of the blood are anticoagulants, sometimes referred to as 'blood thinners'. Warfarin is still one of the most widely prescribed anticoagulants in the NHS. However, the effects of warfarin have to be carefully monitored. If levels are too low then the beneficial effects are not seen and if they are too high then the anti clotting properties put the patient at risk of bleeding. Newer anticoagulants have been approved, but their use is still associated with an increased risk of bleeding, and bleeding complications can be life threatening. A bleeding tendency caused by anticoagulants is not a side effect but the main effect of the drug, and the leading question in the development of anticoagulant drugs has been 'Is it possible to make a potent anticoagulant without a bleeding risk?' Testing this hypothesis with new drugs that are being developed is one of the aims of this new project licence.

About 6,000 people in the UK have haemophilia. They don't have as many clotting factors, so their blood takes longer to clot. As a consequence they may have nosebleeds that take a long time to stop; wounds that bleed for a long time; skin that bruises easily; or pain or stiffness in the joints from internal bleeding, which can cause loss of mobility. Haemophiliacs are injected with medicines that replace the appropriate missing clotting factor. The biggest disadvantage with these medicines is that some people develop antibodies in their immune system, called inhibitors, which make the medicine less effective. These patients then need to take further medicine to overcome this, but these drugs are not very effective. Inhibiting the production of our bodies' own natural anticoagulants that normally act as brakes to limit clotting is an alternative approach we are investigating. Gene replacement therapy is another. This is an experimental technique where genetic material (DNA) is introduced in to a patients cell to compensate for an abnormal gene or to make a beneficial protein, such as

	missing clotting factors.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The short-term benefit will be to further our understanding of the blood coagulation process and whether our approaches to developing new medicines are likely to work when tested in humans. In the long term, successful drugs will offer distinct advantages over current medicines. Blood clots that form in arteries are the most common cause of strokes, and strokes can lead to severe disability or death. It is expected that by 2050, 16 million people will be affected in this way and will cost the NHS £2.2 billion per year. Severe disability or death, due to blood clots that form in one or more deep veins, also affects approximately 1 in 1000 adults each year, especially as they age. Deep vein thrombosis is currently considered the commonest avoidable cause of hospital death. The diagnosis and management of abnormal blood clotting is therefore very important. About 6,000 people in the UK have haemophilia. There is no cure for haemophilia. The current injectable artificial factor medicines have their limitations, including high cost and restricted availability. But the biggest disadvantage is the development of inhibitors, which poses special challenges. The healthcare costs associated with inhibitors can be staggering because of the amount and type of treatment product required to stop bleeding. Haemophiliacs who develop an inhibitor are twice as likely to be hospitalised for a bleeding complication, and are at increased risk of death. Developing a drug that doesn't lead to the development of inhibitors, will offer advantages over current medicines. A drug that is cheap to produce will especially benefit the majority of haemophiliacs who currently have no access to effective therapy and have a life expectancy of only 10 years. Research into gene therapies for haemophilia has the potential to offer a 'one off' treatment by enabling the patient to generate their own missing clotting factors, rather than being given multiple injections of these factors. New medicines that go on to be tested in clinical trials, will guide the improvement of animal models (that are comparable to the human disease) these medicines have been tested in, and justify their value as experimental tools.</p>

<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>All our animal studies use the lowest species possible to answer the scientific question being asked (predominantly rats (3650, which includes 550 genetically altered animals bred in house that are models of haemophilia) and mice (4150, which includes 2250 genetically altered animals that are models of haemophilia), but for some targets we may need to use rabbits (210).</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In many cases research is carried out in animals that are anaesthetised, and from which they won't recover. Under these conditions, we measure blood loss, and the time to stop bleeding from a cut surface, of the tail in rats and mice, or the ear vein in rabbits (this mimics that seen in humans where cuts are made in the skin, and how quickly bleeding stops is timed). To induce blood clots we expose blood vessels in anaesthetised animals and cause damage to a vein or artery by adding a chemical or disturbing it with a focused laser beam. The resultant clot can then be either, looked at under a microscope and measured, or it can be removed and weighed. Giving a drug before injury can test its effect on bleeding and clot formation. In some cases drugs are given to the animal whilst it is awake and small blood samples taken to measure levels of drug in the blood, but these procedures are not expected to cause anything other than minimal pain or distress. Mice and rats with a genetic bleeding disorder, such as those that have clotting Factor VIII missing, are a model of human haemophilia A. As in humans, these animals are likely to experience spontaneous bleeding episodes, that may be painful and these animals are not expected to live as long as normal rodents. The liver makes clotting factors, such as FVIII or FIX, so when measuring how well a novel therapy, such as gene replacement therapy, is likely to work, we may need to compare different routes of administration. This could include giving it directly into the vein that runs into the liver (called the hepatic portal vein), through a surgical procedure carried out under anaesthesia with recovery. If this is successful, then an alternative method to deliver the vector to the liver, we may explore, is by injection into the spleen. The spleen is an organ that lies</p>

	<p>close to the liver and its vein drains blood into the hepatic portal vein. Therefore any vector that is injected into the spleen will end up in the liver, but to prevent the gene becoming incorporated into the spleen on its way through, it may be necessary to remove the spleen. Another method of gene replacement therapy that is emerging as being of clinical importance is to inject the genetic material in a large volume at high speed, called hydrodynamic delivery (HD), which maximises delivery of the gene to the tissues. In mice HD of genetic material, injected via the tail vein to reach the liver, causes the heart to become over filled and the liver to expand but both return to normal. Animals may lie flat immediately following the injection but they quickly become upright again and move about. After this time they may become subdued but responsive to stimuli for about an hour before their usual behaviour returns. To minimise any pain animals are likely to experience we will treat them with pain killers or to kill them by a humane method, if they show signs of distress that do not get better after a short period of time. At the end of any study all animals are humanely euthanised.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Blood coagulation is an incredibly complex process that is under tight regulatory control. Blood flow plays a significant part in the coagulation process, therefore to understand haemostasis fully, and it normally functions in relation to human bleeding disorders, it is important to use the whole animal.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>As part of our research, we use a statistical approach to calculate the minimum number of animals needed for our experiments. We also use appropriate statistical tests to compare the results we get between different treatment groups. Specialist scientists, who are experts in the field of statistics are available to help us with our approaches. In this way we can be confident that any effect of a drug we see is a real effect and did not happen by chance. This means we may not have to repeat experiments.</p>

	<p>Pilot studies that are used to refine procedures and to discover potential problems before the main study begins, uses relatively small numbers of animals based on experience and judgement, but numbers are large enough to provide needed estimates for future sample size analysis. Good planning ensures that within any series of studies we can control any variability that might be introduced. This includes using animals of a similar age/weight range and that have had a similar environment throughout their lives; assigning animals to treatment groups using a tool that selects them at random; testing different batches of test agents in non-animal experiments first; using the same source of reagents; keeping records of observations made and standardising as many components of an animal model as is practicable. Wherever possible, scientists involved in every aspect of an experiment are unaware of which animal received which treatment, and are only made aware of this when all the data has been gathered. This reduces any potential bias that could influence making the effect of a new drug look better than it really is. If an appropriate genetically altered animal is not available commercially, then we manage our breeding programmes to minimise animal wastage. In most cases most excess animals are used to provide blood or tissues to support our work, or are offered to others.</p> <p>Exploring emerging technologies such as HD, to maximise the delivery of molecules (that are not permeable to the cell membrane) to the liver or other tissues, has the potential to reduce the number of animals needed to achieve a positive result when compared to other methods of delivery.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The ideas behind the novel drugs have come from observations made by doctors in patients with bleeding disorders. The animal models of bleeding disorders outlined in this application have been carefully selected to mimic certain aspects of the blood coagulation process (seen in human diseases) that our new drugs are likely to affect. These models were refined under our previous licence to generate robust working procedural methods, but we will continue to</p>

refine these models new ideas are generated. We will mostly use mice or rats but, for some studies we may need to use rabbits. This is because some of the factors which control clotting are not exactly the same in rats and mice as in humans, but they may be in rabbits. Genetically altered rodents that have a bleeding profile, such as haemophilia A mice and rats, are expected to show an increased risk of bleeding. These animals do require careful handling to minimise the incidence of bleeding, especially when carrying out a procedure, such as an injection, and scruffing for injections or blood sampling will be kept to a minimum. We plan to carry out pilot studies to investigate the use of light gaseous anaesthesia when carrying out injections. An alternative, refined method to deliver genetic material to the liver could be by injection into the spleen, if the genetic material is well-suited to be given in this way. The surgical procedure needed to access the spleen is simpler than that to access the hepatic portal vein, so animals are likely to recover more quickly. If giving the genetic material by HD is more successful than giving it via the spleen or portal vein then this will negate the need to carry out a surgical procedure, and animals may not need to be kept for as long (we expect to see the effects of HD to be seen within 4-5 hours, rather than days when given into the spleen or portal vein). We always use best practice for husbandry and special considerations are made where needed, such as providing our haemophilia mice with extra surgical bedding to help minimise the incidence of spontaneous bleeding. Animals are housed in social groups as far as is practical, and if bred in house, then this is done to exacting standards. We seek out new guidelines and information from sources such as the Home Office, the RSPCA, the laboratory animals science association (LASA) and the national centre for the 3Rs (NC3Rs) to maintain awareness of advances in animal welfare.

Project	Provision of an outsourced Drug Discovery platform for diseases with an inflammatory component	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Discovering new and better medicines has become increasingly challenging and sadly many potential new drugs have failed when tested in man. Improved understanding of how diseases start in the body (including the role of faulty genes) and the development of new technology has come together to provide new ways of looking to treat disease. Under this project licence our aim is to provide a service to smart thinking clients by working with them on novel targets they have identified. Combining our knowledge of animal models (and what their	

limitations are) with their understanding behind the target and the disease it is aimed to treat, we should help achieve a better clinical outcome. One such disease we have a real hope in a new treatment being successful for is osteoarthritis (OA), a condition that affects your joints causing pain and stiffness. Our focus is on looking at how other novel treatments for this disease have not been as successful as predicted and on how by teasing apart the progression of disease in the animal models used, may make them more clinically relevant for testing the next generation of related medicines. Secondly, some targets have the potential to treat more than one illness. For example stimulating a mechanism that helps the body rid itself of unwanted molecules may be beneficial in treating both lupus, which is an autoimmune disease in which the body's immune system mistakenly attacks healthy tissue in many parts of the body leading to symptoms such as a feeling of tiredness (it is often associated with a red rash on the face), and in a serious liver disease, that is not caused by over consumption of alcohol but by a build up of fat in liver, called non-alcoholic steatohepatitis (NASH). This disease may be associated with our unwitting desire to consume a fast food diet but once the damage has been done it is difficult to reverse. In these cases of potential multi therapies we will focus on the disease with the biggest un-met clinical need first (NASH) and if we find a potential drug that has a positive outcome in our animal model of that disease then we will go on to evaluate that compound's effects in models of other relevant diseases, such as lupus. The third area we hope to make a difference in is an example of where genetic mutations have identified a link with a lung disease called pulmonary arterial hypertension (PAH). Here the small arteries in the lungs become narrow, making it harder for blood to flow through so the heart has to work harder, eventually becomes weak and may even fail. There is a chance that understanding the outcome of this genetic alteration and how to moderate it with a biological agent could be of benefit to these patients., eosinophilic airways diseases and cancers that are difficult to treat. Discovering new and better medicines has become increasingly challenging and sadly

many potential new drugs have failed when tested in man. Improved understanding of how diseases start in the body (including the role of faulty genes) and the development of new technology has come together to provide new ways of looking to treat disease. Under this project licence our aim is to provide a service to smart thinking clients by working with them on novel targets they have identified. Combining our knowledge of animal models (and what their limitations are) with their understanding behind the target and the disease it is aimed to treat, we should help achieve a better clinical outcome. One such disease we have a real hope in a new treatment being successful for is osteoarthritis (OA), a condition that affects your joints causing pain and stiffness. Our focus is on looking at how other novel treatments for this disease have not been as successful as predicted and on how by teasing apart the progression of disease in the animal models used, may make them more clinically relevant for testing the next generation of related medicines. Secondly, some targets have the potential to treat more than one illness. For example stimulating a mechanism that helps the body rid itself of unwanted molecules may be beneficial in treating both lupus, which is an autoimmune disease in which the body's immune system mistakenly attacks healthy tissue in many parts of the body leading to symptoms such as a feeling of tiredness (it is often associated with a red rash on the face), and in a serious liver disease, that is not caused by over consumption of alcohol but by a build up of fat in liver, called non-alcoholic steatohepatitis (NASH). This disease may be associated with our unwitting desire to consume a fast food diet but once the damage has been done it is difficult to reverse. In these cases of potential multi therapies we will focus on the disease with the biggest un-met clinical need first (NASH) and if we find a potential drug that has a positive outcome in our animal model of that disease then we will go on to evaluate that compound's effects in models of other relevant diseases, such as lupus. The third area we hope to make a difference in is an example of where genetic mutations have identified a link with a lung disease called pulmonary arterial hypertension (PAH). Here the small arteries in the lungs

	<p>become narrow, making it harder for blood to flow through so the heart has to work harder, eventually becomes weak and may even fail. There is a chance that understanding the outcome of this genetic alteration and how to moderate it with a biological agent could be of benefit to these patients.</p> <p>These are just three examples of where we are providing a service to our clients to work towards a common goal of developing new and better drugs. However, because we want to do more we have other collaborations in the early stages of drug discovery that may lead to the treatment of additional diseases such as Alzheimer's disease and Alpha-1 Antitrypsin disease, eosinophilic airways diseases and cancers that are difficult to treat. Because targets for these diseases are developing we are mostly focusing on helping the client with proof of concept studies, developing cell based assays to test their compounds in and then looking at how well these compounds enter the blood stream so we know that drug will reach the right part of the body before going on to test them in the relevant animal model of disease.</p> <p>Whilst drugs are available to help treat all the above diseases none are perfect meaning there is still an unmet clinical need to find better medicines. Animal models that were developed in the past have been invaluable in taking current drugs in to the clinic but they may no longer be the most appropriate ones in which to test the effectiveness of drugs with a novel mechanism of action. Our aim is to provide animal models of disease that will be able to answer the question of whether a drug with a novel mechanism of action will be able to treat that disease when tested in humans.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Advances in our understanding of the science behind diseases that have an unmet clinical need such as OA, lupus and PAH will lead to new and better treatments. By 2050 there will be around 130 million OA sufferers, 40 million of whom will be severely disabled by the disease. There are significant unmet needs in the early diagnosis, monitoring and treatment of the disorder that could bring relief to suffering</p>

	<p>patients. Similarly, whilst the management of lupus has progressed enormously in the last 10 years, there are patients who do not respond to the most widely prescribed drugs and addressing this major unmet need by developing new drugs remains a priority. Non-alcoholic fatty liver disease (NAFLD) has reached epidemic proportions and is becoming the most common cause of chronic liver disease. NASH, a severe form NAFLD, is most likely to become the primary reason why patients will require a liver transplant over the next 10-20 years. An improvement in diet and increasing physical exercise is the first line taken to help these patients but this is known to be of limited effect. Hence, there is an urgent need for new and safe drugs that successfully reverses or prevents progression of liver injury in patients with NASH. Once diagnosed with PAH a patient has a 30% chance of dying within three years. Despite improvements in the diagnosis and management of PAH over the past two decades, with the introduction of targeted medical therapies leading to improved survival, current treatments only manage the symptoms and the prognosis remains poor. By understanding and refining our animal models of OA, lupus and NASH we can learn more about progression of disease and how to design experiments in which to test new medicines in the most appropriate way for each target, and advise on how best to run a clinical trial in humans with the disease. Likewise developing preclinical models of complex inflammatory disorders of the airways and those cancers that are difficult to treat will help with evaluating novel therapies for these diseases. Targets in the early phase of drug discovery our scientific driven approach to the service we provide will help prove whether these targets are worth pursuing by our clients. In all cases we will enable milestones to be met more efficiently for our sponsors (academics, the Pharmaceutical industry, clinicians, venture capitalists) so key 'go' / 'no go' decisions can be made using the minimal number of animals possible and assure a better success rate in the drug discovery process than has been seen previously.</p>
<p>What species and approximate numbers of animals do you expect</p>	<p>We will use normal mice and rats and mice that have been genetically altered to investigate the</p>

<p>to use over what period of time?</p>	<p>disease of interest or the role of a specific gene in the development of that disease. We anticipate we will use 12450 mice and 8250 rats during the five years of this licence. Rats (50) have now been added to Protocol 9 and a new protocol using 50 rats has been included so the total number of rats during the course of this licence has now increased by 1000 to 9250. The numbers of rats and mice in our existing protocol of osteoarthritis has been adjusted down so that the addition of three protocols to study respiratory disorders and cancer, and the addition of rats to an existing protocol, will not increase the total number of animals we expect to use.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The animal models involve either rodents that have a predisposition to exhibit disease or those that undergo a regulated procedure to induce disease. For example we breed mice that have been genetically modified as a model of lupus. These mice spontaneously develop antibodies to their own tissues in the same way as humans with lupus do but they appear outwardly normal. We measure levels of these antibodies in the blood by taking a small sample from a vein in the tail, which causes minimal pain or distress. As these mice age they develop kidney damage, which we can detect by measuring how much protein is excreted in their urine by allowing the mice to urinate onto a special stick that measures protein. Animals are humanely killed before the disease is likely to cause anything other than moderate distress. To model osteoarthritis we either inject an agent into one knee of a rat or mouse that causes inflammation or we carry out a surgical procedure that mimics a tear in the knee caused by a sports injury. Gradually the architecture of the knee is destroyed, as is seen in humans with OA, and as a consequence the animal may experience mild to moderate pain. We monitor this using the same techniques as used in humans. For example by measuring how much weight an animal puts on its affected limb compared to its healthy limb. We know from our experience of looking at histology of the knees how the disease progresses and we do not allow any experiment to go beyond a certain point to ensure that no animal experiences severe pain</p>

or distress. In our model of fatty liver disease we mimic the human condition by feeding normal mice with a diet that is high in fat and putting sugars in their drinking water. This “fast food diet” is similar to that many humans consume who have an unhealthy life style and who eventually develop fatty liver disease. Whilst some of these patients do become very sick none of our mice experience anything other than mild or moderate distress before they are humanely killed and the degree of liver damage assessed. To reproduce PAH in our animal models we either, expose them to air containing low levels of oxygen for up to four weeks and then put them back into normal air or we inject them with a plant extract. In some cases animals are pre-treated with an agent called Sugden that exacerbates the effects of low oxygen levels when they are returned to normal air. These procedures result in changes in the lungs, similar to those seen in humans with the disease, which we measure at the end of a study when the animal is anaesthetised and from which it is not allowed to recover. In some cases we make measurements using non-invasive imaging techniques so we can follow the course of the disease with time. Prior to the non-recovery step in the protocol most animals will suffer no more than mild to moderate distress. However, of those animals that are pre-treated with Sugden, some may suffer a sudden cardiac arrest and prematurely die, just like some humans who are diagnosed with PAH. Death in this way is not anticipated to be particularly painful for the animals. We use drugs or other substances to try and prevent or reverse disease. Our cancer models may use human cells to induce cancer in mice, making them more relevant to studying the human condition. We monitor the development of tumours using calipers and mice are killed once the tumour has reached a pre-defined size or if they show any signs that the animal is no longer able to tolerate the presence of the tumour, such as poor coat condition, reduced movement and/or social interaction. To mirror diseases of the airways in an animal models we expose them to allergens or pathogens that initiate an inflammatory response in the nose or lungs causing the animal to develop non-life

	<p>threatening asthma or increased mucus secretion, which in some cases is associated with nasal polyp-like lesions. The techniques used for dosing with test agents and sampling to measure outcomes are chosen to cause the minimal pain and distress to the animal, whilst achieving the desired outcome. At the end of any programme of work, animals are humanely killed and in most cases, tissues taken and analysed to answer questions around that programme of work.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>All the diseases we are working on have an inflammatory component. Inflammation is very complex with a web of interactions that unfold as the result of provoking an inflammatory stimulus. As such animal models of disease in which there is an inflammatory component are equally complex, with no one model being able to mimic the human condition and there is no <i>in vitro</i> model that recapitulates this complexity. Where they are available, we obtain human cell lines from patients with diseases to help support the hypothesis that a potential new target is likely to lead to the development of a new drug for that disease prior to testing in animals. We have also developed some new techniques in cells and tissues for measuring key biological processes that a target with a novel mechanism of action should affect. When testing new drugs, we can support the use of our animal models by applying these cell/tissue based techniques to the animal model of disease.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of animals we use depends on the desired outcome, the study design, the number of groups needed (including appropriate positive and negative controls (both animals and test agents) and how large a difference we hope to be able to detect. Pilot studies that are used to refine procedures and to discover potential problems before the main study begins uses relatively small numbers of animals based on experience and judgement but numbers are large enough to provide needed estimates for future sample size analysis. We use experienced scientists with a range of skills to</p>

	<p>design experiments and interpret data. Their experience in the challenges of animal experiments helps with determining the minimum number of animals needed to generate data that is meaningful both biologically, and statistically for each measurement made within a study (e.g. the use of careful power calculations to ensure that a significant effect can be detected with the number of animals assigned to an experiment). Good planning ensures that within any series of studies we can control any variability that might be introduced. This includes using animals of a similar age/weight range; testing different batches of test agents <i>in vitro</i> first; using the same source of reagents; keeping records of observations made and standardising as many components of an <i>in vivo</i> model as practicable. Wherever possible our <i>in vivo</i> workers are blinded to any treatment thus reducing bias. Likewise those that carry out downstream analysis is blinded to the treatment. If an appropriate genetically altered animal is not available commercially then we manage our breeding programmes, as far as is reasonably practical so no animals are wasted. In most cases excess animals are used to provide blood or tissues to support target validation or are offered to others. If a programme of work on a particular GA strain is no longer required then the strain would be cryopreserved (frozen at very low temperatures) so they can be re-derived in the future.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>With any new programme of work we scrutinise the literature for information and work closely with our clients to identify which animal model is most appropriate for their target. When new models or experimental designs are being developed we use data from preliminary studies to guide us on refinements, points at which to intervene and what our humane end points should be. Likewise, where signs of harm are difficult to predict, for example when testing novel compounds, we carry out preliminary studies at an expected therapeutic dose and route in small number of animals first (typically 3) and continually monitor them over a 2 hour time period. If acute adverse effects are seen then the dose is titrated down to a no effect level before pharmacokinetic or efficacy studies are</p>

	<p>conducted.</p> <p>We seek input from others, such as clinicians and experts in other disciplines (such as toxicology); look to useful websites such as the NC3Rs, RSPCA and advisory bodies such as FELASA. We also follow the local rules for the animal facilities we use with respect to husbandry, housing, transport and acclimatisation periods.</p> <p>We employ a consultant clinical histopathologist for assessing blind any pathology data in our models. This increases our knowledge of the models and where along the pathway of disease progression novel targets are most likely to be effective. This in turns supports the validity of our models relative to the human condition.</p>
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Project	Provision of Biological Materials
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input checked="" type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The aim of this project is to provide blood and biological products (including organs, brains, lungs and kidneys) from a range of animal species (mice, rats, hamsters, rabbits, chickens, turkeys and dogs) to support scientific research and both diagnostic and regulatory work. This can include ensuring new medicines are safe before release for use and checking the calibration of diagnostic devices used in treatment of both humans and animals.</p> <p>To do this we provide a service to customers, which includes academia, Contract Research Organisations (CROs) and pharmaceutical companies. For whom we produce fresh bloods, plasmas and serums, after assessing</p>

	<p>individual customer requests looking at the purpose of the work to be carried out by the customer and the benefits it may provide.</p> <p>By storing frozen plasma, serum and organs we can then ship them internationally to customers, giving a consistent timely service across different end users working on similar work. This allows researchers to purchase the specific product required as opposed to animals having to travel.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The benefits from conducting this work are dependent on the research projects of the customers ordering: a large proportion of products produced under the previous licences supported regulatory work, which is required under government guidelines and ensures the safety of drugs. Other products go to support calibration of assays and equipment to ensure results from work conducted are validated and that drugs produced are free of viral contamination. Regulations which guide the choice of species selected by customers to perform this testing include: Food and Drug Administration, World Health Organisation and the ICH (International Council of Harmonisation). The data from the assays performed will be used in regulatory submissions to the appropriate regulatory authorities or is used to help form a picture of the potential of putative new drugs to be more efficacious with a better side effect profile than existing therapies in a wide variety of human and animal health indications. These data may not always be positive, and hence, some of these tests may prevent the further development of such entities, preventing the un-necessary use of animals in efficacy and regulatory testing prior to testing in human or animal clinical trials. The scientific benefits directly linked to this licence are dependent on the research projects of our customers; but under previous licences the tissues have contributed to the knowledge of disease processes in man, animals and food crops, understanding of the development of the immune system and its regulation, and extension of the knowledge of neurobiology and associated neurological disease. By</p>

	<p>offering the different products and species from one location we can give consistency across the samples, allowing direct comparisons in the end work performed, even if this is at different locations by different customers. We are able to reduce the movement of animals by shipping blood products to end users across Europe who would otherwise have to transport animals increased distances to produce products themselves. We also can take organs after the death of the animal (for example brains and lungs) and store these until needed. The customers we supply have a preference to outsource this work so they can benefit from the high levels of specific experience and knowledge we provide.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Over the 5 year term of the licence we expect to use up to 160,000 mice, 60,000 rats, 6,250 rabbits, 1250 Hamsters, 170 dogs and 1,060 birds (chickens and turkeys). The majority of animals used will undergo non-recovery procedures (i.e. collection of blood or organs and tissues); carried out under terminal general anaesthesia. However, approximately 250 rabbits, 70 dogs and 160 birds will be used for the repetitive collection of small blood samples. Dogs, birds and a small proportion of the rabbits (5%) would have blood withdrawn from a superficial vein at approximately fortnightly intervals resulting in each animal having approximately 24 samples taken per year. Most rabbits would only have one blood sample taken.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The majority of the animals under this licence will only undergo procedures under non-recovery anaesthetic; these animals will only experience mild discomfort from the administration of anaesthetic such as experienced by human patients undergoing surgery and from being held still as the anaesthetic is introduced. The only difference is that they will not awaken from the anaesthetic and will have death confirmed or be humanely killed at the end of the procedure. Anaesthetic will be introduced either by injection into the veins or by inhalation of gas. For rabbits and dogs sedation may be used</p>

	<p>beforehand which will reduce anxiety and reduce the need for longer periods of restraint. Chickens, turkeys, dogs and rabbits will be kept for repeat blood sampling, and will have approximately 2 blood samples taken a month. These are small volumes that are under 10% of blood circulating volume and will be collected from superficial veins, similar to human blood donations. These animals will only experience minimal restraint during the period of sampling and it is not expected to cause any adverse effects. Where appropriate topical local anaesthetic will be applied to the area before sampling.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The work performed, that uses products produced under this licence, is required under safety and regulatory guidelines; these include testing of drugs (both medical and veterinary) prior to their release to market, as well as ongoing calibration and quality checks of equipment and processes to ensure accuracy of the results that are published.</p> <p>Currently there are no methods to generate animal specific blood products (cells, plasma, serum) without the use of animals.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>By keeping dedicated colonies of dogs, rabbits and birds we are able to take small blood samples across a period of time from the same animals. This reduces the number of animals needed overall and provides a consistent product decreasing the need for retesting.</p> <p>By collecting blood under non-recovery anaesthetic we are able to collect a higher volume of blood per animal compared to collection after the death of the animal. This reduces the numbers of animals used overall.</p> <p>Our customer services department provides a central point to order blood and other biological products from, for a range of customers from small university groups to large contract research companies. This means we can collect different products (blood and organs,</p>

	<p>including brains, heart, liver and lungs) from the same animal and provide to multiple end users. This is frequently done with blood products from birds. All tissues are collected after death from all species.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The choice of animal is determined by the customers' and regulatory requirements, with species such as dogs only used where non-rodent species are required and it is the best model, due to either the research being related to dogs, or due to the similarities in systems that they share with humans.</p> <p>The methods used for the collection of blood samples are based on guidelines of volume and frequency that will cause the least harm to the animals. The processing after collection is designed to get the highest quality and quantity of product so sample sizes can be kept as small as possible; we consider storage methods from across multiple fields including human transfusion services to ensure that we can maintain the quality of stored product.</p> <p>Dogs and birds kept for repeat blood sampling are held in group living conditions, with dogs having access to both inside and outside areas as part of their housing; all are assessed individually and both their behavioural and physiological condition is monitored throughout the time they are held for use in procedures. Rabbits are only kept as repeat donors if the end user requires it, for example we work with a customer who uses fresh rabbit blood cells in human medical diagnostic work and before using the cells from any rabbit they have to validate it in line with ISO 15189 (International standards for medical laboratories). By keeping a donor rabbit they can complete the validation once and then only take small volumes thereafter.</p> <p>For dogs, chickens and turkeys a peripheral vein such as the jugular vein in the neck is used for collection of blood samples, this is a superficial, easily accessible, larger vein which means the time the animal is held for the procedure can be kept to a minimum and adverse effects, even for larger samples are</p>

	<p>rarely seen. For rabbits the marginal ear vein or artery will be used, with the vein mostly used as the samples taken are small and the vein has less chance of bruising. This is an accessible blood vessel that means the rabbit can be held in a natural position for the duration of the sample. For all the animals used in repeat blood sampling procedures the sample time and experience of feeling is similar to a human blood donation or blood test performed medically.</p> <p>Dogs will only be used when the product is required for work that cannot be done without using dog specific materials, currently there is a requirement under EU legislation that drugs are tested in a non-rodent species before release into the medical and veterinary markets. Dogs are used in cases where they have similarities with humans in how they deal with the drugs at a cellular level. Worldwide legislation also requires the use of the same type of species specific plasma or serum product as the test species to support regulatory toxicology work, hence dogs must be used to supply blood products to support toxicology studies in these species.</p>
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Project	Provision of Biological Materials
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input checked="" type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>To supply blood and tissue from rhesus macaques for use in <i>ex-vivo</i> studies.</p> <p>In <i>ex-vivo</i> studies the experiments are carried out on the blood and tissue rather than the living animal. Rhesus macaques are primates and primate blood and tissue are required for three main purposes. These are firstly to investigate primate specific features, secondly to meet regulatory requirements and thirdly to use in health and welfare studies of the primates themselves.</p>
What are the potential benefits likely to derive from this project (how	The blood and tissue taken under this licence is used for a variety of purposes. The main

<p>science could be advanced or humans or animals could benefit from the project)?</p>	<p>use of the blood is for regulatory screening of products destined for human use such as the influenza vaccine. The blood is used to ensure that there is no contamination of the vaccine by other viruses and that the vaccine is safe for use. Other blood may be used to investigate signs of good and poor welfare in the macaques with the potential benefits of improved health and welfare of the macaques. Anatomical studies on brain tissue are used to advance our scientific knowledge of the primate brain.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use up to 120 rhesus macaques for blood and tissue collection under non-recovery anaesthesia over the five year period. These are animals that are being killed for health or colony management reasons. Approximately 40 male rhesus macaques will be used for collection of small volumes of blood under sedation or short-term anaesthesia.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The risk of adverse effects to the animals used in this study is very low. The centre has collected blood from recovery animals over a number of years and has had few adverse effects. The volume of blood collected is kept low to minimise the risk of anaemia and animals are checked over by a veterinary surgeon between blood samples. The expected severity level is mild for recovery animals. At the end of the procedure the non-recovery animals will be humanely killed and the recovery animals will be returned to their social groups. Some of the recovery animals will be reused on the same protocol but no more than six times per year with a minimum of one month between procedures.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Animal tissue and in this case primate tissue is required for a wide range of experiments that cannot be replaced with non-animal alternatives. These include toxicology and studies of brain function.</p>

<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>As a breeding colony we have animals that come to the end of their natural breeding life or are unsuitable for breeding and need to be humanely killed for health, welfare or colony management reasons. By taking blood and tissue under non-recovery anaesthesia we minimise the need for production of additional animals to supply these products and we can also provide tissues from a single animal to multiple users.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The unit's expertise is with rhesus macaques. The products provided by this licence are intended to support and assist the development of methods and experiments that require primate tissue.</p> <p>The animals remain in their breeding groups and are kept in large enclosures with high levels of enrichment. This gives them the opportunity to perform a wide range of natural behaviours including mating and foraging for food, They are cared for by highly experienced animal technicians. As the blood and tissues can be collected at the unit there is no need to transport the animals removing possibility of stress due to transportation.</p>

Project	Provision of Biological Materials	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	1 Year 10 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project is to provide a service for the supply of blood and other biological materials for use as controls in diagnostic testing, quality assurance, and research projects requiring biological materials. The samples supplied from the various farm animal species will be used in evaluating scientific tests for a variety of animal diseases. The project is demand driven and all requests for samples will be ethically approved and only supplied once a written case outlining why the samples are required and why no alternative source is possible. Whenever possible blood collection at post mortem will be used. However there are some tests and projects which require fresh blood free from any post mortem changes.</p>	

	<p>The number of animals used will be kept to a minimum by combining the needs of research groups and other users. The samples will be used to further develop and improve animal diagnostic tests related to the required species for a wide variety of farm animal diseases. Blood collection will be from superficial vessels and the techniques used will be of mild severity with no expected adverse effects. This provides control material for a large number of different tests some of which are statutory and also a number of research projects involving farm animal species.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The main benefits in the licence is allowing peer reviewed research to be undertaken with known control material resulting in a variety of scientific publications and outputs. The tests (including Quality Assurance functions) and products that require blood or sera to underpin vital statutory functions and animal health benefits.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Cattle 50 Sheep 100 Pigs 50 Goats 10 Horses 5 Chickens and Turkeys 200. These use of these animals will be spread over the 5 year life of the licence with lot of the animals being sampled intermittently over periods of months or years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Mild severity, the only effect should be insertion of needle to remove the blood sample. Animals will either be killed by a schedule 1 method at the end or kept alive at the Designated Establishment if they are assessed as not suffering or not likely to suffer as a consequence of the regulated procedures applied. The criteria for this assessment will have been determined by the Named Veterinary Surgeon in advance and records will be kept in a form agreed with the Home Office Inspector. Animals will only be re-used in this protocol if: a) The actual severity of any series of regulated procedures that have previously been applied to the animal, is no more than mild; b) A veterinary surgeon with knowledge of the lifetime experience of the animal or animals has advised that their general state of health and well-being is likely to have been fully restored following the application of the previous series of regulated procedures and that the animal is free of adverse effects arising</p>

	from the previous regulated procedures.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Blood and faeces are the basis for the majority of laboratory tests involved in animal health and veterinary diagnosis. This licence supplies material to tests that are already established for control purposes or in the process of being developed. Wherever possible sample collected post mortem will be used to supply the need for material, however as these laboratory tests are used to diagnose disease in the live animal, there is often the requirement to use fresh material, to avoid post-mortem changes and contamination issues.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of animals used is minimised by re-using them. This allows a small number of animals to provide control blood across the establishment and other research establishments.</p> <p>The experimental design is based on matching demand for blood through the request form to numbers of animals maintained to supply the blood.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The institution and its partner organisation deal with a wide range of farm animal diseases both in a research and diagnostic capacity.</p> <p>Requests for samples from different groups will be combined whenever possible.</p> <p>The department has a group of experienced and knowledgeable staff who are able to perform the necessary techniques quickly and efficiently. Having licence holders who are proficient at the techniques required is essential for good sampling practice, which minimises any animal stress and keeps animal handling to a minimum.</p> <p>Analgesia/anaesthesia will be used where appropriate.</p> <p>The severity limits of this protocol are mild.</p>

Project	Quantifying avian influenza risk at the wild bird-poultry interface
Key Words (max. 5 words)	
Expected duration of the project (yrs)	3 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	Regulatory use and routine production
	Protection of the natural environment in the interests of the health or welfare of humans or animals
	Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To describe and quantify pathways of avian influenza from wild birds to domestic poultry in Great Britain. We will evaluate how and when the disease arrives in the country, how it spreads around the country, how poultry come into contact with it, and whether the virus persists in the British environment during the summer and autumn months ready to re-emerge in the presence of wild birds.
What are the potential benefits likely to derive from this project (how science could be advanced or	Understanding how poultry become exposed to avian influenza (AI) will permit revision of the Government's risk assessment on AI, review of

humans or animals could benefit from the project)?	its policy on poultry housing orders and will allow the development of improved biosecurity advice for farmers. This will potentially prevent the wastage of many thousands of poultry each year, saving the industry millions of pounds.
What species and approximate numbers of animals do you expect to use over what period of time?	We will capture and take blood and cloacal samples from up to 10,000 wild birds over 3 years. All major bird groups, except birds of prey, will be sampled.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Injuries may be sustained and acute stress will be experienced by birds during capture. We expect injuries to be rare and to mostly present as skin abrasions (mild severity). However, serious injury, such as broken limbs and mortality cannot be completely mitigated. Injured and ill birds that are judged by a competent person to be unlikely to make a full recovery will be euthanased. All birds judged fit for release by a competent person will be released back to the wild at the place of capture.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Only birds infected with AI virus pose a risk of AI exposure to poultry. Therefore we need to understand which wild bird species and what proportion of each population are infected with AI virus. This can only be measured in wild birds.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Currently there are no reliable estimates of the proportion of each species that is infected with AI, so we cannot yet estimate the size of the sample that we need. Consequently, during the first period of capture, we will sample as many birds of as many species as we can. The results will be used to calculate the minimum number of each species that we need to sample in order to reliably estimate the proportion of the population of each species that is infected. The minimum number will be our target. Once it is reached, we will not sample any more individuals of that species during that season.

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>It is currently assumed that AI arrives in Britain with migratory water fowl and is then passed on to 'bridge species' such as starlings, which then make contact with poultry. In order to confirm or refute these assumptions we need to measure AI infection in wild birds.</p> <p>The majority of birds that we will sample will be captured during routine bird ringing activities. This will reduce the numbers that we need to catch exclusively for this project. Only people trained, competent and licensed to catch and handle birds will be involved in their capture and only people trained, competent and licensed to take blood and cloacal samples from birds will be involved in their sampling. Sampling will be undertaken under veterinary supervision, and veterinary support will be available allowing us to respond to any cases of injury or illness.</p>
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Project	Radiation combinations for cancer treatments	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The overall plan of the work is to support the discovery and development of novel cancer therapies to benefit human health in the treatment of cancer.</p> <p>To achieve this we will:</p> <ol style="list-style-type: none"> 1. Develop and validate IR treatment protocols for the treatment of superficially implanted human or syngeneic tumours in mice and rats 2. Determine the tolerability of ionising radiation alone or in combination with novel agents 	

	<ol style="list-style-type: none"> 3. Assess the effects of single or fractionated radiotherapy treatment protocols as used in clinical setting, using animal models 4. Once treatment protocols have been validated the models will be used to evaluate and refine combination treatment regimens with novel targeted therapies to direct clinical development 5. Further test novel cancer agents in combination with radiotherapy to look for additive or synergistic effects using either tumour growth inhibition or pharmacodynamic endpoints.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Radiotherapy provides significant benefit being used in over 50% of all cancers. As the patient population treated with radiotherapy is so large, enhancing therapeutic outcome for even a relatively small proportion has the potential to translate into highly significant clinical benefit. To have pre-clinical animal models that will demonstrate and characterise improvements in anti-tumour activity will lead to more relevant potential clinical benefits thus directing clinical development. In this license we will be testing the combination of putative anticancer drugs with ionising radiation with the aim of improving current anti-cancer therapies. With this license we will be able to investigate if new compounds sensitise human tumours to irradiation or if new dosing schedules are better tolerated and/or more efficacious than the current ones. With all this information, new clinical trials can be designed that eventually may change clinical practice.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Only rats and mice will be used within this project licence. Approximately 2000 mice per year and 200 rats per year will be used over the five year life of the project</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at</p>	<p>Adverse effects related to tumour inoculation may cause brief discomfort or pain. Adverse effects will be minimised by: • limiting volumes • choice of appropriate needle size • application of good technique by trained</p>

<p>the end?</p>	<p>licensees The tumour types used are very well tolerated and only one superficial tumour will be used per animal. Tumour size and condition is monitored closely on a daily basis and we will use the least invasive tumour site/line that will achieve the scientific aims and will apply the earliest endpoints to meet the scientific requirement of the study. Animals will be culled if the tumour results in significant pain or distress. Clinical signs related to the pharmacological action of the compound may be seen and mild to moderate signs of toxicity are possible. Animals will be humanely killed if this persists. Local irritation at the site of injection may be observed. Animals will be closely observed on the day of dosing. Animals are observed by trained staff, with referral to the Named Animal Care and Welfare Officer, veterinary staff and Project Licence Holder as necessary. All animals will be regularly monitored for weight loss and general condition. Any toxicity associated with radiotherapy will be minimised by careful monitoring and appropriate lead shielding. In most cases there is no toxicity. Weight loss as a result of repeat anaesthesia may occur and this will be minimised by correct dosing, accurate weighing and good maintenance of body temperature during the period of anaesthesia and the recovery phase. Animals that are used where the immune-system is compromised will be housed in sterile conditions. The protocols are classified as moderate severity. Animals will be humanely culled at the end of the study.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Non-animal alternatives are used in the identification and selection of compounds and generally include measurements of the likely effect of the agent on the target cells or mechanism. Activity in particular cell types however cannot predict the likely <i>in vivo</i> activity given the complexity of issues such as bioavailability, metabolism and elaborate physiological interactions associated with tumour growth.</p> <p>The potential clinical interaction between novel therapies and radiation cannot be fully modelled</p>

	<p><i>in vitro</i>, given the outcome to radiotherapy is governed by a number of pathophysiological factors <i>in vivo</i>, for example tumour reoxygenation. This drives a need to be able to model interactions with radiotherapy in tumour models in animals.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>To maximise the scientific integrity of data generated and to use the minimum number of animals, in house statistical expertise will be applied to all experimental design and analyses. Where plausible the following statistical guidelines will be used to minimise the number of animals required for each procedure:</p> <ul style="list-style-type: none"> ● meaningful biological change and measurable endpoints will be defined ● estimates of biological variability will be used in sample size and power calculations ● animals will be allocated in an optimal way based on estimates of biological variability established from accrued historical databases, pilot studies or published data. ● regular monitoring and updating of biological databases with regular review of group sizes. ● one-sided (rather than two-sided) statistical tests will be used wherever appropriate (e.g. when identifying inhibition rather than change) ● statement of intended statistical analyses and justification for use, if any, of transformed data (e.g. tumour growth data may be analysed on the logarithmic scale if the variance of tumour measurements increases with the mean) ● statistical power will be set to a minimum of 80% (e.g. at least an 80% chance of declaring the defined 'meaningful biological change' as being statistically significant) ● multiple treated groups will be compared against one control to reduce the number

	of studies performed. Group sizes may be weighted to reflect this.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The overall plan of the work is to support the discovery and development of novel cancer therapies to benefit human health in the treatment of cancer. Only rats and mice including immune-deficient strains are used on this licence. Using non-mammalian species of lower neurophysiological sensitivity is not possible since they lack appropriate tissue physiology. Although exact replication of all pharmacokinetic parameters between species is not possible, many features of human PK can be predicted from those observed in small mammalian species unlike effects seen in lower organisms.</p> <p>The most appropriate species and strain of mice and/or rats will be chosen based on previous data that has been used to generate single agent efficacy data. Mice will be used in the majority of studies unless there is a scientifically relevant reason that mice cannot be used, for example, compound metabolism issue with the compound. The choice of strain will be driven by the choice of tumour model. For human tumour lines immune-deficient animals are required to support the growth of the tumour, the least immune-deficient strain required to promote good, reproducible tumour growth will be used. The optimal conditions for tumour growth will already have been developed.</p> <p>For anaesthetic protocols the optimal anaesthetic regime relevant for the species/strain will be developed and used in conjunction with the NVS.</p> <p>Where necessary, pain relief will be used under the guidance of the NVS.</p>

Project	Radiolabelled molecules for cancer imaging and therapy
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research <input checked="" type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project has 3 parts: 1) Optimisation of a technique (targeted radiotherapy (TR)) delivering radioactive molecules to cancers to destroy them (2) Characterisation of novel radiolabelled probes that can be used to detect cancer (3) Measure the efficacy of anticancer drug combinations and the effect of treatment on the uptake of radiotracers used in medical imaging.</p> <p>1) Cancer patients often present with metastatic disease where cancer cells have spread from the primary cancer to establish new cancers elsewhere in the body. Whilst radiotherapy is the most effective anticancer</p>

	<p>treatment for cancers that cannot be surgically removed it cannot be used to control metastasis. Currently chemotherapy drugs, which distribute around the body, are used to treat metastases but cancers and their metastases almost always develop resistance to chemotherapy. Targeted radiotherapy (TR) involves injecting radioactive drugs targeted to cancer cells which seek out primary tumours and metastases and use their radioactive payload to locally irradiate cancer tissue. In this project we will produce molecules capable of delivering the necessary radiation dose to tumours and their metastases. They will be tested on biological models for efficacy and safety. Results from this study will provide requisite data for a full clinical study.</p> <p>2) Early cancer detection is critical to long term survival. Positron emission tomography (PET) is the most sensitive medical imaging technique for cancer detection. Currently patients are administered with a radioactive tracer called FDG which is concentrated by cancers facilitating detection of the cancer in the body by the PET camera. Although FDG is very useful its uptake is not restricted to cancer so novel cancer imaging agents are under development. These need to be tested to determine their cancer targeting potential and suitability with respect to where they locate in the body and how long they stay in the body.</p> <p>3) Combinations of drugs can increase the anticancer effect compared with giving a single drug but cancer response varies between patients. When a cancer is responding to drug treatment, the uptake of a radiotracer by the cancer tends to decrease (this is measured in patients using a PET camera). Here we will test how combination treatments modify the uptake a radioactive tracer to justify using PET in patients to detect response to drug combinations.</p>
<p>What are the potential benefits likely to derive from this project</p>	<p>1) The anticancer efficiency of targeted radiotherapy (TR) is limited by the poor</p>

<p>(how science could be advanced or humans or animals could benefit from the project)?</p>	<p>distribution of the TR molecules within cancers due to regions in cancers where blood flow is poor and due to variation in the amount of TR-target that different cancer cells display. To overcome these problems this project will determine the optimum chemical makeup of the TR molecules. This optimisation will provide the most suitable molecules for a subsequent clinical study. 2) Medical imaging techniques including PET which detects radioactive molecules (tracers) that are attracted to cancers are proving to be very useful in cancer detection and in identifying response to treatment. Novel tracers are being developed which are attracted to cancers but less so to non-cancer tissue to more precisely identify cancers and so reduce uncertainty in cancer diagnosis. 3) Changes in the uptake of tracers during treatment (compared with before treatment) can signal response or non-response at an early time point. Where patients are shown not to be responding to a treatment combination it can be changed to a more effective one.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>1) Project 1 should take 2-3 years and will use about 250 mice in order to optimise our targeted radiotherapy molecules. 2) Project 2 will be ongoing as new tracers are produced (maximum of 4). Each study will need 50 mice (total of 200). 3) Project 3 will examine treatment efficacy and the effect of treatment on the uptake of the tracer and require 200 mice. This study will take 2-3 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Tumours (cancers) will be induced in some mice by subcutaneous (under the skin) injection of cancer cells into the flank. Initial injection may be associated with some inflammation. Tumours will be grown to a maximum size of 15mm (longest dimension) which will not hinder movement nor will they spread as the animals will be humanely killed after a few weeks. This type of procedure is generally assigned a moderate level of severity. Some animals will be injected with clinically relevant doses of radioactive molecules that target the cancers and are</p>

	<p>not expected to have any adverse effects. Some mice will receive anticancer drugs that target signalling pathways in cells and are not expected to exhibit any side effects (mild level of severity). At the end of each experiment mice will be killed by a humane method and tissues collected.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>1) NC3Rs website has been examined for possible cancer models that could replace animals. A 3D model that is less than 1mm in diameter was found. However we need ones that are 10-15mm in length as we need to demonstrate that the targeted radiotherapy molecules distribute within a solid tumour and produce a uniform dose distribution throughout. The dose is deposited (killing cancer cells) up to 10mm away from the decaying nuclide so the tumours need to be 10-15mm in length to facilitate uptake within a cell kill range. This is in common with comparable studies in the literature. No alternative models for the development of targeted radiotherapy molecules could be found.</p> <p>2) To determine how a novel imaging agent distributes within the body, how long it stays in the circulation and its excretory route are essential information before a novel cancer imaging agent would be allowed to be used in the imaging of a patient. To determine how the uptake of tracers is influenced by treatment response is initially tested <i>in vitro</i> using isolated cancer cells. However <i>in vivo</i> many other factors influence what happens to the tracer so <i>in vitro</i> findings need to be verified <i>in vivo</i> prior to clinical translation.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>1) <i>In vitro</i> studies using cells grown in culture can be used to determine the sensitivity of cancer cells to types of cytotoxic radionuclide and to drugs. We will use these methods to inform on the levels of anticancer agents likely to cause tumour regression. Pilot studies based on these findings and the literature for comparable studies will reduce</p>

	<p>the number of animals required to determine sensitive anticancer agent doses.</p> <p>2) To ensure that the <i>in vivo</i> studies are justified tracers will be screened for specific binding to cancer cells before they are tested <i>in vivo</i>.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Xenograft (cancers derived from human cancers and grown in animals) cancer models are grown in immunocompromised mice. The mice will be frequently checked to ensure that injections of cancer cells, anticancer drugs, radioactive tracers do not produce any unexpected adverse effects. Formation of a lump within 24h after injection or increased licking of the injection site would give an initial indication of an (unlikely) adverse effect. The animals would be expected to show no adverse effects but would be closely monitored for changes in appearance.</p> <p>At the end of procedures the animals are humanely killed and tissues analysed</p> <p>orsomeofthestudieswewillrequirealargeblood samplewhichcanmosthumanelybeacquiredby cardiacpunctureunderterminalanaesthesia.</p> <p>Where oral gavage is used to administer drugs flexible tubing will be used to decrease the discomfort experienced by the animals.</p>

Project	Radiolabelled molecules for cancer imaging and therapy	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project has 3 parts: 1) Optimisation of a technique (targeted radiotherapy (TR)) delivering radioactive molecules to cancers to destroy them (2) Characterisation of novel radiolabelled probes that can be used to detect cancer (3) Measure the efficacy of anticancer drug combinations and the effect of treatment on the uptake of radiotracers used in medical imaging.</p> <p>1) Cancer patients often present with metastatic disease where cancer cells have spread from the primary cancer to establish new cancers elsewhere in the body. Whilst radiotherapy is the most effective anticancer treatment for cancers that cannot be surgically removed it cannot be used to control</p>	

	<p>metastasis. Currently chemotherapy drugs, which distribute around the body, are used to treat metastases but cancers and their metastases almost always develop resistance to chemotherapy. Targeted radiotherapy (TR) involves injecting radioactive drugs targeted to cancer cells which seek out primary tumours and metastases and use their radioactive payload to locally irradiate cancer tissue. In this project we will produce molecules capable of delivering the necessary radiation dose to tumours and their metastases. They will be tested on biological models for efficacy and safety. Results from this study will provide requisite data for a full clinical study.</p> <p>2) Early cancer detection is critical to long term survival. Positron emission tomography (PET) is the most sensitive medical imaging technique for cancer detection. Currently patients are administered with a radioactive tracer called FDG which is concentrated by cancers facilitating detection of the cancer in the body by the PET camera. Although FDG is very useful its uptake is not restricted to cancer so novel cancer imaging agents are under development. These need to be tested to determine their cancer targeting potential and suitability with respect to where they locate in the body and how long they stay in the body.</p> <p>3) Combinations of drugs can increase the anticancer effect compared with giving a single drug but cancer response varies between patients. When a cancer is responding to drug treatment, the uptake of a radiotracer by the cancer tends to decrease (this is measured in patients using a PET camera). Here we will test how combination treatments modify the uptake a radioactive tracer to justify using PET in patients to detect response to drug combinations.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>1) The anticancer efficiency of targeted radiotherapy (TR) is limited by the poor distribution of the TR molecules within cancers due to regions in cancers where blood flow is poor and due to variation in the amount of TR-target that different cancer cells display. To overcome these problems this project will determine the optimum chemical makeup of the TR molecules. This optimisation will provide the most suitable molecules for a subsequent clinical study.</p> <p>2) Medical imaging techniques including PET which detects radioactive molecules (tracers) that are</p>

	<p>attracted to cancers are proving to be very useful in cancer detection and in identifying response to treatment. Novel tracers are being developed which are attracted to cancers but less so to non-cancer tissue to more precisely identify cancers and so reduce uncertainty in cancer diagnosis. 3) Changes in the uptake of tracers during treatment (compared with before treatment) can signal response or non-response at an early time point. Where patients are shown not to be responding to a treatment combination it can be changed to a more effective one.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>1) Project 1 should take 2-3 years and will use about 250 mice in order to optimise our targeted radiotherapy molecules. 2) Project 2 will be ongoing as new tracers are produced (maximum of 4). Each study will need 50 mice (total of 200). 3) Project 3 will examine treatment efficacy and the effect of treatment on the uptake of the tracer and require 200 mice. This study will take 2-3 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Tumours (cancers) will be induced in some mice by subcutaneous (under the skin) injection of cancer cells into the flank. Initial injection may be associated with some inflammation. Tumours will be grown to a maximum size of 15mm (longest dimension) which will not hinder movement nor will they spread as the animals will be humanely killed after a few weeks. This type of procedure is generally assigned a moderate level of severity. Some animals will be injected with clinically relevant doses of radioactive molecules that target the cancers and are not expected to have any adverse effects. Some mice will receive anticancer drugs that target signalling pathways in cells and are not expected to exhibit any side effects (mild level of severity). At the end of each experiment mice will be killed by a humane method and tissues collected.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>1) NC3Rs website has been examined for possible cancer models that could replace animals. A 3D model that is less than 1mm in diameter was found. However we need ones that are 10-15mm in length as we need to demonstrate that the targeted radiotherapy molecules distribute within a solid</p>

	<p>tumour and produce a uniform dose distribution throughout. The dose is deposited (killing cancer cells) up to 10mm away from the decaying nuclide so the tumours need to be 10-15mm in length to facilitate uptake within a cell kill range. This is in common with comparable studies in the literature. No alternative models for the development of targeted radiotherapy molecules could be found.</p> <p>2) To determine how a novel imaging agent distributes within the body, how long it stays in the circulation and its excretory route are essential information before a novel cancer imaging agent would be allowed to be used in the imaging of a patient. To determine how the uptake of tracers is influenced by treatment response is initially tested <i>in vitro</i> using isolated cancer cells. However <i>in vivo</i> many other factors influence what happens to the tracer so <i>in vitro</i> findings need to be verified <i>in vivo</i> prior to clinical translation.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>1) <i>In vitro</i> studies using cells grown in culture can be used to determine the sensitivity of cancer cells to types of cytotoxic radionuclide and to drugs. We will use these methods to inform on the levels of anticancer agents likely to cause tumour regression. Pilot studies based on these findings and the literature for comparable studies will reduce the number of animals required to determine sensitive anticancer agent doses.</p> <p>2) To ensure that the <i>in vivo</i> studies are justified tracers will be screened for specific binding to cancer cells before they are tested <i>in vivo</i>.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Xenograft (cancers derived from human cancers and grown in animals) cancer models are grown in immunocompromised mice. The mice will be frequently checked to ensure that injections of cancer cells, anticancer drugs, radioactive tracers do not produce any unexpected adverse effects. Formation of a lump within 24h after injection or increased licking of the injection site would give an initial indication of an (unlikely) adverse effect. The animals would be expected to show no adverse effects but would be closely monitored for changes in appearance.</p> <p>At the end of procedures the animals are humanely</p>

	<p>killed and tissues analysed</p> <p>For some of the studies we will require a large blood sample which can most humanely be acquired by cardiac puncture under terminal anaesthesia.</p> <p>Where oral gavage is used to administer drugs flexible tubing will be used to decrease the discomfort experienced by the animal.</p>
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Project	Radiosensitisation of bladder tumours and normal tissues
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X Basic research
	X Translational and applied research
	Regulatory use and routine production
	Protection of the natural environment in the interests of the health or welfare of humans or animals
	Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Bladder cancer is the fourth commonest cancer in UK men and when it spreads to the bladder wall muscle (30% of patients) it is only curable in about half of patients with currently available treatments. We wish to identify drugs which can be added to radiotherapy treatments for bladder cancer or modifications to diet, which would improve the survival in patients following treatment but at the same time not add to the side effects expected when giving radiotherapy alone. To do this, we have to use mouse models to:</p> <ol style="list-style-type: none"> 1. identify drugs which combined with radiotherapy are more effective against tumours located just

	<p>under the skin,</p> <p>2. see if a high fibre diet might be as effective as adding a drug,</p> <p>3. finding drugs and dietary effects which cause minimal effects to the normal tissues surrounding the bladder when combined with radiotherapy,</p> <p>4. to test the most promising treatment combinations in mice which have the tumour growing in the bladder,</p> <p>5. to test strategies to prevent tumours spreading into the bladder wall muscle</p> <p>6. to test FLASH radiotherapy which is ultra-high dose rate and is thought to spare normal tissues from radiation whilst still being effective in treating tumours.</p> <p>7. to test if feeding normal mice and germ-free mice with the gut bacteria from mice and humans alters their responses to radiotherapy.</p> <p>If successful, such treatments could be tested in humans in clinical trials.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>It is now generally recommended that patients having radiotherapy for their bladder cancer also have a drug or drugs added to make the treatment more effective. However, most bladder cancer patients are elderly and are not fit enough to receive these drugs or do not tolerate the current treatments well. There is therefore an urgent need to find drugs or modifications to diet which are suitable for these patients. We can deliver focussed radiotherapy in mice which will allow us to study these agents in a setting which is relevant to the human situation. This will hopefully mean that we can identify and test new drugs to add to radiotherapy, which can be taken forward to clinical trials in patients, and which are likely to improve tumour cure while not adding to the side effect burden. We are developing another form of test, using groups of organised cells called organoids, which are small mini-guts grown in a dish, to test potential drugs to add to radiotherapy. If successful, this could ultimately replace the use of our current test that requires mice to be irradiated and for their guts to be examined under the microscopy. Before</p>

	<p>we get to that stage, the organoid method would hopefully reduce the numbers of animals needed, by screening out compounds that are not going to be useful. Mouse models have an important role to play in the development of biomarkers to predict which patients are most likely to benefit from adding chemotherapy to radiotherapy, as we can remove tissues from them after death to look at various factors which could be associated with treatment. Although we have access to a large number of patient samples which can be studied like this, their use is limited in this regard as these patients have not been treated by the agents of interest.</p> <p>Feeding mice with dietary fibre supplements, or gut bacteria/faeces and/or antibiotics will allow us to see if altering the gut microbiome could improve responses to radiotherapy, which might be applicable to humans also.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use mice and expect to use approximately 5,600 over five years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The mice have a <5% risk of dying from a general anaesthetic. Tumours in the bladder may block the tubes to the bladder and prevent the mouse from passing urine. Tumours may spread to the lymph nodes and other organs. Mice may develop side effects from the irradiation delivered in terms of toxicity to the bladder and bowel, and skin in the case of superficial tumours. Mice could develop toxic side effects from the agents used in imaging (<1%) or from the test drugs, although mice be exposed to the minimum harm possible and will be killed humanely if there is any sign of this happening. At the end of the experiments, the mice will be killed humanely.</p> <p>Feeding mice with fibre is not expected to have serious side effects for the mice. Furthermore, antibiotics are not expected to have serious side effects, and altering the gut microbiome is not expected to harm the mice either.</p>
<p>Application of the 3Rs</p>	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Growing bladder tumour cells in dishes allows us to see if drugs can work in combination with radiotherapy to kill more tumour cells than radiation alone. However, this method does not allow us to test whether the drugs are also having an adverse effect on the normal tissues which would normally surround the tumour area to be treated in humans. Tumours also develop complex surrounding structures to feed and support them in animals and humans, and these cannot be simulated in dishes in a laboratory.</p> <p>We are now looking into using organoids in the laboratory, but these are not yet at the stage to replace mice. We cannot use lesser animals such as zebra fish, as work in them cannot then be used to develop human clinical trials.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>By doing small test experiments with only a few animals, this will let us decide which are the most important larger experiments to perform, which are likely to give us successful results. This will mean that animals are not wasted in experiments which are unlikely to give useful information.</p> <p>We will keep the numbers of animals used in the early test experiments to an absolute minimum, usually 2 or 3 per group.</p> <p>In the larger experiments, we will use a 'factorial design' which means that because of the statistics involved, fewer animals are needed per group to get a meaningful result.</p> <p>Because of new sophisticated methods of imaging the animals, when a tumour is growing inside, rather than having to kill an animal to examine the tumour, animals can be imaged over time, and this means far fewer animals are needed for each experiment.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms)</p>	<p>We use mice as they are the animal species where most is known about how tumours respond to drugs and radiation.</p> <p>In many of our experiments we will inject the tumours cells under the skin in a part of the body that does not cause a nuisance to the animal, and we will not allow the tumours to reach a size that affects the behaviour of the mouse or makes it ill.</p>

to the animals.

We use careful injection techniques in all our experiments to minimise the chance of infection.

In some experiments we will create tumours in the bladder wall, and this will be done under anaesthetic by injection under ultrasound imaging, using a very small needle. Again the tumours will not be allowed to grow so large as to cause the animal to suffer. In a third, genetic model, mice develop bladder tumours after drug treatment, which will allow us to investigate tumours which develop in the bladder similarly to human tissues, and the effect of treatments on prevention of progression of tumours into the muscle. Tumours will not be allowed to grow so large as to cause the animal discomfort. These mice may develop a skin condition caused psoriasis, which will need to be carefully monitored and treated. The latter two experiments will be more relevant to the human drug/radiotherapy situation.

We will use imaging in some mice to better understand the behaviour of the tumour, but we have limits to the numbers of scans that can be performed in each mouse and also complicated scans will be done under general anaesthetic.

We check carefully for blood in the urine to detect this at an early stage, to minimise the discomfort to the mice.

The drugs will be given in the smallest amount of liquid that is practical and where possible in a liquid that matches the body composition of the animal, to minimise the effects on the mouse.

We deliver our radiotherapy very carefully using specialised equipment to treat only the minimum amount of normal tissue required for our experiments.

We are careful to make sure that the mice are eating palatable food and receiving enough food to keep their weight stable.

We will cause the mice the minimum harm possible, and they will be killed humanely if they show signs of distress.

Project	Reagent Production in Support of Diagnostic Tests	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	<p>The objective of this project is to produce reagents for either direct use in diagnostic tests or the improvement of them.</p> <p>The Unit producing these reagents is responsible for producing approximately 300 different polyclonal antibodies and 50 antigens for diagnostic work.</p>	
Why is it important to undertake this work?	<p>The tests directly supported by or developed as a result of this project support disease diagnosis and improvement of health of farmed animals. Some of the tests also cover zoonotic organisms such as E.coli, salmonella and lyssaviruses which can have significant impact on human health.</p> <p>The Unit producing these reagents is responsible for</p>	

	<p>producing approximately 500 different diagnostic reagents. Approximately 60% of these reagents are polyclonal antibodies, 11% are antigens and the remainder are kits and other reagents.</p>
<p>What outputs do you think you will see at the end of this project?</p>	<p>The availability of high quality diagnostic reagents will be maintained in order to support the work of both animal and human health and animal welfare through the supply of reagents to the Organisation's national diagnostic capacity for disease diagnosis and import/export testing in order to control diseases that are exotic to the UK.</p> <p>For example the antibodies against different salmonella serotypes will allow epidemiological investigation of disease outbreak, preventative action and indirectly support publications and other communications.</p> <p>Reagents are also sold commercially for similar reasons.</p>
<p>Who or what will benefit from these outputs, and how?</p>	<p>The work of this project is ongoing in the short and long term. It supports both animal and human health and animal welfare through the supply of reagents to the Organisation's national diagnostic capacity for disease diagnosis and import/export testing in order to control diseases that are exotic to the UK. Reagents are also sold commercially for similar reasons. Production of reagents are managed in order to ensure a continuous supply using a stock management system with trigger levels set appropriately to allow for lead times.</p> <p>These reagents are also supplied to several research groups therefore minimizing the numbers of animals required.</p>
<p>Will this work be offered as a service to others?</p>	<p>Yes</p>
<p>How will you look to maximise the outputs of this work?</p>	<p>This is a service licence with production of these biological reagents is carefully linked to demand.</p>
<p>Explain why you are using these types of animals and</p>	<p>The animals selected have been proven to be the best model to produce the material of the best specificity</p>

<p>your choice of life stages.</p>	<p>and sensitivity for testing e.g. chickens for Marek's Disease antibody, rabbits for E.coli and salmonella. To produce antisera the animal has to have competent immune system, this varies from starting with day old chicks (for the Marek's Disease antisera) to rabbits that are free living and a few weeks old. In some cases the organisations such as the O.I.E (world animal health organisation) dictate a particular model should be used.</p> <p>Marek's disease antigen production is done in chickens as this is natural host for the disease, with the experiment starting using chicks that are approximately two weeks old.</p>
<p>Typically, what will be done to an animal used in your project?</p>	<p>Protocol 1 Polyclonal antiserum production in rabbits</p> <p>Programmes vary with type of antibody required to be produced. Typical programmes are below, slight variations may occur due to practical reasons these will not affect the overall ethical cost to the animal.</p> <p>For short (somatic) programme which lasts 19 days, 5 inoculations followed by exsanguination under terminal anaesthesia.</p> <p>For long (flagella) programme which lasts 35 days, 6 intravenous inoculations followed by exsanguination under terminal anaesthesia.</p> <p>For the Adjuvant (somatic) programme which lasts 43 days, 4 inoculations either subcutaneous or intramuscular followed by exsanguination under terminal anaesthesia.</p> <p>Protocol 2 Production of Marek's Disease Virus Antisera</p> <p>Vaccination of 1 day old chicks live Marek's Disease vaccine - followed by 4 boosts with small amounts of Marek's Disease virus. After 90 days the birds will be exsanguinated under terminal anaesthesia.</p> <p>Protocol 3 Production of Marek's antigen</p> <p>Chickens will be infected by intra-muscular, subcutaneous or intra-abdominal injection with a vaccine strain of Marek's disease virus at a minimum of two weeks old. They will be euthanased by schedule 1 method when the feather follicles swell, indicating</p>

	suitable antigen harvest.
What are the expected impacts and/or adverse effects for the animals during your project?	<p>Protocol 1. As the inocula have undergone inactivation procedures, no adverse effects are anticipated. With the protocol using adjuvants there may be some localised swelling or inflammation around the injection site.</p> <p>Protocol 2. An attenuated commercial Marek's Disease vaccine is used for the first inoculation, this is followed by inoculations of small amounts of attenuated Marek's Disease virus for the rest of the inoculations, no adverse reactions were observed in the previous studies in 2019 or earlier in 2018.</p> <p>Protocol 3. Adverse effects expected to be limited to lethargy and inappetance and swelling of the feather follicles . If this persists more than 24 hours the birds will be euthanased.</p>
What are the expected severities and the proportion of animals in each category (per animal type)?	<p>Rabbit - all mild severity</p> <p>Chicken - mild severity for antisera raising, 50% moderate for the MDV antigen raising (rest mild).</p>
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	<p>For antibody production it is not possible to produce suitable antibodies using in-vitro systems as the complexity of the animal's immune system is required, particularly as the reagents are used in the diagnosis of disease in animals and high sensitivity and specificity is required. However work on replacement of salmonella serotyping work is being undertaken, which requires antibody produced by this licence to genetic sequencing techniques that do not. This licence is structured so as demand reduces so will the production of antisera.</p> <p>The generation of Marek's disease antigen in chickens under this licence is considered a stop gap whilst the cell culture method is further refined to improve quality so it is able to replace this work under this licence.</p>

<p>Which non-animal alternatives did you consider for use in this project?</p>	<p>Use of recombinant antibodies from genetically modified cell lines.</p> <p>In-vitro (cell culture) production for Marek's Disease Virus.</p>
<p>Why were they not suitable?</p>	<p>Recombinant antibodies target single antigenic epitopes and lack the ability to mimic the polyclonal antibody responses that occur in the field (and target multiple antigenic epitopes). Therefore the use of recombinant antibodies would result in the test lacking the broad activity or sensitivity required for field diagnostic test use.</p> <p>There are currently various issues with the cell culture Marek's antigen production including achieving virus titre and matching specificity and sensitivity. In-house procedures are in development to address this, using chick embryo fibroblasts for 11 day old embryos.</p>
<p>Enter the estimated number of animals of each type used in this project.</p>	<p>rabbits: 1000</p> <p>other-birds: -</p>
<p>How have you estimated the numbers of animals you will use?</p>	<p>The number of animals used under the previous project licence is related to the level of reagent demand from customers and have been used as a guide to future requirements. The level of demand is constantly monitored using an Integra stock management system enabling production to be planned so that minimal waste occurs and optimum stock levels are maintained. Additionally close contact is maintained with customers to ensure that reagents are supplied to them in appropriately sized volumes to minimise wastage. As a result the number of animals used is the minimum required to support testing requirements. The estimated number of chickens to be used in this project have been reduced from 775 to 530.</p> <p>Where possible commercially available reagents are bought in e.g. Dourine IFAT slides have been recently identified and validated for use meaning we do not need to use animals to produce these in-house.</p>

<p>What steps did you take during the experimental design phase to reduce the number of animals being used in this project?</p>	<p>For both antisera and antigen production the key to reducing numbers is to maximise the amount and titre of the material collected from each animal. The outputs of each batch are monitored and changes made to optimise the protocols if required. For example in the last licence the use of killed Salmonella inoculum with the addition of Montanide adjuvant raised the titres of the ~40 factors used in the A-S screening serum. This has meant that the final product can be diluted 100% and resulted in a significant reduction in the number of rabbits used from 72 to 36 per year.</p>
<p>What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?</p>	<p>These reagents are also supplied to several research groups therefore minimising the numbers of animals required.</p>
<p>Which animal models and methods will you use during this project?</p>	<p>For antibody production rabbits and chickens are used predominantly as they have a proven ability to generate good immunological responses with high specificity of the resulting antibodies. This means there is less cross reactivity, so more accurate tests.</p>
<p>Why can't you use animals that are less sentient?</p>	<p>To produce antisera a competent immune system is required, this involves using fully developed animals with associated sentience. The process takes several weeks and terminal anaesthesia is used when the animals are bleed out, which stops sentience and maximises yield of blood/antisera.</p> <p>For antigen production live sentient animals are used only when cell culture or embryonated eggs cannot make antigen of suitable quality to give tests suitable specificity or sensitivity.</p>
<p>How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?</p>	<p>I have regular contact with the NVS, NACWO and NIO through various forums and use of the library function which can scan for relevant publications. In developing the Marek's Disease work, I have been in contact with researchers outside of the organisation who specialise in Marek's Disease work.</p>
<p>How will you refine the procedures you're using to minimise the welfare costs</p>	<p>As well as pre-start meetings involving the NVS, NACWO and animal care staff to ensure current knowledge is brought to bear, all projects are followed up by a wash up meeting. All aspects are discussed,</p>

(harms) for the animals?	was the project a success, what went well and if there was anything that could be done better. If there are any suggestions for refining the procedure they will be considered and if appropriate, incorporated into the protocol.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	HO The Harm–Benefit Analysis Process HO Guidance to ASPA HO Code of practice OIE (World Organisation for Animal Health) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. RSPCA Guidance on Welfare of rabbits and chickens LASA Guidelines on substance administration NC3Rs web site

Project	Reciprocal tumour-host interaction in cancer metastasis and therapy response	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cancer consists not just of malignant tumour cells but also many number of normal host cells that are not malignant. These include cells that provide nutrition and growth factors to the tumour cells and that form an essential support to the tumour as it progresses to be able to escape from the local environment to a distant site. This process is known as metastasis and is the primary cause of death among cancer patients. Unfortunately overall survival for these patients with metastasis has not changed for twenty years because this stage of disease	

	<p>cannot be operated surgically and is often resistant to anti-cancer therapies. This indicates that the current medicines are ineffective for treatment of metastatic disease and this demands development of new medicines in order to effectively cure the disease.</p> <p>The natural history of cancer is that they progress from benign (not life threatening) to malignant (life threatening) stages. Our and others studies have indicated that these transitions to the most dangerous stages of the diseases is speeded up by the normal cells in the tumour environment. Our previous research has indicated that killing these non-malignant cells in the tumour can inhibit tumour cell metastasis. Our experiments have mainly focussed upon the sub-populations of a type of blood cells called macrophages that reside within the tumour. In particular we have indicated that these tumour-associated macrophages are important for the spreading of cancer cells. At each step of this process macrophages provide support to the cancer cells. Consequently our general experimental plan is to use mouse models of cancer to define the molecules involved in the tumour cell-macrophage interaction and the relationship of these cells with other components/cell types of the tumour microenvironment. Thus while it is now established in the majority of pre-clinical models that the tumour microenvironment regulates metastatic progression the actual basis of these effects still largely remain to be explained. This project aims to determine the molecular basis for these tumour-stroma interactions using genetic and imaging methods, which will indicate targets that may lead the development of novel therapeutics directed to the tumour microenvironment and may ultimately be used in humans. The goal of this animal based research therefore is to develop an understanding of the cellular mechanisms responsible for the development and spread of cancer in an attempt to define possible therapeutic strategies in humans.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or</p>	<p>Through these studies, we will be able to reveal the role of tumour surrounding normal cells (stromal cells) in cancer metastasis and therapy</p>

<p>humans or animals could benefit from the project)?</p>	<p>resistance. This project also allows us to identify factors that mediate tumour-stroma interaction and affect cancer cell behaviour and efficiency of anti-cancer therapies. Such information will provide novel strategies for targeting stromal cells to treat cancer metastasis and therapy resistance. Comparison of stromal cell functions between healthy tissue and tumour tissue gives us essential information to generate more specific and therefore less toxic therapeutic strategies. Investigation of pro-tumorigenic functions of stromal cells will also provide important information for cancer researchers to develop novel prognostic markers to predict disease outcomes, diagnostic markers to follow disease progression and select optimal therapy to prevent cancer progression to the more deadly forms. The potential benefit of this research is to provide the basis for cure of metastatic disease and therapy resistance in a wide range of cancers.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mouse 126,000 over 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Both experimental and genetically engineered mouse (GEM) models of cancer develop benign to malignant tumours. This may cause moderate discomfort and can become severe in rare cases given extended time. However, their development is tightly controlled to minimize the suffering while allow scientific information to be collected. Furthermore, the tumour-bearing animals are monitored for their clinical signs, and are euthanized upon humane endpoint. All surgery and injection methods are well established and we have enough experience to minimize infection and health problems that may arise from these methods. So far, intravital imaging is the only method to reveal tumour cell behaviour and dynamic interaction with stromal cells in the complex environment. To obtain clear images, we will insert imaging window over the tumours, which will cause pain to the mice and possibly to induce post surgical inflammation but only in rare cases. However, pain is controlled by general anaesthesia and</p>

	<p>analgesics, and risk of infection is minimized by good surgical and aseptic techniques. The insertion site is monitored for signs of inflammation and infection, and antibiotics will be given if necessary. All the animals used in the experiments will be euthanized at the end point.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We use animal models in these experiments because tumour progression involves progressive genetic changes that cannot be modelled adequately by cells in culture. Furthermore, the complex tumour environment contains many different cell types whose interactions can only be found <i>in vivo</i>. For these reasons only animal models can be used. We chose mice as an experimental animal because cancer in these animals has been studied for many years giving us a good foundation for our experiments. Furthermore our group and others have carefully documented tumour progression in this species for several different tumour types. We have some mice in which human cancer cells or tissue fragments can be grown, which allows us to study them in a fashion that is not possible in culture. We have developed and are using a few <i>in vitro</i> assays to study certain aspects of the cell-cell interaction <i>in vivo</i>. We will review and incorporate alternatives throughout the project duration whenever possible.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>To understand molecular mechanism and identify potential therapeutic targets, our study requires various single and compound genetically engineered (GE) strains, which requires complicated breeding strategies. To minimize the number of animals involved we have now identified the most efficient breeding crosses to generate mice of the correct genotype. To reduce the required numbers of GE animals, we will transplant bone marrow or mammary gland from GE animals to wild type recipients because it can provide enough animals with same genetic mutations in blood cells or other cells without further breeding. We will also use repeated non-invasive <i>in vivo</i></p>

	<p>imaging and intra-vital imaging to monitor tumours and stromal interaction, which will greatly reduce the number of mice to be required.</p> <p>We ensure the experimental plans are designed using statistical principles to test our specific hypotheses and thereby only use the numbers of mice necessary. Group size will be determined based on our experience and published studies and power analysis based on preliminary data generated using minimal number of mice whenever not certain.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use mouse tumour models that have been developed to mimic the progression of the disease in humans as much as possible to understand the disease mechanism and identify potential effective therapeutics to treat metastatic cancers. The welfare cost is metastatic tumour in these mice. Our research group has developed many of these models and thus have extensive experience of the clinical signs. This allows us to effectively use humane end points to collect necessary scientific data while avoid severe health problems in the animals during the experiments. We will ensure that all animals receive the highest standard of care, and animal suffering is kept to a minimum by close monitoring, in consultation with NACWO and NVS, of health status and clinical signs using a scoring system agreed by NVS and Home Office Inspector. We will use our refined genetic modification techniques to improve the welfare of our animals. We also have developed methods to examine tumours repeatedly using non-invasive and non-terminal methods that minimise the numbers of mice used.</p> <p>Furthermore, we continue to monitor technical advances and to innovate novel techniques in an attempt to reduce the impact of experimental cancer on the animals. If suitable models occur, we will test and adopt in consultation with NVS and Home Office Inspector.</p>

Project	Recombinant Vaccines for Infectious Diseases	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Vaccines are amongst the most cost-effective tools in reducing the burden of infectious diseases. In the past century, widespread vaccination campaigns led to the eradication of smallpox in most parts of the world. However infectious diseases continue to cause significant morbidity and mortality. The WHO estimates that 23% of deaths worldwide are due to infectious diseases, with the highest burden in children under 5, where 64% of deaths are infectious-disease related (<i>World Health Statistic 2015</i>). Sadly the majority of these deaths continue to occur in developing countries. Malaria alone caused almost ½ million deaths in 2015, with the highest burden of diseases in children under 5 in</p>	

	<p>sub-Saharan Africa where it accounts for 67% of all malaria related deaths (<i>WHO 2015 Malaria Report</i>). In addition to the known big killers (malaria, tuberculosis, Influenza and HIV), emergence and re-emergence of dangerous pathogens have been identified as a serious threat to human health.</p> <p>The purpose of this project is to develop vaccines against malaria, tuberculosis, Influenza and emerging/re-emerging/neglected diseases. The work is divided into 4 stages</p> <ol style="list-style-type: none"> 1) Develop new vaccines and vaccination regimens to enhance the immune response 2) Test the efficacy of these vaccines against parasite, bacterial or viral challenge 3) Identify new vaccine antigens 4) Understand the kinetics and mechanism in response to vaccination and infection <p>The last stage includes understanding of how other factors, such as the microbiome and diabetes, affect the immune response and as a consequence protective immune responses induced by vaccination.</p> <p>There are currently 425 million adults with diabetes mellitus and this number is expected to rise to 629 million by 2045. People with diabetes have 2.3-4.3 times increased risk of TB and an increased risk to other infectious diseases. However there is very little information regarding the effect of subclinical and clinical diabetes on vaccine immunogenicity and efficacy. We will explore the impact of hyperglycemia on vaccine efficacy and the immunological mechanisms behind it. This information is essential for vaccines in development as they will be to be administered to an increasing population of people with diabetes or to people that will go on to develop diabetes.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The benefits of this work include 1) The development of vaccines against new emerging pathogens and major diseases traditionally viewed as very difficult targets. 2) Improvement to vaccination regimens to induce better and</p>

	<p>more robust immune responses 3) Increased knowledge about the immune response to vaccination and ways to improve immunity induced by these vaccines 4) Improved understanding of the immune response to infection by these pathogens (malaria, tuberculosis and Influenza) which will positively impact on future vaccine design. 5) Improved understanding of how the microenvironment can effect the immune response to vaccination. 6) Improved understanding of how a co-morbidity such as diabetes can impact the immune response to vaccination. As the viral vector and recombinant vaccine technology investigated is broadly applicable to development of vaccines for other diseases requiring robust cellular and/or humoral immunity, this could lead to the development of vaccines against other communicable and non-communicable diseases for both human and veterinary medicine. In addition, better vaccine design can only be achieved through improved understanding of the immune response to infection, as this can identify the type of immune response required and potential antigenic targets. The increasing incidence of diabetes and the susceptibility of diabetic people to infectious diseases is now appreciated. The majority of diabetic cases live in low and middle-income countries where infections such as malaria, TB and HIV are already prevalent. Defining the differences in the induction of immune responses between diabetes and health is essential for vaccines currently in development, as these vaccines need to be effective in people that have, or may develop diabetes in the future. If differences are detected data generated will have a significant impact on vaccines under development in many ways. For example, vaccine clinical trials will have to be performed on people with diabetes similarly to other risk groups such as HIV-infected people.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use a maximum of 31850 mice and 100 rats over 5 years. On average we have 20 active PIL holders per year with each using approximately 300 mice per year</p>
<p>In the context of what you propose</p>	<p>The majority of mice will be purchased from a</p>

<p>to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>commercial supplier and be vaccinated with a vaccine intramuscularly on two separate occasions. Mice are typically killed 2 weeks after the last vaccination and the immune response measured in an in vitro assays. On some occasions it will be necessary to challenge mice with malaria, tuberculosis or Influenza to demonstrate the efficacy of our vaccines. These mice may get sick as a result of the infectious challenge, however the disease course is well documented and mice are killed when moderate symptoms of disease develop. For the majority of mice challenged with an infectious organism, the scientific endpoint of the experiment is reached before mice show signs of systemic disease (liver-stage malaria challenge and tuberculosis). All mice are killed at the end of each experiment. To maintain our colony of mosquitoes and to provide a source of malaria parasites for challenge experiments, it will also be necessary to allow mosquitoes to feed on anaesthetised mice. To investigate the role of CD8+ T cells in mediating protection from malaria at the single cell level, we will perform multi-photon live imaging experiments. Whole mouse imaging will also be used to measure malaria parasite burden in the liver or vaccine distribution/longevity. As one main of the main hurdles in the development of vaccines against human diseases in the inability of the species that infect humans to infect rodent models (particularly malaria species). We will use humanised mouse models that can be infected with malaria in experiments to identify new malaria antigen targets. Individuals with Type II diabetes have an increased susceptibility to infectious diseases, with a 3x increased risk of developing TB, work on this project aims to develop diabetes models to study the impact on immune responses to vaccination. There is an increasing body of literature demonstrating the role of the microbiome (commensal gut bacteria) on the development of diseases, therefore we wish to study the impact of the microenvironment on the immune response to vaccination and development of efficacious immune response.</p>
<p>Application of the 3Rs</p>	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The immune response to vaccination and infectious disease involves multiple, complex systems interacting in a physiological environment often involving antibodies, T cells and cells of the innate system and therefore cannot be replicated in tissue culture. For pre-erythrocytic malaria there is currently no <i>in vitro</i> predictors of <i>in vivo</i> efficacy, although a number of exploratory <i>in vitro</i> assays are under assessment in our laboratory that might assist towards this goal. We will continue to investigate <i>in vitro</i> assays and <i>in vitro</i> organ culture systems.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Throughout the project we will employ several strategies to minimise the number of animals used:</p> <ol style="list-style-type: none"> 1) Prior to experimental work statistical power calculations will be performed allowing use of the smallest number of animals needed to provide satisfactory analysis of the data. We will continuously evaluate and update our statistical approaches and group sizes. 2) Past experience enables selection of experimental time points that maximise the amount of data while using the minimum number of time points. 3) Many experiments necessitate the inclusion of control groups to enable the comparison of new vaccines to a gold standard or most-promising vaccine to date. To minimise the repeated use of control groups, as many test conditions as possible will be included in an experiment. However the overall size of the experiment will be limited so as not to compromise the scientific integrity of the experiment. 4) Where possible the data from each individual animal will be maximised through the collection of multiple tissue samples at endpoints and/or sequential sampling from the same animals across a time course, eg blood sampling or whole imaging during sporozoite challenge. 5) The number of excess mice generated through breeding will be minimised by constant

	and careful monitoring of breeding programs.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We have chosen mice for these studies since they are the most characterised species for detailed immunological analysis. Mice have proved to be excellent indicators of immunogenicity enabling the clear assessment of novel vaccines and vaccination regimens for improvements. For isolation of primary hepatocyte we will also use rats that are larger and provides higher numbers of viable cells.</p> <p>To minimize suffering we only use well established disease models and the majority of mice are killed before systemic disease develops. Monitoring is tailored for each disease and is increased around the peak time of illness to ensure animal suffering is minimised. During an Influenza challenge when protected mice lose weigh before overcoming the infection and putting weight back on, water and food (often as mash) is placed on the cage floor to support animals during their most vulnerable period.</p> <p>We continue to investigate ways to refine our techniques to minimize animal suffering. Our previous experience ensures we can reduce the number of blood samples to only the key timepoints post-vaccination. As the primary focus of our program is clinical deployment, we primarily use adjuvants that have been approved for use in humans and have minimal side-effects.</p>

Project	Recovery of peripheral nerve function	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Loss of mobility and control of bladder or gut are all major concerns for patients with nerve injury. One solution could be peripheral nerve interfaces, devices that are implanted into cut or surgically teased nerves so that activity in the nerve fibres can be recorded or stimulated. In this project interfaces are being developed for bladder control, for restoring movement in amputees and for controlling visceral functions via the vagus nerve.</p> <p>This project aims to develop interfaces suitable for human use, to prevent the scarring reaction that currently limits their useful life, and to develop wireless communication from</p>	

	interfaces to receivers outside the body.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The aim ultimately is to restore function to human and canine patients that have lost bladder control after spinal cord injury, or who have lost limb function through nerve injury or amputation, although only rat and mouse models will be used in this early study. A nerve interface is used to pick up signals for limb or bladder control, and this is used to drive stimulators or nerve block to prevent unwanted bladder emptying, empty on command, drive robotic limbs, or control muscle contraction in a paralyzed limb. In addition vagal nerve recording and stimulation is being developed for control of the immune system, gut and other organs.
What species and approximate numbers of animals do you expect to use over what period of time?	625 adult rats 625 adult mice Over five years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The procedures are of moderate severity or, where possible, carried out exclusively under humane, terminal anaesthesia. The main adverse effects from nerve surgery are sensory changes that on very rare occasions lead to animals biting their toes. This can be minimized by choosing strains that do not do this and by humanely killing the animal if it is observed. Animals will be humanely killed at the end of the procedures, and most will be examined by histology.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The aim of the licence is to develop nerve interfaces for limb, bladder and visceral control. This can only be done by implanting prostheses into nerves in which there is electrical impulse activity, driving muscle contractions and bladder control. Tissue culture experiments would be meaningless because there would be no nerves to interface with, and no activity to record. In addition we need to develop methods to stop the scarring reaction that currently limits the useful life of prostheses. This only happens in animals with

	a working inflammatory system and a scarring reaction.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The main method for minimising numbers is careful preparatory work in pilot experiments with small numbers of animals, or terminal experiments on a few animals. By using fully developed methods, and behavioural experiments that we have fully validated in previous work we can get highly repeatable results, so the groups of experimental animals can be small.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rats are the best experimental animal for nerve interface development, because the nerves are reasonably large, there is sufficient space in the body or under the skin for connectors and interfaces, and the patterns of nerve impulses in response to movement are similar to those in humans.</p> <p>Mice are used when it is important to use genetically modified animals, particularly in experiments in which we are working out which mechanisms in the immune system are responsible for scarring in response to prostheses.</p> <p>All experiments are done on one side of the animal only, which means that there is little disability and no loss of bladder control.</p> <p>By choosing the correct strains of animals we can minimize the tendency of animals to bite their toes after nerve surgery.</p>

Project	Regeneration and cancer in epithelial tissues	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	3 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	How cells decide to multiply themselves, or not, is still not understood. Normal tissue growth and development involves strictly controlled cell replication. When a wounded or damaged tissue regenerates, extra cell divisions occur to help rebuild the tissue. However, uncontrolled cell divisions can produce tumours. We aim to understand how cells make these decisions in tissues. We are particularly interested in an important group of genes that are well known to control these decisions in insects, but have not yet been fully examined in the mouse. Since mice are closer to humans than insects, investigating these genes in mice is important to understanding human tissue regeneration and	

	human cancer.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The genes we are interested in are some of the most promising new cancer genes to be discovered in the past few years. The work on these genes has been mostly done in insects, where they are fundamentally important to controlling when and where cells multiply. Thus, investigating these genes in mice promises to advance our understanding of human tissue growth, regeneration and cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice only and the project will require approximately 10'000 animals over the term of the licence
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will use genetic engineering to knockout several genes, or combinations of genes, in the epithelial tissues of the mouse. We will then examine the knockout mice, where tissues may grow slower than normal. We will also examine knockout mice that are regenerating their tissues after wounding, which we expect may be compromised in the knockouts compared to normal mice. Finally, we will examine tumour formation in normal versus knockout mice. Tumours will be induced by either a genetic modification or via a chemical carcinogen. We do not anticipate harms during breeding, although animals sometimes die unexpectedly during this process at a low frequency of less than 1%. Experimental knockout mice can sometimes develop unexpected symptoms, so will be carefully monitored to ensure they are healthy and pain-free. Any signs of distress will be attended to immediately to ensure the animals do not suffer. However, we do not expect any moderate or severe symptoms to occur in our skin-specific conditional knockout mice. Moderate symptoms may occur in other tissues, in which case the animals will be monitored for signs of suffering and euthanised appropriately. To examine tissue regeneration, we will need to induce small wounds of 6mm or less to test the ability of the knockout mice to heal those wounds. We expect only mild severity of suffering for these experiments. To examine tumour formation, we will need to induce small tumours in epithelial tissues that

	<p>will not be allowed to grow to a large size that causes the animal severe distress or pain. We expect mild or moderate severity for these experiments.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Our laboratory is combining experimental analysis in mouse with mammalian cells grown in culture as well as using the fruit fly <i>Drosophila melanogaster</i>. All the molecules and molecular pathways that we are studying in mice are also present in <i>Drosophila</i>.</p> <p>In our research pipeline, we are starting our analysis of the YAP protein and other proteins in the Hippo pathway in <i>Drosophila</i> before confirming our results in mammalian cell culture and later in mice, <i>in vivo</i>. Indeed, mouse tissues are closer to human tissues than are the tissues of insects and the genes and proteins are much more similar, for example both mice and humans have two Hippo effector proteins, YAP and TAZ, with slightly different functions, whereas <i>Drosophila</i> has just a single one, Yorkie.</p> <p>Ultimately, we must perform these experiments in mice to establish the relevance of these genes to human development and disease. Our work in <i>Drosophila</i> is on-going in parallel with the mouse work, and will continue to inform the mouse experiments to ensure efficient use of animals. In addition, we are using mammalian cells grown in culture to test drug molecules for desired effects. However, cells grown in culture are still very different from a whole organism, so ultimately these results need also to be confirmed in mice.</p> <p>In parallel, we also seek to replace mouse experiments by conducting more work in organoid cultures, which are essentially miniature, highly simplified organs. Whilst the gut organoids do originate from mouse guts and thus do require an animal for their generation, the proliferative capacity of the cells means that the cultures can be expanded and used in multiple experiments beyond what a single mouse gut <i>in situ</i> may be used for.</p>

<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will minimise the number of animals used firstly via the use of inbred mouse strains, which reduce variability. Secondly, mouse colonies will be tightly monitored by experienced staff to avoid overbreeding and regulate animal numbers. When designing a programme of work we will always estimate the number of animals required with the probability to obtain the proper genotype in order to avoid overbreeding as well as keeping in mind statistical principles. Wherever possible mouse littermates will be used as control animals.</p> <p>We will also aim to maximise the amount of data we can obtain from each mouse, for example, multiple tissues will be collected from a single knockout animal and each tissue will be divided into multiple samples which are processed in different ways to yield different types of data.</p> <p>Once we will acquire significant results for a specific genotype and conclude we no longer need to perform further experiments, this specific line will be frozen down and no longer bred.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Our animal units are well equipped and their technical staff is well trained to make sure that mice are sufficiently provided with food, water and their litter is changed often. In addition, mice are provided with extra bedding. All the mice are monitored for any sign of discomfort, harm or health distress and actions will be taken to tackle any issue arising, from isolating a wounded animal to its euthanasia if its burden cannot be alleviated.</p> <p>The protocols we wish to use to generate and analyse knockout mice are highly developed and routinely used within our institute. Therefore, their potential welfare costs are well known and described and strategy has been put into place to prevent any harm unrelated to the experimental manipulation.</p> <p>All the designed Programs of Work, an experimental plan required prior to undertaking any mouse experiment and approved by our inhouse animal welfare specialists, will contain a detailed list of potential adverse effects and we</p>

	<p>will put in place ways of monitoring the animal for its general welfare and more precisely for the specific listed adverse effects. Mice will also be monitored for adverse effects and euthanised at the appropriate stage to minimise suffering. We will use anaesthesia, antibiotics and analgesics where necessary. We will set humane endpoints for our studies so that mice do not experience unnecessary suffering.</p> <p>When performing a new experiment only a reduced number of animals will initially be treated to limit any unforeseen complication. Observation of the animals during the initial experiment will then inform us on any effect on the welfare of the animal and the experimental design will then be tailored to prevent or minimise any unnecessary burden.</p>
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Project	Regeneration in the nervous system	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To identify how ageing and disease impair myelin regeneration in the central nervous system (CNS, which comprises the brain and the spinal cord), by adult stem cells and to develop new therapies to promote myelin regeneration. The fibres of nerve cells (axons) are wrapped in layers of fatty membrane (myelin) which protect them and allow them to transmit electrical impulses very rapidly. Myelin in the central nervous system (CNS – which comprises the brain and the spinal cord) is produced by cells called oligodendrocytes. Oligodendrocytes and myelin are lost in diseases such as multiple sclerosis (MS). If myelin is damaged (demyelination) and not restored, the nerve	

	<p>fibres will not work properly and will eventually die. The loss of nerve fibres is irreversible, and its accumulation due to myelin regeneration failure accounts for the currently untreatable progressive phase of MS. Although stem cells in the brain are capable of making new oligodendrocytes that restore myelin, this regenerative process declines with age. Currently, there is no therapy promoting myelin regeneration. To meet this urgent unmet clinical need, it is necessary to know exactly what causes this failure and how to overcome it.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The project will advance the understanding of 1) myelin regeneration in the nervous system, 2) the factors and conditions that influence this process, and 3) why myelin regeneration fails in diseases and ageing. Our discoveries may be harnessed to develop new regenerative therapies in the treatment of MS.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This project will use rats and mice. We expect to use up to 13,600 mice (including breeding genetically altered mice) and 2,700 rats over a five-year period. The actual number of mice is expected to be lower than the above figure because some of the mice bred under breeding protocols will be used in the experimental protocols on this licence.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We will predominantly use animal models that involve creating a very small area of injury in the CNS. Most of these models require surgical procedures, which usually take 30-60 minutes under anaesthesia. More than 90% of the animals will recover spontaneously without showing apparent deficits in normal functions. A small proportion (<10%) of rats undergoing brain injury may exhibit a significant loss in movement control and balance, which will be killed humanely. Some animals will be subjected to experimental interventions such as delivering drugs by injection or implantation of slow releasing minipumps, and imaging under a relatively long period of anaesthesia. These procedures likely lead to considerable discomfort, stress and even pain to the animals, indicated by signs such as reduced activity, subdued behaviour. Animals receiving certain</p>

	<p>substances (e.g. compounds to be tested) may suffer considerable weight loss due to toxicity and affected food and water intake. We will make sure the animals undergoing procedures to have adequate pain relief as standard, e.g. during and after surgery. If animals are identified suffering considerably from experimental procedures (reaching the established end points) indicated by e.g. loss of mobility, sharp reduction of body weight, signs of distress or pain not readily alleviated, they will be humanely killed. Some animals in this project will be subjected to calorie restriction or exercise or behavioural tests, which are expected to cause no more than temporary stress until the animals have acclimatised. <u>Certain strain related defects may develop in genetically modified animals such as malocclusion, the overgrown of misaligned front teeth in rodents which affects normal eating. Although these animals may be maintained healthy by trimming the overgrown teeth, we will only carry out the procedure if we cannot obtain healthy alternatives to minimise stress and incidence related to the procedure.</u> All animals used in the project will be killed under anaesthesia or humanely using another method and at end of studies, tissues are harvested for further analysis.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>CNS (brain and spinal cord) myelin regeneration is a complex biological process involving many interacting cell types locally and also affected by factors of whole body (e.g. from blood). At present, there is no non-animal substitute that can accurately replicate CNS regeneration. Although non-protected animal alternatives (non-vertebrates) may be useful for studying fundamental cellular processes, they do not exhibit the same level of complexity in the CNS, i.e. their nerve fibres do not have myelin sheaths. For this reason, mammals are the only option from which we can gather enough information to meet the objectives of the project. We therefore use mainly mice for our work and a lesser number of rats.</p>

	<p>There are some alternative techniques that can be considered as a partial substitute: using cultured cells isolated from animals; using human induced pluripotent stem cells (cells capable of generating any cell type in the body) and using synthetic fibres to replace axons. However, these techniques are still in their infancy and cannot completely substitute the use of animals. We will however, conscientiously explore the opportunity to develop and maximise the use of these techniques wherever possible.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will ensure our experimental design is sound, and we aim to use the minimal number of animals that will give statistically meaningful data.</p> <p>Experimental groups will be randomly selected to consist of animals with same age, sex and strain. The number of animals in each group will be determined by a statistic principle based on existing data from similar studies or early phase so called 'pilot' experiments. In addition to randomisation, proper controls and sample sizes, we will avoid the introduction of bias that may otherwise influence interpretation of results by “a blind” approach, so that the researchers are hidden from the information and identities of the animals (e.g. control or treated) during the experiments until all the data have been obtained.</p> <p>Where possible, repeated non-invasive tests (e.g. imaging, behavioural tests) will be performed on the same animal to maximize the efficiency and robustness of data acquisition, reducing overall animal usage whilst simultaneously ensuring there is no increased harms to animals used. We will continue to use cell and tissue culture in our studies to obtain preliminary data before using animal models. This ensures only the most promising experiments are progressed and performed on animal models.</p> <p>Where possible, we will use human tissue and cells with appropriate ethical permissions in our work which will reduce the use of animals.</p> <p>We will ensure our materials, e.g. control tissues and data are shared with other researchers to</p>

	eliminate the unnecessary repetition elsewhere of the same experiment using animals.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The animal models proposed were chosen because of the minimal functional loss that they induce. Among the models available, we will always choose the one which is least harmful to the animals, gives the clearest results and is the most consistent.</p> <p>In conducting animal work, we will always ensure most appropriate handling and housing, minimising the impact of single housing of animals (which is at times scientifically necessary) and other sources of stress. We will take appropriate measures (pain relief and anaesthesia) to minimise pain in the animals undergoing procedures. We will limit the numbers of procedures on individual animals to prevent cumulative and unnecessary harm. Whenever feasible and scientifically appropriate, we will choose the least harmful procedures for our tests.</p> <p><u>Non-specific developmental defects or adverse signs may occur in genetically altered mouse lines, such as retarded growth, or 'runt', and malocclusion, overgrowth of misaligned front teeth. We will always ensure proper selection of breeding animals to avoid the presence of such traits in the background based on their recorded health status in the 'family tree', so that the occurrence of the defects may be eliminated or minimised.</u></p> <p>Before testing a new compound which not previously been used in animals we will perform small scale 'pilot' experiments with low numbers of animals to determine effective dosage and toxicity to minimise potential harm on larger scale studies.</p> <p>We will continue to improve the method of detecting the impact of adverse effects of procedures on animals, using a consistent, objective measurable way to record pain, stress and functional deficits e.g. utilising 'scoring' systems where appropriate.</p> <p>We will ensure adequate training of all</p>

	<p>researchers who carry out animal work and continue to develop effective measures to reduce pain and discomfort for animals under procedures such as incorporating pain relief agents into food supplements after surgeries as an alternative to giving by injection, and work with animal technicians to strengthen our monitoring system.</p>
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Project	Regulation miR-29 targets in wound repair	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The incidence of non-healing skin ulcers and the need of improved skin regeneration is continuously increasing in our society. For the development of efficient strategies to improve skin repair it is essential to understand the mechanisms underlying normal and impaired healing. For my project, I am seeking to further describe functions of small ribonucleic acids (RNA) molecules, which I identified as regulators of the top layer of the skin called the epidermis. Upon wounding, the cells of the epidermis (mostly keratinocytes) are responsible for covering the wound site with a new layer of skin that protects our body from infection entry and water loss. Keratinocytes can sufficiently grow and move into</p>	

	<p>the wound site only in a healthy skin. The overall goal of this research is to improve normal growth of keratinocytes and thereby, repair of the top layer of skin. Thus, it is important to find molecules that regulate and ensure a fine-tuned control over the normal growth of keratinocytes. Small RNAs, like miR-29s, have been already described as regulators of normal skin growth. Importantly, because of the small size and chemical properties of short nucleic acids, microRNAs (for example, miRNAs-29, or in short, miR-29) can be used for molecular therapy. miR-29s have already been tried in the clinic to improve skin condition in patients with inappropriate skin growth, and thus can potentially be rapidly used for molecular therapy of other skin diseases, including wounds. However, it requires knowing all possible molecules that interact with miR-29s inside keratinocytes, and all intra- and intercellular processes, which may be regulated by miR-29s in the epidermis. I will study the effect of miR-29 on growth and movement of keratinocytes and will use substances to inhibit miR-29. This will allow development of the new strategy to achieve a successful regeneration of the skin during wound healing, after massive burns, and in reconstructive surgeries. I will study the function of miR-29s in murine wound healing model, necessary to mimic the complexity of the regeneration in a full body model. The long-term goal of the project is to utilize miR-29 to improve skin regeneration in patients suffering from large acute wounds, bedsores, and ulcers.</p> <p>Our main objectives are to:</p> <ol style="list-style-type: none"> 1. Identify the underlying molecular mechanisms that contribute to dysregulated wound healing. 2. Identify potential new therapeutic strategies to promote healing in humans and animals.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>We hope this study will develop the potential for gene and cell-based therapies to aid patients with large-area wounds. In addition, we hope this study will benefit animals with wounds.</p>
<p>What species and approximate</p>	<p>Over a 5 year period: 2720 mice (2400 for</p>

numbers of animals do you expect to use over what period of time?	breeding purposes and 320 for experimental procedures)
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals will be monitored for adverse events using score sheets developed in conjunction with the NVS and NACWO. This will allow objective measurement of clinical signs associated with the adverse event to determine when the humane end-point has been reached. This study is designed to understand how microRNAs control normal wound environment and how this mechanism can be potentially used to improve wound environment. Anaesthetised mice will two receive small (6 mm diameter) wounds on their skin so that we can compare the processes involved in wound healing in wild-type (normal) mice with the miR29a deletion. We will apply the short microRNA-like nucleotides to the wounds that we believe will enhance wound healing, in order to find the best treatment for large-area wounds. After surgery, mice will be provided with pain relief and monitored closely for any signs of distress. Distress in mice after this type of surgery is very rare, however, if there is any indication of suffering that cannot be controlled by pain relief, the animal being humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have to use animals in this study because understanding how different types of skin cells interact with wound healing in a pathological environment must be studied in the complete physiological setting. That way, we get an accurate picture of this process. Mice offer the best animal model which can be genetically altered to accurately model the systemic response to skin damage in humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By reading the scientific literature, we will avoid repeating anything that has already been done. For example, previous studies found very little correlation between wounds within the same animal, suggesting that use of wound biological replicates is acceptable for the accurate estimation of the histological measurements. Thus, we will use two wounds per animal to reduce the number of animals required.

	<p>By consulting with colleagues that have expertise in our area, we will refine our experimental design. This will reduce the chances of inducing an adverse effect and reduce the number of animals needed to accomplish the objective. To plan for our animal work, we have consulted a statistician to establish the minimum number of animals required for each study.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The species and models we have chosen are based on how well they model systemic response to wounding in humans, their sensitivity (they are the least sentient models we can use for our study), how well-characterised they are, and our expertise. The animals will be given anaesthesia and analgesia when they undergo wounding. They will also be given pain killers so when they wake up they will not have any discomfort. Mice will be watched closely to make sure they do not show any signs of being in pain or becoming ill. If they appear to be in pain or appear unwell, veterinary advice will be sought.</p>

Project	Regulation of axonal transport in neurons in health and disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Our aim is to identify how substances are transported within nerve cells. This essential process, which is called axonal transport, provides a delivery system that connects one end of a nerve cell to the other. Nerve cells are vulnerable to defects in axonal transport, resulting in neurodegenerative diseases, such as motor neuron disease, Alzheimer's disease and pathologies affecting peripheral nerves.</p> <p>We plan to use a combination of imaging, chemical (i.e. drugs) and genetic approaches to define the mechanisms of axonal transport and t</p>	

	<p>relevance to neurodegeneration. We will determine the consequences for axonal transport of mutations that cause neurodegenerative diseases by performing experiments with nerve cells taken from healthy and genetically-altered, disease-modelling mice. We will also do this in living animals using a special type of microscopy that allows us to analyse the movement of components transported in intact nerves. We will generate novel disease-modelling mice in which axonal transport is compromised and we will identify the consequences on animal survival and wellbeing. These experiments are essential to understand how and why axonal transport is impaired in disease, and will help us to formulate new remedies for neurodegeneration. Indeed, we will treat mice displaying the symptoms of human neurodegenerative conditions, such as motor neuron disease and Alzheimer's disease, with novel drugs which restore axonal transport and assess whether these treatments improve overall symptoms and survival.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Our work will help uncover the causes of nerve cell death in neurodegenerative conditions, such as motor neuron disease and Alzheimer's disease. Understanding how nerve cells survive or die is key for a better diagnosis of these human diseases and to generate more specific and effective therapies able to stop progression of these pathologies. Information gained in this research project will also have impact in other areas of human medicine, such as the development of novel gene therapy approaches to treat neurodegenerative diseases, as well as an improved understanding of how certain toxins attack the nervous system.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We plan to use mice and a small number of rats in our research programme. Even though we are frequently using mouse embryonic stem (ES) cells and human reprogrammed stem cells (hiPSC), which do not require animal use to generate different types of nerve cells, mice remain the most reliable source for naturally developed nerve cells. Many mouse models of human neurodegenerative conditions are available and these mice provide us both</p>

	<p>healthy and sick neurons for our studies. These mice allow us to test the role of alterations of axonal transport in neurodegeneration and the effects in restoring this process on nerve cell survival. Mice therefore remain the best species of choice for our research programme. In view of our scientific targets and the size of our laboratory, we anticipate that we will breed and maintain less than 10,000 mice in the next five years. Additionally, we plan to use 50 rats in selected experiments to validate the results obtained in mice.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Our research programme requires the generation and maintenance of mice in which axonal transport is compromised. We will determine the consequences of these alterations in cells and tissues, starting from development until late in life. Cell death will be determined. We will perform tests to monitor the ability of these animals to move, to sense touch, pain and other external stimuli, and their ability to interact with the environment and to record memories. Mice will also be used to monitor alterations in the transport of cellular components along their nerves. We will test the effect of drugs that have been shown in nerve cells in culture to modulate axonal transport positively or negatively (chemotherapeutics), and use the former group to reverse the deficits in this process in mouse models of neurodegeneration. We will measure the effects of the drugs on the symptoms of neurodegeneration, including nerve cell death. The severity of the harms in this licence is moderate. Examples of adverse effects are: abnormal positioning of the limbs, deficits in the ability of mice to move freely, which may ultimately result in paralysis, progressive nerve cell death, male sterility, lethality in the womb or early life in rare cases. Health conditions of the animals will be carefully monitored to adhere to moderate severity levels. Mice showing any unexpected harmful phenotype will be promptly culled.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p>	<p>Animals are required as a source of nerve cells to test the effects of impairments in axonal</p>

<p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>transport both during development and in adulthood. Although invertebrate models have been used previously to study this phenomenon, the mouse remains the system of choice to understand how impaired axonal transport contributes to nerve cell death and to evaluate the effects of novel drugs repairing these deficits in disease. This is due to the similarity in how mouse and human nerve cells work, and the vast range of mouse models closely mimicking human neurodegenerative diseases currently available.</p> <p>We have explored alternatives to replace animals in our experiments, such as the use of cell lines, and neurons derived from mouse embryonic stem (ES) cells and human reprogrammed cells (hiPSC) from patients. ES- and hiPSC-derived nerve cells effectively replace nerve cells obtained from mice in some analyses, such as drug screens and preliminary tests to assess the consequences of removing genes on axonal transport and nerve survival. However, mice (and rats) are irreplaceable for testing the effects of these mutations on the development of the nervous system, its maintenance in adulthood and to test the effects of novel drugs repairing axonal transport during the progression of neurodegenerative diseases.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Established protocols for nerve cell differentiation using ES (mouse) and hiPSC (human) cells allow us to substantially reduce the number of animals required for our research; for example, these cells have been used to test large numbers of drugs for the ability to repair deficits in axonal transport.</p> <p>A further reduction in the number of animals used in our research is achieved by using viruses that specifically travel to nerve cells. These viruses either cause the disruption of specific genes, or enable the production of altered proteins, which cause human neurodegenerative diseases, in healthy or genetically-altered mice, allowing us to induce the desired negative impact on nerve cells only in the minimum number of animals required for the analysis rather than employing extensive</p>

	<p>breeding programmes.</p> <p>The use of optical windows for imaging will allow the repeated observation of a nerve over time in a single animal, which will decrease the variability of our measurements, ultimately reducing the total number of mice and rats used in our studies.</p> <p>We also grow nerve cells in small chambers that contain miniaturised tunnels, which direct the growth of nerve cells, better mimicking the conditions found in the body (e.g. nerve to muscle connection) and facilitating imaging. This system increases the percentage of successful experiments, therefore decreasing the overall use of animals and cells isolated from them.</p> <p>Experiments will be designed in consultation with statisticians to minimise the number of animals required and results will be assessed together using specialised computer software to assess significance, data spread and will facilitate the identification of novel treatment options.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse remains the animal of choice to understand the mechanisms controlling axonal transport because the process is very similar in mice and humans, and several reliable neurodegenerative mouse models are presently available that show deficits of axonal transport associated with neurodegenerative disease symptoms.</p> <p>Animal care and experimental protocols are well established in our laboratory and through collaborators, minimising the use of animals and their suffering. Pain relief will be used whenever possible.</p> <p>We will use mouse models in which gene loss or expression of a disease protein is induced by a specific drug or a selected virus only in cells that we have chosen, thus minimising disease symptoms.</p> <p>Humane endpoints have been introduced to restrict animal suffering strictly within moderate limits, both for established disease models and newly established mouse lines. Local paralysis</p>

	<p>will be used as humane endpoint for the challenge with paralysis-causing agents, such as tetanus and botulinum neurotoxins. Furthermore, we will use a range of dosages inducing a slow appearance of symptoms, making it easier to avoid unexpected adverse effect or symptoms exceeding the established limits.</p>
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Project	Regulation of brain function from early life to old age	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Stress is an increasing problem in today's lifestyle and is an important environmental factor associated with age-related memory decline and the increased incidence of mental health disorders such as depression. Results from the UK Mental Health Foundation's 2018 study sampling 4619 adults, revealed 74% of people felt so stressed in the past year that they have been overwhelmed or unable to cope. Although a little stress is beneficial in the short-term, prolonged stress can have detrimental effects on the brain and body. Stress in middle age may accelerate memory decline with subsequent ageing and increase the risk of dementia. Early life stress (while in the womb) may also have long lasting effects	

	<p>that lead to memory and mood problems into adulthood and old age. Glucocorticoids (steroid hormones) produced in the adrenal glands and released following stress play a key role in the process. This project aims to increase our understanding of how glucocorticoids modify brain function (especially memory and mood) with a focus on ageing and early life. We will investigate the effects of genetic and behavioural or environmental changes that may alter glucocorticoid levels and test drug treatments to improve brain function.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This research will increase our knowledge and understanding on the mechanisms by which stress or glucocorticoids particularly in early life, midlife and old age leads to memory and mood disorders. Such insights may lead to future development of novel drugs for the treatment of age-related memory impairments (or brain fog) including dementia and stress-related brain disorders (e.g. Post-Traumatic Stress Disorder). The knowledge gained may benefit public health and social care by providing research data for the media to implement stress management strategies and as a basis to introduce other interventions that promote healthy brain ageing and mental health.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice – 9100 over 5 years (around 60% will be for breeding genetically altered animals). Rats – 1100 over 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The main output measures from this project are from behavioural testing of the experimental animals. Mice or rats of different genetic strains, elderly or following a stressful event are placed in different types of mazes and their memory function assessed. In general, these have no anticipated adverse effects and the expected level of severity is moderate. Chronic stress exposure will impair memory and, in our experience, may cause some weight loss (usually ~10%) but this will not be allowed to decline further than 20%. In young adults, the adverse effects of chronic stress on memory are reversible as the animal recovers. However, stress exposure in</p>

	<p>pregnant animals can have long term effects on the behaviour of the offspring such as increased anxiety. Chronic stress in middle aged or older animals can also have long term effects on anxiety and depressive-like behaviours even months after the end of stress exposure. Pain relief will be provided following surgical procedures to deliver drugs to the brain and best practice guidelines will be applied to minimise any surgical complications. Problems arise with elderly animals much in the same way as elderly humans – loss of hair, reduced levels of activity and mobility, spontaneous tumours, ulcerated feet. These are sometimes treatable by the vet, and where not, the animals will be humanely killed. At the end of the experiment, animals will be humanely killed and blood, brain and peripheral organs collected for laboratory assays.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Animals are required because the effects of stress, measurements of behaviour and steroid levels within specific brain regions cannot be modelled in vitro. The study of the effects of stress on brain function during ageing means that we need to use the whole animal in our experiments. Where possible, we will use post-mortem human brain tissue samples or cell cultures to analyse altered genes or proteins of interest to complement the in vivo animal studies.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of animals used is based on our previous experience with similar study designs and procedures and also on calculations to determine optimum group size and ability to detect an effect if there is one. Where possible the minimum number of animals will be used. Furthermore, multiple measurements of blood steroid levels, behaviours and body weights will be taken from the same animal over time, thus reducing the number of animals needed to provide strong enough data to answer our question. Non-invasive in vivo brain imaging carried out in the same animal before and after interventions also reduce animal numbers.</p>

	<p>Study design is based on current best practice and, where necessary, following discussion with statisticians and/or bioinformaticians.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice and rats will be used because their brain anatomical structure, physiology and function (including learning and memory) are similar to humans. Most of the work will be in mice as the alteration of genes of interest are more widely studied and better characterized in this mammalian species. The Alzheimer's disease (AD) mouse models to be used develop AD-like pathology with ageing similar to the human condition and is the most appropriate to examine the combined effects of stress and ageing on the development of AD pathology. In all our experiments we are mindful of the need to reduce suffering, and appropriate refinement to protocols including the use of pilot studies and in vitro cell culture experiments will be incorporated where possible. The general health of the animals will be monitored as they age (e.g. gait, visible tumours, teeth, activity, coat condition, posture etc). Animals will be checked for visual abnormalities in appearance or behaviour on a daily basis. If mice show any of above abnormalities, veterinary advice will be sought. Mice that show signs of marked deterioration with age related problems (e.g the development of tumours) will be humanely killed.</p>

Project	Regulation of early heart development in vertebrates	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Cardiovascular disease is the biggest killer in industrialised world and is a major medical concern. An efficient way of treating heart failure will be to replace the diseased tissue with new, healthy tissue, ideally from the same patient. Whilst this is not possible now, an emerging field of heart repair, or cardiac regenerative medicine, is developing the necessary knowledge and procedures that will make it possible.</p> <p>Our research focuses on early embryonic development of the heart. This research requires the use of animals. Even though heart muscle cells can be made from embryonic stem</p>	

cells in culture, providing a powerful in vitro model, there is still a requirement for a complementary in vivo model. As it is difficult to study early heart development in mammalian embryos, we are using frog embryos instead. Frog embryos are our choice model, as they are easy to obtain in large numbers, they develop rapidly under very simple culturing conditions, and also because they do not require heart for early development, so any experimental manipulation or mutations affecting the heart will be tolerated for several days. At the same time, frogs are vertebrates that share many features of development with mammals, including us, so most findings obtained from studies on frog embryos will be of broader significance. We use frog embryos to understand how heart muscle cells develop in the vertebrate embryo; what is the recipe for producing heart, instead of, for example, brain or muscle? We are trying to understand what are the first molecular signals for heart development and what are the key early genes that are mediating heart development.

If we understand the details of the process of making a heart muscle cell in the embryo, we may be able to use this knowledge to create heart muscle cells ourselves, from human embryonic stem cells, or from almost any type of adult cell that is first reprogrammed to be like an embryonic stem cell.

An additional outcome of our research might be a better understanding of the mechanisms that cause congenital heart disease, the most common form of birth defects.

We almost exclusively obtain embryos for our work from South African frogs (*Xenopus*) by natural matings. Egg-laying is induced by injecting human Chorionic Gonadotrophin (this was the basis for an early version of pregnancy test), a mild procedure that is repeated 3-4 times per year for each female animal. The animals are kept in a purpose-built facility with constant water purification and temperature control, and they are kept at optimal density (these are social animals and do not thrive under low density or isolation). Our colony has several hundreds of frogs, which provide many thousands of

	embryos for our work each year.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Improvements in cardiac regenerative medicine: by providing better directed differentiation protocols to supply new myocardium. Also better understanding of the mechanisms that cause congenital heart disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Frogs <i>Xenopus laevis</i> and <i>Xenopus tropicalis</i> . 1200 adults and 6,000 larvae over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Our routine use of adult animals in this project is to produce wild type or genetically altered embryos which involves an injection procedure of very low severity for the toads. The project involves establishment, breeding and maintenance of genetically altered lines, which are not expected to produce major adverse effects. Adults may be genotyped by having toes or tails clipped but this does not cause any long lasting harm to the animals. The majority of the work under this project is performed on embryos and therefore the severity is low.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Research on early heart development requires embryos. Non-animal alternatives such as production of cardiomyocytes from human embryonic stem cells cannot yet fully replace this requirement.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Adult animals are used repeatedly to provide gametes after optimal recovery time. Embryos are obtained primarily by matings instead of in vitro fertilisation. Both practices act to reduce the number of animals that would have been used otherwise.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to	The frog <i>Xenopus</i> is the lowest complexity vertebrate model used for studying embryonic development. At the same time it is a vertebrate that shares many features of development with mammals, including us, so most of findings that we obtain from this non-mammalian model will

minimise welfare costs (harms) to the animals.	be of broader significance. Minimising harms is primarily through the use of general anaesthesia during toe and tail clipping and regular monitoring following the procedure.
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Project	Regulation of Energy Balance	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>We currently face an obesity epidemic together with an increasing ageing population; obesity and ageing are two major risk factors for a number of life-threatening diseases such as cancer, type 2 diabetes and cardiovascular disease. Calorie restriction (CR; ie. dieting) is the primary self and physician prescribed treatment. However, successful weight loss is often short-term and long term dieting and sustainable weight loss appear to be unachievable to many. Maintenance of a steady body weight requires energy intake and energy expenditure to be in equilibrium. This is known as energy balance, which is a tightly regulated, complex system. The body is fine tuned to recognise when energy supply is low and when we diet the body preferentially uses energy from our fat stores to make up the negative energy</p>	

	<p>imbalance, however, lean tissue is also utilised. Similarly, when an individuals' energy levels are in surplus both fat and muscle are stored. Changes in dietary composition ie. the source or amount of protein, carbohydrates or fat, also appear to alter the loss/ gain of body fat and lean tissues. While we know the coordination of energy balance is centrally controlled, we do not have a clear understanding on how the body recognises different dietary components. The aims of the project is to identify key genes and hormones involved in this response and ultimately design diets where specific macronutrients promote changes in body composition and link these changes to the to the key genes and hormones involved.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The identification of key genes and diets that promote fat loss while maintaining lean mass will be of benefit to several public health related issues such as obesity and its co-morbidities (cancer and type 2 diabetes).</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>To address the highly variable response to dieting between individuals we will use mice as a model. Similar to humans the mouse also responds with varying degrees of weight loss (or gain) on a diet. Knock-out mice models may be generated from the identified genes which will allow the validation of their role in controlling energy balance. Aged mice up to 2 years may also be used for studies investigating the specific gene involvement in the life extending response to calorie restriction. Over the 5 year project licence we estimate the use of 3500 mice.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>CR is known to improve not just health but also lifespan, so although the mice will lose some weight this is not harmful. All dietary manipulations are tested for palatability before use. Measuring food intake can be done easily by weighing the food left each day. Animals will be handled and weighed daily. Some animals will need to be left to get old, but any animal experiencing age-related disease inflicting pain such as arthritis will be removed from the study. However to measure energy expenditure accurately we implant a transmitter. The surgery should not result in a greater than moderate severity, because pain is controlled by use of pre- and post-surgery pain killers and anti-inflammatory drugs. Once implanted</p>

	transmitters are non-invasive and do not affect the activity or behaviour of the mice. Blood sampling will cause very short term needlestick pain. Administration of compounds affecting energy balance will be done via injection or the surgical implantation of small pumps subcutaneously, again using pain killers. In some instance these will be replaced once. To measure the changes in individual tissues, hormones and genes the mice are humanely culled at the end of study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible <i>in vitro</i> studies will follow up work from the animal research, ie specific response to compounds in muscle and fat cells can be done using cell culture. However, is vital to understand how diet, hormones, genes interact and this cannot be done <i>in vitro</i> . Unfortunately there are no feasible alternatives to measure whole body response other than to use the mouse model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	From 1 single mouse information on numerous levels are obtained from body weight to activity to gene expression levels. Study plans are rigorously designed to obtain the maximum information from the minimum number of animals. As part of the design we seek statistical advice to ensure animal numbers are adequate to meet statistical significance.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	While mice are the ideal model for looking at the phenotypic responses to manipulations of energy balance, we will use knockout models to ascertain the function of any genes of interest we identify. Any suffering throughout these protocols is minimised and appropriate analgesia and anaesthetics used where necessary. We have experienced staff and advice from NACWOs and NVS are sought where necessary. All animal procedures and analytical assays are designed to be as non-invasive as possible and the majority of our data are collected using non-regulated procedures. Where absolutely necessary surgical procedures are used with guidelines on aseptic techniques strictly adhered to. All techniques as well as the follow assays are refined to cause the least stress to the animals.

Project	Regulation of Glomerular Barrier Function in Health and Disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The overall focus of this research is to determine how the kidney filters are maintained in health and disrupted in disease. The experiments I propose will improve our understanding about the biology of kidney filters before applying this knowledge to test therapies that will prevent or stabilise the effects of kidney disease in animal models. These studies will be the prelude to human studies of new treatments for kidney disease.</p> <ul style="list-style-type: none"> • We aim to find early biomarkers for kidney disease. We have already shown that mice with kidney disease show very 	

	<p>early changes in the kidney filters using a powerful technique called proteomics and we can now test whether these biomarker changes provide an early warning about disease.</p> <ul style="list-style-type: none"> • We aim to find the molecular mechanisms that cause kidney disease to progress in severity. We have shown that disrupting the ability of the kidney filters to respond to mechanical forces can affect the progression of glomerular disease and we now want to find ways of protecting the kidney filters from these forces. <p>Using the knowledge we acquire from the above aims we expect to identify and test new therapies for kidney disease in zebrafish and mice.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Chronic kidney disease (CKD) is a huge public health concern, affecting more than 10% of the global population and substantially increasing their mortality. When kidneys fail, renal replacement therapy with dialysis or transplantation is necessary but costs are escalating and replacement therapies are not universally accessible. Strategies to improve early detection of CKD and targeted therapy to prevent disease progression would have significant impact on improving human health. This research programme aims to identify early disease biomarkers and also new therapies for early intervention in CKD. As such this research could have significant impact on the early detection and treatment of kidney disease.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice 7000 over five years 4000 adult zebrafish</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at</p>	<p>The project has a 'Moderate' severity level. We will use breeding programmes so that, typically, clinically healthy parents (each carrying a mutant gene) are mated to produce litters containing animals with two mutant genes. The latter animals will have kidney disease.</p>

the end?	Glomerular injury will be induced by removing kidney tissue or the administration of substances such as chemicals, peptides or antibodies. Therapies such as chemicals, peptides, antibodies or non-harmful virus vectors – which deliver genetic material into cells, will be delivered to animals. In experiments, when the interventions are mild or moderate, we will cautiously follow the progress of mice in the 8 months after birth. Should signs of ill health become apparent, the animal will be killed by a humane method.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We aim to determine whether new therapies can prevent or treat kidney disease. Cell culture models alone provide limited insights into mechanisms of kidney disease and response to therapies. Currently there is no alternative to using live animals for preclinical models. In addition the administration of treatments to whole animals will ensure that we can detect any (albeit unanticipated side effects) on other organs.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>In order to minimise the numbers of animals in our experiments we will use carefully determine the number of animals that are needed for experiments together with University statisticians. In the majority of cases we will need to start with pilot studies using less than five animals in each experimental group. Important experimental results will be repeated or validated via an alternative follow-up experiment to minimise the likelihood of spurious nonreplicable results.</p> <p>Sources of variability will be considered at all stages of the experimental design. For mice we will consider the genetic background and sex of the animal carefully when designing the experiment and choose animals that are appropriate to address the specific research question for a particular experiment. We will also consider the variability of experimental observers and where possible will allocate one observer to each animal experiment.</p>

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>At present, the mouse represents the best or most refined species with which to test the efficacy of new therapies for glomerular disease. It has a kidney of similar structure and anatomical complexity (e.g. with glomeruli and branching collecting ducts) to human organs. However our inclusion of zebrafish studies will allow us to refine the number of studies in mice. Our experiments are proposed in mice after they are born when they will be closely monitored. Particular attention will be paid to their weights and behaviour. Should these parameters deviate markedly and/or persistently from normal, mice will be humanely killed. For all experiments in animals we will use good experimental conduct with the appropriate use of post operative analgesia for surgical interventions and the appropriate species specific management of animals during and post anaesthesia.</p>
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Project	Regulation of glucose homeostasis in vivo	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Diabetes is a multisystem disease, involving insulin secretion deficit in response to elevated blood glucose, and insulin resistance in multiple tissues, including the liver, the adipose tissue, the brain, the placenta (for gestational diabetes), and the enteroendocrine cells of the intestines. Also diabetes complications cause stroke and heart attacks, kidney failure, neuropathy and limb amputation and blindness due to retinopathy. More specifically this project aims to understand the molecular mechanisms behind pancreatic beta cell failure during the progression of obesity and insulin resistance in order to provide invaluable data to healthcare professionals and pharmaceutical companies to	

	<p>develop new tools and drugs to limit the devastating effects of this disease. Another objective of this project is to implement, develop and validate the technique of islets transplantation in the anterior chamber of the eye for serial imaging which will provide an instrumental tool for following the damages caused to the islets by diabetogenic insults and the potential recovery induced by new drugs and treatments.</p> <p>Although originally developed as a weight loss intervention, bariatric surgery can improve various metabolic co-morbidities, particularly type 2 diabetes. Mechanistic research has demonstrated that changes in gastrointestinal physiology can play a role in the effects of surgery on diabetes. The exact mechanism of action, however, remains unclear. This project aims to develop the three main bariatric surgery models in rodents and explore the mechanisms of diabetes remission facilitating the group's expertise, including the islets transplantation in the anterior chamber of the eye.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Diabetes is one of the most challenging socio-health emergencies of the third millennium. About 350 million people worldwide are estimated to be diabetic (50% of whom are undiagnosed), but this number is rapidly increasing due to aging populations and sedentary lifestyles, with the prospective of exceeding 500 million cases in 2030. Every year, 1.5 million deaths can be directly attributed to diabetes. In Western countries, the economic cost of diabetes can exceed 15% of the budget of national health systems. Therefore, impact of innovative methodologies and technologies for diabetes management can be extremely high. This basic research is trying to identify molecular signalling pathways involved in protecting the human body against the devastating effects of over-nutrition, physical inactivity and obesity, contributing to the development of type 2 diabetes and its crippling complications. Moreover, the most recent guidelines accept that bariatric surgery, and more specifically gastric bypass, causes diabetes remission in 80% of the cases, making the operation the closest we have been to a</p>

	<p>long-term treatment. It is therefore essential to decipher this observation to find the missing link between pancreas, liver and the gut so as to be able to create more specific drug targets that could allow us to replicate the effect of bariatric surgery. Overall, the immediate beneficiaries will be academics working in the field of cell biology, but this research has the potential to interest pharmacological drug companies and to translate into clinical research by identifying potential drug targets to protect the pancreatic beta cell and the liver against glucolipototoxicity. The patients suffering from diabetes are ultimately going to benefit from this research.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The licence allows for 11700 mice and 6700 rats over a period of 5 years, spread over 6 research groups. The numbers have been adjusted according to the newly calculated numbers for Protocol 8.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Animals will be rendered diabetic either by genetic modification, administration of drug or a high fat Westernised diet. The severity of diabetes will be investigated by serial blood tests and glucose (sugar) administration either orally or by injection. It can also be necessary to perform some surgery on selected animals. All the procedures in this licence except 8 are classified as either mild or moderate and are done under local, general or terminal anaesthesia where appropriate to minimise stress and suffering of the animals. Procedure 8 is classified as severe and the number of animals that will undergo bariatric surgery will be closely monitored and humanely killed if suffering or distress is observed (assessed by lack of grooming, eye/ nose dark discharge and pigment, feeding behaviour, decreased bowel movements, urination, blood in faeces in the first 3 days) All the animals will be humanely killed at the end of the procedures. We will always consult with the vet and animal care staff to make sure we are up to date in applying the most refined methods.</p>
<p>Application of the 3Rs</p>	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The maintenance of normal blood glucose requires the interplay between hormonal secretion from the islets of Langerhans and hormonal action on peripheral tissues such as the liver, the skeletal muscle and adipose tissues. Neuronal and endocrine outputs from the gut in response to changes in hormonal signalling and nutrient availability also modify the net effect on blood glucose. Such complex interrelations cannot be reproduced <i>in vitro</i> and require a whole living organism.</p> <p>For bariatric surgery, this project will also require the use of a number of normal and diabetic animals to form surgical and control cohorts. This is essential as it is currently the only way we can replicate different types of gastric bypass and assess the progression of diabetes re emission day by day. Moreover, it has been demonstrated in the past that hormonal mechanisms associated with glucoregulatory gut function are not always direct eg. Gut- liver- pancreas axis for glucose homeostasis. It is therefore impossible to fully facilitate cell lines and explants to replicate the effect of bariatric surgery <i>in vitro</i> without an initial <i>in vivo</i> assessment. This type of research will require extensive access to post-operational intestinal tissue in specific days during the study, which is not feasible with human subjects who undergo bariatric surgery. Cell lines will be used in potential insulin sensitivity and receptor activation studies, to ensure a significant reduction of animals where necessary.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Animal usage is based on careful power calculations, performed with G*Power. For example, for glucose tolerance tests, we may need a total requirement of 22 animals (11 per genotype) per experiment. This requirement is based on a typical standard deviation in the measurement of blood glucose of ~4 mmol/L. So to detect abnormal glucose tolerance (20% difference compared to a normal 30 min peak of ~ 26 mmol/L during glucose tolerance tests in 12-week old WT C57BL/6 mice on high fat diet in our hands), the effect size $d = 1.3$, requiring a group of 11 mice per group to detect a change at a significance level (α) of 0.05 with 80 %</p>

	<p>power. [Campbell,M.J., Julious,S.A., & Altman,D.G. Estimating sample sizes for binary, ordered categorical, and continuous outcomes in two group comparisons. <i>BMJ</i>311, 1145-1148 (1995)]</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the lowest vertebrates in which genetic manipulation can be successfully achieved and where diabetes studies are well documented. Rats give a better yield of blood and tissues per animal than mice and could be preferred if the relevant strain is available.</p> <p>Both species are well acclimated to live in cages and laboratory conditions.</p> <p>All the procedures in this application are done under local, general or terminal anaesthesia where appropriate to minimise stress and suffering of the animals.</p> <p>A researcher with over 6 years of extensive microsurgery training and 2 years of experience in intestinal microsurgery will be performing the bariatric procedures. The researcher has already optimised the procedures in previous work appointments, in terms of pre, intra and postoperative care and will be consulting with the NVS on optimal animal care.</p>

Project	Regulation of Heart Development: The Role of Gene Regulatory Networks and Cell Signalling	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim at a better understanding of the processes regulating the building of heart muscle. We specifically aim at validating easily detectable cell-surface markers (or indicators) to enrich heart progenitors (source cells for building heart muscle) to improve methods for generating functional heart muscle in the laboratory, eventually for medical applications.	
What are the potential benefits likely to derive from this project	Improved methods for isolation of cardiac progenitors (heart source cells) will allow	

(how science could be advanced or humans or animals could benefit from the project)?	generating heart muscle cells (cardiomyocytes) suitable for therapeutic applications. Ultimately this has applications for regenerative medicine to generate heart cells with high efficiency, as well as potential use for drug discovery in cardiomyocyte genetic defect models.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate that up to 1250 mice will be used during 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The proposed protocols are well established and these interventions will generally result in only minor changes from which the mice will return to normality very rapidly. Every proposed single manipulation will have little adverse impact on adult mice. We will need to use regulated procedures to make males infertile and give females hormone treatment (similar to IVF in humans) to make them more fertile. We will also alter the genetic makeup of some mice to study heart development in the embryo, which may cause some of these embryos to develop a heart defect. The specific effect caused by the proposed targeted genetic alteration will be based on the literature and the data from experimental heart muscle cells made in the laboratory. All mice will be humanely killed at the end of the study and their tissues analysed.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>This project ultimately aims to replace animal experiments by improving the experimental generation of heart muscle cells in the laboratory. Much of the analysis work will be carried out on isolated tissues rather than whole live animals. For this particular project it is necessary to use animals in order to validate the protocols and tools used to make heart muscle cells more similar to real heart muscle cells and therefore eventually more useful for clinical use. This cannot be done using cultured cells or computer simulations alone.</p> <p>We will also use theoretical modelling approaches with the help of computers to replace animal experiments where possible and</p>

	to help us formulate better supported scientific questions for our animal experiments (see refinement).
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Making experimental genetic mouse models with defects in the PROTOGENIN gene is a central aspect of this project. We will use the most advanced technology (i.e. CRISP/R, which uses significantly less animals than the conventional method based on embryonic stem cell gene targeting). Studies will be robustly designed to ensure the appropriate number of animals are involved. Statisticians will be consulted when appropriate.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse is the most suitable model as it is the least sentient (or consciously aware) small mammal whose genome can be readily manipulated whilst the majority of its anatomical and physiological features are shared by humans.</p> <p>Where substances are given to animals to study molecular mechanisms of heart cells, the levels will be adjusted based on all available information to avoid any unintended consequences. These substances will be given using methods which have the lowest impact on the mice.</p> <p>All those involved in the care and procedures carried out on the mice are very experienced. Surgical procedures are carried out under aseptic conditions and the mice will receive appropriate care including pain relief where required.</p>

Project	Regulation of inflammation in wound repair	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	3 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Diabetic patients and animals have severely impaired skin wound healing and humans often develop chronic skin wounds. This also can occur in elderly patients. Complications from chronic wounds costs the health service over 10% of their annual budget and is devastating for these patients' lives. By comparing factors in skin wounds from diabetic and aged animals with normal skin wounds, we can begin to understand what is important for efficient skin wound healing and how to promote impaired skin wound healing. In particular, immune cells from diabetic and elderly patients and animal models are altered and inhibit skin wound healing. However, this process is poorly</p>	

	<p>understood and the key factors that control these cells are not known. Many pro-inflammatory factors are much higher in diabetic and aged chronic skin wounds compared to normal skin wounds, but whether they are causative or a consequence of altered immune cells is not known.</p> <p>Our objectives are to (1) determine differences in how genes and other factors contribute to altered immune cell behaviour in diabetic and aged rodents and how this impacts skin wound healing, and (2) test whether we can manipulate these factors to improve healing in diabetic and aged skin wounds. The results of this study will be important in future therapeutic development for chronic wounds.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The results of this project are intended to: 1. Identify the factors that contribute to altered immune cell behaviour in diabetic and aged rodent models. 2. Contribute to scientific knowledge related to chronic skin wounds. 3. Identify potential new therapeutic strategies to promote healing in diabetic and elderly humans. The potential benefits of this study include the development of potential drug, gene and cell based therapies to aid patients with chronic skin wounds and reduce the need for limb amputation.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Over a 3 year period: 1600 mice (approximately 1000 for breeding purposes and 600 for experimental procedures) 160 rats (approximately 100 for breeding purposes and 60 for experimental procedures)</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>This study is designed to understand how inflammation is controlled in a normal skin wound and what might be different in a diabetic or aged skin wound. The wounding protocol is considered moderate severity and the breeding and bone marrow transplant protocols are considered mild severity. In some experiments we may need to use oral administration or injections of drugs to elicit a particular condition. This is well-tolerated in rodents and will only cause momentary discomfort. Wounds of 10 mm diameter or less will be made on the back and</p>

the effect of manipulating different factors during wound healing will be tested. Adverse effects include stress and discomfort following the procedure. In some cases the substances we treat the mice with may interfere with wound healing or cause the animals to become ill unexpectedly. To mitigate these possibilities, after surgery, animals will be provided with pain relief and monitored closely (twice a day) for any signs of distress. Distress in rodents after this type of surgery is very rare, however, if there is any indication of suffering we will seek veterinary advice or make a decision on the condition of the animal using established scoring criteria. If the animal does not show improvement after 24 hours, the animal will be humanely killed.

In some studies we will exchange bone marrow from one mouse/rat to another mouse/rat in order to determine the effects of the diabetic or aged environment on how bone marrow cells develop and behave. To do this we condition a recipient with a dose of radiation that will allow for the donor's bone marrow to replace the original. The animals do not feel anything during the radiation treatment and they are given replacement bone marrow following their treatment, so they should only feel mild and momentary discomfort during this injection. These animals will be monitored twice a day for radiation sickness or anaemia. This is rare, but if they show signs of this then they will be humanely killed. Animals may also lose weight due to some damage to their digestive tract from the radiation, however, this should only be transient. Animals will be weighed every other day to monitor this, and any animals showing abnormal weight loss will be humanely killed. After 8 weeks' recovery, blood sampling will be performed and should only cause momentary discomfort. Some animals may be wounded to track cells to their wounds. They will receive anaesthesia and pain medication as described above.

Strategies to minimise adverse effects due to our treatment, as well as minimise the number of animals needed for these studies include testing the effects of the factors we are putting on the wounds in cell culture first. In this way we

	will be able to identify the most promising candidate factors without using animals. This will reduce the chances of inducing an adverse effect, and reduce the number of animals needed to accomplish the objective. Animals will be humanely killed at the end of each study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have to use animals in this study because understanding how immune cells interact with skin wound healing in a pathological environment must be studied in the complete physiological setting in order to get an accurate picture of this process. Mice and rats are the least sensitive animals that accurately mimic disease in humans (in this context we understand 'sensitivity' to be the animal's ability to sense and respond to the world around them). The use of animals that can express fluorescent proteins facilitates the tracking of cells. We cannot use humans for these experiments because we would not be able to modify their genes nor track the cells from the bone marrow.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By conducting experiments in cell culture (in vitro) first, we will identify many of the factors that may regulate immune cells. We will also test potential therapeutic treatments in cell culture models of wounds first. To plan for our animal work, we have consulted a statistician to establish the number of animals required for each study. Also, where possible, we will use two wounds per animal to reduce the number of animals required in balance with refinement. In addition, live imaging experiments will be used, which will allow repeated measures to be taken and thus reduce the number of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to	By keeping up-to-date with the latest scientific literature and conference proceedings, as well as consulting with colleagues that have expertise in our area, we will continuously refine our experimental design. The species and models we have chosen are based on how well they mimic diabetes and ageing in humans, their sensitivity (they are the least sensitive models

the animals.	we can use for our study), how well-characterised they are, and our expertise. The animals will be given anaesthesia so they will not feel anything when they undergo wounding. They will also be given pain killers so when they wake up they will not have any discomfort. They will be watched closely to make sure they do not show any signs of being in pain or becoming ill. If they appear to be in pain or appear unwell, and do not show signs of recovery in 24 hours, they will be humanely killed.
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Project	Regulation of keratinocyte proliferation and differentiation	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The objective of the project is to discover how the properties of cells called keratinocytes are controlled via the genes they express and by signals that they receive from other cells. Keratinocytes are the cells that form multilayered epithelia, such as the outer covering of the skin and the lining of the mouth. Multilayered epithelia act as a protective barrier between our bodies and our environment and are frequent disease targets. By investigating the ways in which cell division and maturation are controlled, we aim to obtain new ways of preventing or treating benign skin conditions such as psoriasis and eczema,</p>	

	and also cancers of the skin and oral cavity.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Tumours of the epidermis, called basal cell carcinomas and squamous cell carcinomas, are the most common tumours in humans. Psoriasis affects 2% of the population and eczema is also very common. Tumours of the oral cavity are less common but have a 5 year survival rate of only 50%. By gaining new understanding of the properties of keratinocytes we can potentially prevent or treat these and other diseases of multilayered epithelia.
What species and approximate numbers of animals do you expect to use over what period of time?	89,000 mice over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The expected adverse effects are the development of skin wounds, inflammation and cancer. In most cases the severity will be mild. However, in some situations, such as tumour development, the severity will be moderate. At the end of each experiment mice will be humanely killed and their tissues will be subject to analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible we replace mice with studies of cells that are grown in culture in the laboratory. However, there are three situations in which cultured cells cannot be used: (1) when the properties of the tissue need to be studied for over 3 weeks, which is the limit for maintaining keratinocytes in culture; (2) when communication between keratinocytes and the multiple different cell types within the tissue, such as nerves and blood, needs to be analysed; (3) when the effectiveness of drugs that might act indirectly on keratinocytes needs to be tested.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To prevent unnecessary breeding we keep stocks of frozen mouse sperm and embryos. In planning our experiments we perform statistical analysis of the minimum number of mice required to observe a clear outcome. We share necropsy samples with other research groups so

	<p>that they can obtain data without having to breed their own mice. It is anticipated that advances in non-invasive imaging technology will potentially reduce the number of animals used in this licence.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the lowest form of mammal that can be used to study normal and diseased tissue and are the only mammal in which genetic modification works reliably. Whenever possible, we minimise harm to mice by carrying out procedures for the shortest time periods. When a new procedure is involved, training is first carried out on dead animals. We perform pilot experiments with the minimum number of mice and mildest conditions predicted to have an effect.</p>

Project	Regulation of leukocyte recruitment during acute and chronic inflammation	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Inflammation is a protective response to tissue injury and infection. White blood cells (immune cells) recruited during the initial response need to be removed from the tissue to allow the response to stop. Errors that prevent inflammation from being stopped occur in a number of chronic inflammatory disease (e.g. rheumatoid arthritis [RA]) leading to long term tissue damage. Here we will look at how immune cell derived agents (e.g. PEPITEM) and tissue-resident cells (e.g. fibroblasts or mesenchymal stem cells) control the movement	

	<p>of immune cells in health and disease.</p> <p>Initially we will study these using laboratory based models incorporating human cells from healthy subjects and patients with different types of inflammatory arthritis. We will confirm these findings using animal models of health and arthritis to address the 3 following questions:</p> <p>Is immune cell movement changed by:</p> <ol style="list-style-type: none"> 1) Treating mice with adiponectin-PEPITEM axis modifying drugs? 2) Treating mice with cell therapy (e.g. MSC)? <p>Tissue-resident cells (fibroblasts) from patients with acute resolving or chronic persistent inflammation?</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This work will advance scientific knowledge surrounding what controls the movement of immune cells from the blood, into and through the tissue during inflammation and how this goes wrong in disease. It will highlight to what extent PEPITEM, mesenchymal stem cells, or fibroblasts control these processes and whether these offer new therapeutic targets and/or agents</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice Approximately 1800 over 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Mice will get moderate inflammation within the joints of the front and back limbs. When pain becomes apparent in these animals they will be administered with routine analgesics to manage this. Before pain and inflammation pass a moderate level, animals will be killed to prevent any on-going discomfort. Certain models of arthritis in the mice will require between 1 and 3 injections containing reagents that induce arthritis. These will cause mild discomfort and animals will be monitored closely throughout procedures and killed where adverse effects are identified to prevent any on-going discomfort. Some animals will undergo surgery for tissue/pump implantation. Pain will be pre-emptively managed with routine analgesics to</p>

	<p>manage this. Before pain and inflammation pass a moderate level, animals will be killed to prevent any on-going discomfort. We have taken precautions to reduce any suffering from other procedures including anaesthesia, therapeutic agents, and blood withdrawals by keeping the number of these procedures to a minimum.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Murine animal models are highly effective in driving our understanding of the pathophysiology of inflammatory disease. They allow us to perform in vivo studies that would be otherwise physically or ethically impossible in human cohorts and provide a translational link between basic and clinical research. In this study, murine models are essential to address the objectives outlined within this proposal. First and foremost, only in these animal models of the disease can we determine whether influencing immune cell recruitment will protect against clinical symptoms of arthritis. Essentially, this will provide the validation and rationale to examine this in human inflammatory disease such as RA.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>A key strength of our work is that it combines both human and animal models so that each can be used to inform the other and therefore minimize an over reliance on mouse models of disease.</p> <p>Carefully refined statistical analysis will ensure that we use the least number of animals to provide a meaningful answer to our research questions. Pilot studies throughout using small experimental numbers will ensure that no large experiments will go ahead unless they will provide meaningful results.</p> <p>Using non-invasive strategies such as imaging of joints or other tissues in the same animal over time will also reduce the number of animals required allowing statistically significant differences to be obtained from less mice.</p>

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the best model for the study of persistent disease because:</p> <ol style="list-style-type: none"> 1. The main components of their immune system is shared by humans; this is essential where immune responses as opposed to the function of individual genes is being studied and thus will produce satisfactory results 2. An extensive range of reagents is available for analysis of immune responses 3. They are the most acceptable animal model that shows the least degree of neurophysiological sensitivity and will suffer the least pain, suffering, distress, or lasting harm. 4. There are no other alternatives to this work. <p>We are employing models that have been refined and streamlined as much as possible by our collaborators REDACTED</p>
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Project	Regulation of malaria immunopathology
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Malaria is a devastating global disease that affects over 200 million people and causes over 500,000 deaths per year. In recent times it has become increasingly clear that in life threatening cases of malaria the patient's own immune system is responsible for causing much of the tissue injury that occurs. The work to be undertaken under this licence aims to elucidate the underlying immunological mechanisms responsible for the tissue damage and thereby identify therapeutic interventions that could be used to prevent it.
What are the potential benefits likely to derive from this project	The benefits of this work will be to advance understanding of the mechanisms responsible for

(how science could be advanced or humans or animals could benefit from the project)?	causing severe malaria in patients and to identify and evaluate new therapeutic approaches that could be used to improve the treatment of the disease. It is expected that more effective treatments for malaria sufferers will be identified as a direct result of the studies undertaken in this project.
What species and approximate numbers of animals do you expect to use over what period of time?	This study will utilise approximately 6600 mice over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of studies undertaken are not expected to result in mice showing any outward signs of disease. In a small number of studies, disease would develop in a proportion of animals if the infection was allowed to run its full course however, these animals will be culled at an early stage before the full disease develops. At the end of the study all of the mice will be culled. The overall severity of this work is classified as mild.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>To reduce the numbers of mice used, we will ensure that we obtain as much of our data as we can from alternative sources. To this end a significant part of this study will be conducted using non-animal based investigation conducted using blood samples obtained from malaria sufferers and healthy human volunteers. These samples will be obtained through collaborations with clinicians in Gabon and Mozambique. Whilst these studies will produce valuable data as to the underlying causes of severe malaria, they will not alone enable the specific factors responsible to be identified or for treatment aimed at countering these to be assessed. Consequently, in order to undertake this work there is no alternative to the use of animals.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Our laboratory has extensive experience using mice to model malaria, which has allowed us to calculate the minimum number of mice which will be required for each experiment in order to gaining meaningful data.</p> <p>In addition to this, we will use experimental</p>

	<p>approaches which help to minimise the number of animals used. These will include recording multiple experimental readouts, to gain as much information from each animal used, reducing the need to repeat experiments to gain new insights. We will also harvest multiple mouse tissues for each animal at the end of each study and preserve this for use in future analysis.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse model of malaria used in this study is the most suitable for dissecting the host responses to this disease. The mouse has the lower sentience of species suitable for this study. Its immune system is well defined and provides a good model of the human immune system. In addition, mice deficient in certain components of their immune system are available and provide a valuable tool for this research. Reagents to manipulate the mouse immune system are also readily available.</p> <p>The majority of animal studies will be performed using a malaria model that does not cause the animal to become clinically ill. For a small number of studies, particularly those testing the effectiveness of treatments to prevent severe malaria, a more severe disease model may be used however, the mice will be killed at an early stage before the full disease develops.</p> <p>A comprehensive welfare assessment scoring system has been developed that will be used to assess the wellbeing of all mice used in infectious studies. The criteria set by this scoring system ensure that mice are detected early in the development of the disease and killed before significant disease develops.</p>

Project	Regulation of membrane transport in cardiac health and disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The heart is a pump that contracts and relaxes rhythmically from birth to grave. Contraction and relaxation is achieved simultaneously in all the cells of the heart as a result of the movement of ions across the surface membrane of a cell. Contraction expels blood and relaxation allows the heart to refill with blood ready for the next beat, so understanding how ions are moved and how this can go wrong is central to understanding how the heart works and diseases arise. We have discovered a new way in which this process of ion transport is controlled in cells of the heart. This is a	

	<p>chemical change made to certain proteins that changes the way they work and where they go in a cell. All of the main routes for ions to enter and leave the cell undergo this new regulatory mechanism that we have found.</p> <p>This programme of work will investigate the role of this chemical change in controlling a protein highly relevant to cardiac function. The project will investigate what the chemical change does to this protein in the heart, and how this goes wrong in disease. It aims to determine the cellular control mechanisms and find out how the chemical changes control how proteins work in both healthy and diseased hearts.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Movement of ions across cell membranes is essential for all sorts of processes in the cardiovascular system, and is known to go wrong in certain diseases. The research will make a fundamental difference to our understanding of how ion transport works, and may uncover new treatment targets in heart disease. Such treatments would reduce the socioeconomic burden of heart disease in the UK and reduce the morbidity/mortality of patients suffering from heart disease.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Over a period of 3 years, it is expected that 600-1000 mice (WT and transgenic mice expressing 'targeted' genes encoding ion transporters) will be subject to regulated procedures (one or more of the regulated procedures detailed within this license). The research project requires a colony of mice with both copies of the ion transporter gene targeted to change its chemical properties. It is therefore estimated that up to 1500 mice may be required in order to produce/maintain the homozygous transgenic mouse colony throughout the duration of the project. The total number of mice to be used within this project is estimated at 1500, meanwhile it is estimated that 20 rats will also be used to complete the body of work (i.e. cell-related experiments). Rat hearts contain 10x more tissue than the hearts of mice therefore using 20 rats (instead of 200 hundred mice) within the project will keep animal numbers to an absolute minimum and comply</p>

	with the N3CRs guidelines.
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Mice will be kept in a tightly controlled environment with excellent welfare conditions. The expected levels of severity vary throughout the project depending on the procedures the mice are subject to and vary from mild to moderate to severe. Mice may have telemetry devices implanted to monitor their vital signs e.g. heart rate and blood pressure throughout the course of the project which is deemed to be of a moderate severity. Additionally, these mice may undergo ligation of the aorta to induce hypertrophy of the left ventricle which may result in heart failure (although heart failure is unlikely to occur during the short time we are investigating these mice for post-ligation/banding (9 weeks) as heart failure usually occurs over a longer period of time). This procedure requires complex microsurgery and would be classed as a 'severe' in terms of the level of severity. Our expertise and care ensures that pain, suffering and unexpected deaths are minimised. The duration that the animals will be exposed to cardiac disease will be the minimum required to obtain sufficient data about the acute and chronic changes in cardiac structure and function. These mice will also undergo minimally/non-invasive imaging and/or pressure-volume loop analysis under general anaesthesia to determine the level of damage to and cardiac function of the heart and are expected to experience little adverse effects in response to either. The blood vessels of the mice may also be cannulated for the administration of substances or the withdrawal of blood, however, this would likely be at the very end of the protocol and would be done under terminal anaesthesia. Post-mortem, organ/tissue/cell assessment will be performed in order to establish changes to gene/protein expression that occur after disease. It is at this time that detailed structural and biochemical analysis will be conducted. At the end of the study, animals will be killed humanely by anaesthetic overdose or by cervical dislocation where death will be confirmed by severing of the femoral artery. Any animals which fall ill unexpectedly will be humanely killed</p>

	immediately.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>It is difficult to obtain viable human heart muscle including suitable non-diseased heart muscle for comparison purposes. There is considerable variation in age, medication and underlying pathology of any obtainable human tissue and there is the likelihood of progressive disease being present. It is also not possible to investigate the processes at well-defined time points after a single incidence of damage. Heart muscle cells do not divide, so cannot be grown in cell culture like other tissue types. It is simply scientifically invalid to use other types of cells grown in culture as models of cardiovascular disease. Cultured cells are usually unable to contract, don't resemble cells from the heart, and do not make connections with each other, so lack all of the structural features of a living beating heart. Substantial prior and continuing cell experiments will inform and limit the number of animal experiments required and where possible as much information from one animal will be obtained. Despite this, experiments involving cells present limitations in terms of the physiological information that can be derived. It is therefore necessary to appropriately acquire tissues from animals at times in order to gain a comprehensive understanding of the role of ion transporters in the heart and any other organs/tissue which may be physiologically relevant</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>This lab has considerable expertise in minimising the number of animals required for experiments whilst ensuring the generation of robust data as evidenced by our publication track record. We will use advice from statisticians in our Institute where required. Sample sizes will be set from our knowledge of the literature, previously performed experiments and statistical analysis.</p> <p>Before we start any experiments we determine how many animals that experiment might need based on a 'power analysis'. This takes into account the likely magnitude of the effect that</p>

	<p>will be seen in the experiment, and the likely variability within the experiment. Based on this, we can calculate the minimum number of animals that should be investigated.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Less sentient species i.e. fish are not appropriate for the current body of research as it is not possible to replicate the disease model we wish to investigate in such species nor is it possible to determine the cardiac phenotype of these animals with sufficient precision using the current technologies available i.e. PVL analysis. The mouse is therefore the least sentient species in which it is possible to replicate the disease model we seek to investigate while allowing us to conduct in depth cardiac phenotyping of this model.</p> <p>The mouse and rat are the lowest mammalian vertebrate group with which the scientific community have been able to fully characterise the alterations in cardiac structure (including ventricular remodelling) and function in disease models such as left ventricular hypertrophy. The models chosen closely resemble the pathological changes in human heart disease. There is no alternative to using these models, however, we will continue to utilise our current laboratory animal and organ/tissue/cell data to inform whether particular aspects of the project's severe procedures are required or whether the information we seek can be best obtained using protocols of lower severity. Wherever possible, experimental design will be 'longitudinal' (making several observations as disease develops in a single animal – for example using telemetry or echocardiography), rather than focussing on experimental endpoints that involve a terminal procedure (for example, the insertion of a pressure catheter into the left ventricle to measure cardiac function). We will constantly review the literature for ways to refine the severe disease models.</p> <p>Distress and suffering of any animal will be minimalised by conducting regular visits to the animals which have recently undergone surgery, completing post-operative monitoring forms, administering analgesia as and when</p>

	appropriate and liaising with the vets (when necessary) to raise any concerns and to discuss the welfare of any animal which is demonstrating signs of distress and suffering.
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Project	Regulation of neuronal and neuroendocrine function across the life course	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project seeks to increase scientific understanding of how nerve cell signals and hormones influence the development and functioning of the brain through identifying the changes to gene activity which they produce and learning more about the biological and behavioural consequences of these changes. In addition, we wish to identify novel small molecule drugs that may alter the functions of the proteins encoded by some of the genes affected by these nerve signals and hormones, and which may be of value in treating nervous system disorders.	

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This work will lead to increased understanding of how the vertebrate nervous system grows and develops. Our proposed studies will also shed light on the functions of genes implicated in human nervous system disorders. The proposed small molecule screens may provide novel drug leads that could be of value in developing much-needed new therapies for neurological disorders.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Animal usage will be kept to a minimum. Most of the adult fish required for this project will be used for breeding purposes to produce genetically altered embryos and larvae that will then be used in experiments, at developmental stages prior to the onset of independent feeding. For each gene of interest, a stock of ~120 adult fish are needed to provide a reliable supply of embryos and larvae. We estimate that ~4000 adult fish will need to be bred per annum. Adult fish are killed humanely at the end of their breeding life (~2 years of age). Overall, we expect to use 25,500 fish over the period of this project licence (5 years).</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Breeding stocks of genetically altered fish are not expected to exhibit major physical or behavioural abnormalities. Fish recover well from the biopsy procedures used to obtain DNA samples from fish. Fish exhibiting any unexpected adverse behavioural responses to compound treatments will be humanely killed. Any fish exhibiting signs of abnormal balance, physical posture, a failure to feed or to breed, and other physical manifestations of ill-health such as the presence of tumours, will be humanely killed. All animals will be humanely killed at the end of each experimental procedure.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Crucial hallmarks of the developing vertebrate nervous system are the orderly assembly and integrated functioning of nerve cells as components of neural circuits in the whole animal. This process is not fully understood, and it cannot currently be modelled with any accuracy in vitro using cultured neurones or in silico models.</p>

	<p>Understanding of how neural circuits are built and how they function together absolutely requires in vivo animal studies. Most experiments will be performed on embryos and larvae under the age of protection. A few experiments will use juvenile or adult fish. Alternative model vertebrates, such as the chick and mouse are of higher neurophysiological sensitivity than zebrafish, and they do not have the combination of genetic and pharmacological tractability, as well as the optical clarity for live and fixed tissue imaging, that the zebrafish possesses. For these reasons, the zebrafish an excellent replacement for the mammalian models that have traditionally been used for understanding the development and function of the vertebrate nervous system.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will maintain sufficient numbers of adult zebrafish to ensure that a reliable supply of embryos and larvae are available for the proposed experiments. For chemical treatment and behavioural studies, we will perform small scale pilot studies to inform the power calculations that will be needed to enable the definitive experimental studies to reach statistical significance. Where appropriate, molecular data obtained from in vivo studies will be confirmed in cultured mammalian cells.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The zebrafish provides great analytical power for understanding how the vertebrate brain is built and how it works. The human and zebrafish genomes are highly similar, so insights learned from zebrafish genetic studies are likely to be relevant to understanding human brain development and function. Many sophisticated genetic tools are available to manipulate gene function in the whole animal, and the optical transparency of externally developing zebrafish embryos and larvae allows individual cells and their behaviours to be observed in the intact animal in real-time, with great precision and resolution. The chemical permeability of zebrafish embryos also allows in vivo drug discovery research using compounds that are diluted directly into the water. Zebrafish aquaria and husbandry techniques are developed and implemented with fish health and welfare foremost in mind. Aquarium and veterinary staff involved</p>

	<p>with the project are involved with relevant aspects of research aimed at improving welfare and ensuring standards of best practice are improved. All new experiments on protected animals are assessed internally by Individual Study Plans, and pilot studies are initially undertaken to assess feasibility and inform larger scale experimental design, ensuring that fish usage is kept to a minimum.</p>
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Project	Regulation of the immune response to tumours and pathogens																
Key Words (max. 5 words)																	
Expected duration of the project (yrs)	5 Years 0 Months																
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<table border="1"> <tr> <td data-bbox="730 638 751 728"><input checked="" type="checkbox"/></td> <td data-bbox="751 638 1401 728">Basic research</td> </tr> <tr> <td data-bbox="730 728 751 817"><input checked="" type="checkbox"/></td> <td data-bbox="751 728 1401 817">Translational and applied research</td> </tr> <tr> <td data-bbox="730 817 751 907"><input type="checkbox"/></td> <td data-bbox="751 817 1401 907">Regulatory use and routine production</td> </tr> <tr> <td data-bbox="730 907 751 1075"><input type="checkbox"/></td> <td data-bbox="751 907 1401 1075">Protection of the natural environment in the interests of the health or welfare of humans or animals</td> </tr> <tr> <td data-bbox="730 1075 751 1164"><input type="checkbox"/></td> <td data-bbox="751 1075 1401 1164">Preservation of species</td> </tr> <tr> <td data-bbox="730 1164 751 1254"><input type="checkbox"/></td> <td data-bbox="751 1164 1401 1254">Higher education or training</td> </tr> <tr> <td data-bbox="730 1254 751 1344"><input type="checkbox"/></td> <td data-bbox="751 1254 1401 1344">Forensic enquiries</td> </tr> <tr> <td data-bbox="730 1344 751 1480"><input type="checkbox"/></td> <td data-bbox="751 1344 1401 1480">Maintenance of colonies of genetically altered animals</td> </tr> </table>	<input checked="" type="checkbox"/>	Basic research	<input checked="" type="checkbox"/>	Translational and applied research	<input type="checkbox"/>	Regulatory use and routine production	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals	<input type="checkbox"/>	Preservation of species	<input type="checkbox"/>	Higher education or training	<input type="checkbox"/>	Forensic enquiries	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
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<input type="checkbox"/>	Maintenance of colonies of genetically altered animals																
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The aim of our project is to understand how tumours and pathogens escape from the immune system and to develop new immunization tools to combat these diseases. We are using the mouse tumour models in order to address therapies in a wide range of human tumours in clinical trials that we are about to initiate. The treatments that we develop in mice will be used in treatments for Melanoma skin cancer, Lung cancer, colon cancers and bladder cancer.</p> <p>Cancer:</p> <p>White blood cells of the immune system have</p>																

the ability to kill tumour cells and protect the body from cancer. However, tumours can fight back; as tumours grow and try to avoid the body's immune system they change their surrounding in order to promote their own growth. In doing so, the tumours can suppress the immune system. For example, tumours can consume the nutrients in their surrounding area in order to starve invading immune cells. Tumour cells can also display molecules on their surface that instruct the immune cells to stop working. It would therefore be great if we could combine ways of boosting the immune response against cancer while at the same time prevent the tumours from fighting back.

Influenza (flu) virus epidemics and emerging pathogens:

Most vaccines against influenza virus rely on generation of antibodies in the body of the vaccinated person. These antibodies are proteins that adhere to the surface of the virus and neutralise it. Unfortunately, the surface-characteristics of influenza virus changes very rapidly and vaccines that generate antibodies to one influenza virus rarely protect people to a new influenza virus that has changed characteristics. Furthermore, new viruses can emerge that humans do not have immunity to, and new vaccines must be developed to target these pathogens.

In addition to antibodies, the immune system has killer cells that can recognise, and kill, flu-infected cells thus preventing the virus from spreading and therefore eliminating the disease. This part of the immune system is slightly less susceptible to changes in the flu virus because it recognises parts of the virus that change less frequently. This means that generation of an immune response to one flu virus is more likely to protect the person against other viruses. Vaccines that work best to induce such immune killer cells are "attenuated" viruses; they are alive but their ability to cause disease is disabled. The key is to develop a virus that is attenuated sufficiently so that it does not cause disease but it is viable enough to trick the immune system to think there is a real infection. We have developed

	<p>such a vaccine and believe that it is safe enough not only to immunise in the normal injection route but also as an inhaled vaccine into the lungs where it is much more effective at raising a protective immune response.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>1) Discovering new compounds that can be used in immunization to jump-start the immune system. 2) Identifying and understanding the ways that tumours fight back against the immune system. 3) Discovering compounds that can subdue the tumours' ability to suppress the immune system. 4) Testing new antitumour treatments before applying them to people. 5) Developing new vaccines against influenza virus and other emerging infectious pathogens.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Approximately 7,200 mice will be used per year over a five-year period. More than a third of these will simply be used for breeding and maintaining the many strains that are required for this research. The rest will be used to address many aspects of the aims described above</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In most cases, mice will be immunized by injections or inhalation of a vaccine, together with antibodies and/or drugs. The mice will then be injected with tumours or infected with viruses to test whether the vaccine treatment can protect them. In the majority of cases mice will only receive a few injections, and a few blood samples will be taken.</p> <p>Tumour models: i) In most tumour experiments, tumour cells will be injected under the skin. Such tumours are not expected to cause harm since they do not metastasise or interfere with the animals' natural behaviour. ii) Some tumours are injected into the bloodstream of the mouse. The development of these tumours is monitored by periodical blood sampling. The danger with these tumours is that they may get lodged in essential organs and disrupt the animals' normal physiology. However, based on our knowledge of these tumours this is a very rare event, but the mice will be carefully monitored for signs of</p>

abnormal behaviour and appearance during such experiments. iii) In some experiments melanoma cells are injected into the blood and 2-3 weeks later the appearance of tumours in the lungs is examined. These experiments are not allowed to exceed the time limit of 3 weeks. Very rarely animals may present problems breathing and when that happens they will be removed from the experiment and culled to prevent suffering. In addition, some of the mice that have tumours will be injected with a light-emitting compound. The mice will then be anaesthetised and their bodies scanned in an imaging machine. Since this is done under anaesthesia, the procedure will not stress the animals. However, repeated anaesthesia carries a small risk of anaesthetic death and we minimise this by carefully monitoring the animal's breathing rate and making sure that it is completely recovered before repeating the procedure. Influenza model: Infecting mice with influenza virus makes them ill. If the mice do not receive immunisation they may lose up to 20% of their weight within 5-7 days. If this happens the mice will be humanely killed to prevent further suffering. It is necessary to produce this disease in order to see whether our vaccines provide effective protection. Other pathogens: Some mice will be infected with other non-lethal pathogens such as vaccinia virus, attenuated adenovirus, non-pathogenic strains of bacteria or Fungi such as Aspergillus and Candida. Animals will experience symptoms equivalent to the common cold as the immune response kicks in, but these are all infectious organisms that are cleared within a few days, so symptoms disappear within 24-48 hours. Animals experiencing symptoms such as lack of movement or problems with breathing will be killed humanely. Irradiation In few instances animals will be irradiated to kill their blood cells and then replacing the cells with cells from another animal (bone marrow transplant). Animals which are irradiated in this way experience some discomfort as evident from a lack of grooming. Animals are also more susceptible to infections during the 3 weeks after irradiation and so are given antibiotics to prevent infection. During this time animals are

	<p>kept under close watch to intervene if their health deteriorates. Injection of Immuno-modulating substances. In several cases, animals will be administered compounds that stimulate or inhibit the immune system. These may cause a short term (24-48 hours) general ill health that will resolve within that time. Animals will be observed closely for signs of ill health and will be humanely killed if symptoms persist. In some instances, the application of substances will be intranasal (done under short term anaesthesia), which may affect the animal's breathing rate. Animals are observed closely to make sure there is no immediate adverse effect. The same is true of intranasal administration of infectious organisms (attenuated flu vaccines).</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The majority of our work is done without the use of animals. This is possible by using samples that we obtain from patients undergoing surgery or as part of the analysis of samples from patients in clinical trials. We also use cell lines that we grow in the laboratory conditions (in vitro) and allow us to some extent to mimic physiological behaviors.</p> <p>Experiments that are done in mice are only done where they examine questions that can not tested outside a live organism.</p> <p>The animal experiments are often designed on the basis of information that we assembled from computer analysis of published information and from data that we obtained from the laboratory (in vitro) studies.</p> <p>We chose mice as an animal model to experiment with because mice are the lowest vertebrate group in the evolutionary tree from which suitable models of immune responses are available.</p> <p>Many of the experiments are carried out in genetically altered mice that allow us to identify the role of specific genes in generating an immune response. At present, such genetic</p>

	<p>alterations have only been prepared in mice.</p> <p>By using mice, we are also building on a vast body of information that has accumulated from laboratories around the world so that we can focus on our questions without having the "re-invent the wheel" by having the use less well studied organisms.</p> <p>We use in vitro methods as far as possible, but these methods cannot reliably mimic the multi cellular environment that determines the action of the immune cells or neutralizing antibody.</p> <p>In order to examine the usefulness of vaccination methods in the setting of the complex interactions between the tumour and the many types of immune cells, we have to use a whole organism.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Experiments in animals are done only after all other options have been exhausted.</p> <p>For some T-cell activities we have in vitro assays which can be used to screen reagents such as antibodies and enzyme inhibitors, before we use them in vivo. This is done in order to whittle the number of reagents down and reduce the number of mice that are used.</p> <p>We use the minimum number of mice that allows us to gain a clearly identifiable effect so that experiments do not need to be repeated. No animals are used that do not add to the conclusions we reach. To achieve this we minimise the number of animals that are bred. We consult statisticians in order to determine the minimal number of animals that need to be used for each experiment in order to get a statistically significant result. We also coordinate with our colleagues to make sure that mice that are not required by us can be used by others and vice versa.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise</p>	<p>In choosing an animal model we decided to use mice for several reasons, the first being that the immune system of mice has been extensively studied and well defined and this allows us to build our research on top of a large body of knowledge.</p>

<p>welfare costs (harms) to the animals.</p>	<p>Another reason is that the immune system of mice is more similar to that of humans compared with the immune system of less evolved animals. Mice are therefore the best balanced-choice for research that is both well-defined and applicable to human disease. Furthermore, the research tools for analysing mice are much more developed compared with any other animal model.</p> <p>The design of the experiments with animals takes careful account of the welfare requirements of animals. In the case of mice this means housing these social animals in groups and providing them with environmental enrichment such as tubes to tunnel through, objects to chew on, and nesting material. Mice are housed in individually ventilated cages with woodchip bedding, and an ample supply of food and fresh water. Mice are inspected at least once every day by people who are trained to identify changes from their normal behavior.</p> <p>During experiments, we use volatile anaesthetics, delivered by inhalation, which act more quickly and disperse more rapidly than injectable anaesthetics. This reduces the anxiety which slow recovery from anaesthetics may cause. Where necessary we treat the mice with painkillers (analgesics) either before a procedure and/or following a procedure.</p> <p>Some experiments are expected to cause ill health, for example when we give the animals influenza virus in order to assess the potency of new vaccines. In most cases, flu symptoms will last a limited period until the immune system eradicates the virus; however in mice that do not get the vaccine treatment the health of the mice may deteriorate, in which case the animals are humanely killed to prevent suffering.</p> <p>In procedure where adverse effects are a possibility, we use preventative measures to eliminate any anticipated problems. For example: Animals that are irradiated for the purpose of bone marrow transplantation are given antibiotics for three weeks while their immune system is regenerating. In other experiments, when tumours are implanted on</p>
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	<p>animals, a location is chosen that is least disruptive for their normal behavior. Normally this is in the rear end of the back where any tumour growth would not interfere with the movement of the mouse and where there is plenty of loose skin underneath which a tumour can grow without generating pressure on any other organ. In experiments where increased dehydration is anticipated, subcutaneous saline injections can be used as a supportive measure.</p> <p>In order to reduce the animals anxiety, the minimum possible number of injections are used, the smallest possible volumes are injected and the least invasive route of injection is chosen.</p> <p>Where anaesthesia is used, we try to combine several procedures under the same anaesthetic duration.</p> <p>When surgery is involved, we will use appropriate aseptic techniques, monitor the animals before during and after the procedure and pay special attention to the husbandry of animals while they recover.</p>
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Project	Regulation of tumour growth and metastasis by sodium channels																
Key Words (max. 5 words)																	
Expected duration of the project (yrs)	5 Years 0 Months																
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<table border="1"> <tr> <td data-bbox="727 638 751 728"><input checked="" type="checkbox"/></td> <td data-bbox="751 638 1402 728">Basic research</td> </tr> <tr> <td data-bbox="727 728 751 817"><input checked="" type="checkbox"/></td> <td data-bbox="751 728 1402 817">Translational and applied research</td> </tr> <tr> <td data-bbox="727 817 751 907"><input type="checkbox"/></td> <td data-bbox="751 817 1402 907">Regulatory use and routine production</td> </tr> <tr> <td data-bbox="727 907 751 1075"><input type="checkbox"/></td> <td data-bbox="751 907 1402 1075">Protection of the natural environment in the interests of the health or welfare of humans or animals</td> </tr> <tr> <td data-bbox="727 1075 751 1164"><input type="checkbox"/></td> <td data-bbox="751 1075 1402 1164">Preservation of species</td> </tr> <tr> <td data-bbox="727 1164 751 1254"><input type="checkbox"/></td> <td data-bbox="751 1164 1402 1254">Higher education or training</td> </tr> <tr> <td data-bbox="727 1254 751 1344"><input type="checkbox"/></td> <td data-bbox="751 1254 1402 1344">Forensic enquiries</td> </tr> <tr> <td data-bbox="727 1344 751 1480"><input type="checkbox"/></td> <td data-bbox="751 1344 1402 1480">Maintenance of colonies of genetically altered animals</td> </tr> </table>	<input checked="" type="checkbox"/>	Basic research	<input checked="" type="checkbox"/>	Translational and applied research	<input type="checkbox"/>	Regulatory use and routine production	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals	<input type="checkbox"/>	Preservation of species	<input type="checkbox"/>	Higher education or training	<input type="checkbox"/>	Forensic enquiries	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
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<input type="checkbox"/>	Maintenance of colonies of genetically altered animals																
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The goal of this research is to gain a greater understanding of a type of ion channel that exists on the surface of breast cancer cells, called a VGSC, or 'voltage-gated sodium channel'. This sodium channel opens when there is a change in the cell's membrane voltage and allows sodium ions to flow into the cell. We have found that VGSCs are present on breast cancer cell lines cultured in the laboratory, where they help the cells to move and invade. These proteins are therefore potential new targets for the treatment of invasive breast cancer. The plan is to study the role of VGSCs in regulating breast cancer																

	metastasis in mice. We use therapeutic drugs to inhibit VGSC activity, and genetic approaches to switch VGSC genes on or off in breast cancer cells.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Breast cancer is a leading cause of death worldwide. The major cause of mortality in breast cancer is metastasis, the process by which cancer cells spread from primary tumours to secondary sites. There is an urgent need for more effective treatments to combat metastasis. VGSCs allow sodium ions to pass into/out of neurones. Drugs that target these channels are used in patients in order to treat epilepsy, abnormal heartbeat and pain. VGSCs have been detected in a range of human cancers. The main potential benefit of this work is that VGSCs may be alternative targets in breast cancer diagnosis and therapy. VGSC-targeting drugs already in clinical use might also be effective in breast cancer treatment. By better understanding the role of VGSCs in regulating metastasis, we should be able to design new, better treatments in order to reduce and/or slow breast cancer metastasis.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, 1800 animals over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The surgical procedures experienced by the mice in this project will be of no more than moderate severity. A number of the mice used in this project will develop breast cancer. The tumours may grow to a size big enough to cause minor discomfort, in which case we will give the mice pain-relieving medication. Mice will be put to sleep at the end of the experiment and their tissues will be banked for analysis and to reduce the need to use further animals.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal	Our laboratory uses <i>in vitro</i> models as investigative tools whenever possible, and a large part of this work uses <i>in vitro</i> tissue culture techniques. However, at this point it is necessary to use animals to answer questions

alternatives	about VGSC function during metastasis <i>in vivo</i> . Metastasis is a complex, integrative process that cannot be accurately mimicked using cell lines or computer models alone. Cancer studies in animals yield valuable insights into our understanding of metastatic breast cancer.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We use <i>in vitro</i> models and clinical datasets wherever possible in order to limit the number of animals used for this research. We use state-of-the-art imaging approaches, which enables us to generate large amounts of data on tumour growth and metastases from individual mice, reducing the numbers needed. Finally, we collect and bank tissue from the animals used in this project and share these samples with the breast cancer research community in order to reduce the number of animals used in breast cancer research.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are unique in their ability to accept, develop and accurately model breast cancers. These models involve the injection of human cancer cells into the breast of female mice, or using genetically modified mice that spontaneously develop breast cancer. The tumours may grow to a size big enough to cause minor discomfort, in which case we will give the mice pain-relieving medication. The tumour, metastases and the response to treatment, can then be seen using specialised imaging systems and microscopes. Some of the mice will be given a non-toxic dose of a VGSC-inhibiting drug with or without other chemotherapy. Some of the mice used in this project are immunodeficient so that they can accept human tumours. They will be kept in individually ventilated cages to avoid infections.</p>

Project	Regulation, mechanism and targeting of platelet receptors in thrombosis and haemostasis																
Key Words (max. 5 words)																	
Expected duration of the project (yrs)	5 Years 0 Months																
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<table border="1"> <tr> <td data-bbox="531 710 563 804"></td> <td data-bbox="563 710 1396 804">Basic research</td> </tr> <tr> <td data-bbox="531 804 563 898">X</td> <td data-bbox="563 804 1396 898">Translational and applied research</td> </tr> <tr> <td data-bbox="531 898 563 992"></td> <td data-bbox="563 898 1396 992">Regulatory use and routine production</td> </tr> <tr> <td data-bbox="531 992 563 1117"></td> <td data-bbox="563 992 1396 1117">Protection of the natural environment in the interests of the health or welfare of humans or animals</td> </tr> <tr> <td data-bbox="531 1117 563 1211"></td> <td data-bbox="563 1117 1396 1211">Preservation of species</td> </tr> <tr> <td data-bbox="531 1211 563 1305"></td> <td data-bbox="563 1211 1396 1305">Higher education or training</td> </tr> <tr> <td data-bbox="531 1305 563 1400"></td> <td data-bbox="563 1305 1396 1400">Forensic enquiries</td> </tr> <tr> <td data-bbox="531 1400 563 1480"></td> <td data-bbox="563 1400 1396 1480">Maintenance of colonies of genetically altered animals</td> </tr> </table>		Basic research	X	Translational and applied research		Regulatory use and routine production		Protection of the natural environment in the interests of the health or welfare of humans or animals		Preservation of species		Higher education or training		Forensic enquiries		Maintenance of colonies of genetically altered animals
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What's the aim of this project?	Platelets are small cells in the blood involved in prevention of bleeding and controlling inflammation and other processes in the vasculature. The overall aim of this project is to deepen our understanding of how platelet activation is controlled by tyrosine kinase-linked receptors in haemostasis and thrombosis as these are targets for development of novel anti-platelet drugs.																
Why is it important to undertake this work?	Heart attack and stroke are two of the leading causes of death in the developed world. These cardiovascular events happen when a clot made of blood components and cells blocks blood flow to parts of the heart (heart attack) or brain (stroke). This process is termed thrombosis and it is often started by the switching on (or activation) of a cell type in the																

	<p>blood called the platelet.</p> <p>The usual job of platelets is to stop bleeding when blood vessels are damaged. They do this by sensing the environment around them through proteins known as receptors on their surface. When damage is recognised by platelet receptors, an activation signal is sent into the platelets which causes them to become sticky. Activated platelets can then stick to the blood vessel wall and clump together with other blood cells and proteins to form a clot that blocks the site of damage and prevents blood leaking out. This process is termed haemostasis and it is tightly controlled in healthy vessels so that clots only form and seal areas of damage. Thrombosis occurs when platelet receptors recognise a powerful damage signal that starts uncontrolled clot formation and results in a clot so large that it interrupts the flow of blood.</p> <p>As platelets play a central role in thrombosis, people at risk of thrombotic events are currently given drugs like aspirin to limit platelet activation. Unfortunately, because of how they work, these drugs cause the side effect of excessive bleeding in some people and in others they are not effective at preventing thrombosis for reasons that we do not fully understand. Therefore, improved drugs and new treatment combinations are required which are better at preventing thrombosis and/or cause less bleeding complications. Development of these treatments requires more information about platelet receptors, including how they function and how they are regulated, so that thrombosis can be specifically and effectively targeted.</p> <p>Therefore it is important to undertake the work in this project so that we can further build the basic research information base required for the design and development of new drugs that inhibit platelet activation (anti-platelet drugs) for the treatment of thrombotic diseases.</p>
<p>What outputs do you think you will see at the end of this project?</p>	<p>We expect the following outputs by the end of the programme of work in this project:</p> <ul style="list-style-type: none"> - publication of new insights into platelet receptor function in thrombosis and haemostasis in scientific journals - identification of potential new drug targets for thrombotic disease
<p>Who or what will benefit from these outputs, and how?</p>	<p>Immediate benefits: This work will further our understanding of the mechanisms that induce and limit platelet activation by a special class of proteins called</p>

	<p>tyrosine kinase-linked receptors, in particular the receptors named CLEC-2, PEAR1 and GPVI. This will inform the direction of future research in our group and other groups around the world as we share this knowledge with scientists and medical doctors through conference presentations, and publication of the data in scientific journals using the ARRIVE guidelines on animal experiment reporting.</p> <p>Short term benefits: This work will further our understanding of the mechanisms that induce and limit platelet activation by a special class of proteins called tyrosine kinase-linked receptors, in particular the receptors named CLEC-2, PEAR1 and GPVI. This will inform the direction of future research in our group and other groups around the world as we share this knowledge with scientists and medical doctors through conference presentations, and publication of the data in scientific journals using the ARRIVE guidelines on animal experiment reporting.</p> <p>Medium term benefits: In the medium term, inhibitors of platelet receptors or tyrosine kinases will be further tested by clinical trials in patients, in association with academic institutions and hospitals in Europe, as well as in Pharmaceutical Companies developing drugs.</p> <p>Long term benefits: The long term outcome of this research will be new anti-platelet drugs, benefiting the Pharmaceutical Industry, medical doctors and most importantly patients around the world.</p>
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	We will share results and conclusions from this project with scientists and medical doctors around the world through presentations at conferences and publication in scientific journals.
Explain why you are using these types of animals and your choice of life stages.	Mice are the animal of choice as there are a large number of genetically altered mice available and because of the knowledge built up over 20 years of research. We will use adult mice, usually between 8 – 12 weeks of age.
Typically, what will be done to an animal used in your project?	Typically mice will be bred on protocol 1, where they will have tissue sampled for genotyping, before being used in experimental protocols 2. In protocol 2, animals will be terminally anaesthetised and blood drained (exsanguinated) or they will undergo a bleeding time experiment or

	<p>thrombosis experiment before being culled by a Schedule 1 method.</p> <p>In addition some animals may be administered substances that alter the function of platelet receptors and/or have small amounts of blood taken from a superficial vein prior to terminal procedures on Protocol 2.</p>
What are the expected impacts and/or adverse effects for the animals during your project?	The animals may experience weight loss (due to loss of appetite) following the administration of drug to alter platelet function and mild discomfort due the injections (which in part will be due to the handling). The mild discomfort should be only last a short time while that of weight loss will be mild and not sustained.
What are the expected severities and the proportion of animals in each category (per animal type)?	<p>The major severity category is mild. Less than one in twenty of the animals will experience a moderate level of severity due to:</p> <ul style="list-style-type: none"> - cumulative severity of administration of substances that alter platelet receptor function by intraperitoneal and intravenous injections once a day for up to 5 days. - potential for some of the substances to cause mild weight loss. - potential risk of bleeding with some substances whilst platelets are inhibited / depleted. This has not however been observed in our experience, causing little to no harm.
What will happen to animals at the end of this project?	used-in-other-projects
Why do you need to use animals to achieve the aim of your project?	We are not able to monitor the complex pathways of haemostasis and thrombosis in a cell based test. We cannot yet make platelets in the laboratory that have the same properties of platelets in human. We cannot carry out genetic experiments to modify human platelets due to the absence of a nucleus.
Which non-animal alternatives did you consider for use in this project?	The fundamental reason why the use of animals is required to understand these processes is that at present no methods in the laboratory exist to model platelets. Platelets lack a nucleus so cannot be grown outside of the body.

	Megakaryocytes (the platelet mother cell) and megakaryocyte cell lines do not produce sufficient numbers of platelets of the same level of reactivity as those produced in animals
Why were they not suitable?	<p>Where possible, experiments in cell line models have been used to model GPVI, PEAR1 and CLEC-2 receptor pathways. However these are limited due to different proteins being present in these cell lines and because they do not give information about platelet function.</p> <p>Human platelets will be studied, however they cannot replace platelets from genetically altered mice, since (i) inhibitors against every protein in platelets are not available, and because of concerns of 'off-target' effects of inhibitors; (ii) we have performed an extensive genetic study of patients with bleeding disorders but have not found any in the UK who do not have GPVI, CLEC-2, PEAR1 and their associated proteins.</p>
Enter the estimated number of animals of each type used in this project.	mice: Over 5 years, we would expect to use no more than 28,000 mice in total - 8,000 animals for scientific protocols and 20,000 to breed genetically altered strains required.
How have you estimated the numbers of animals you will use?	The major use of animals is in the generation of genetically altered mice and their littermatched controls. Because of increasing use of mice deficient in more than one gene, this can involve extensive breeding. The general plan of experimental design involves the undertaking of a series of studies to investigate cellular and functional processes of genetically altered platelets and megakaryocytes in response to stimuli. The majority of these studies will give clear-cut answers from between 3–5 experiments, although in some situations, we may need to increase this value. For example, when more than one gene contributes to a pathway (the effect on loss of one gene may be relatively small) or where tests are known to be associated with a wide variation in response such as flow-based studies on collagen surfaces. For studies to investigate haemostasis and thrombosis in mice, we will use established statistical tests to determine the statistical significance of a result. Where appropriate, we will use power calculations and will consult with an external statistician to ensure that we are using the appropriate number of mice.
What steps did you	Reduction will be achieved by first performing experiments

<p>take during the experimental design phase to reduce the number of animals being used in this project?</p>	<p>on human platelets and transfected cell lines through the use of pharmacological inhibitors to identify proteins that alter receptor function. We will also limit in vivo studies in mice to conditions where there is clear evidence of the role of a protein in regulating function.</p> <p>We have refined our techniques for use of small amounts of blood from mice. This has involved development of new tests that require very small numbers of platelets, such as flow cytometry and static adhesion assays. In addition, because we are a relatively large group, we are able to allocate as many as six people at a time to study platelets and megakaryocytes from a single mouse. We also store tissue for later protein analyses. Thus, through the combination of this experience and allocation of people, we are able to keep the numbers of mice to the minimum required to answer a particular question.</p>
<p>What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?</p>	<p>We have refined our techniques for use of small amounts of blood from mice. This has involved development of new tests that require very small numbers of platelets, such as flow cytometry and static adhesion assays. In addition, because we are a relatively large group, we are able to allocate as many as six people at a time to study platelets and megakaryocytes from a single mouse. We also store tissue for later protein analyses. Thus, through the combination of this experience and allocation of people, we are able to keep the numbers of mice to the minimum required to answer a particular question.</p> <p>Administration of any substances to alter platelets that we do not have experience with will be first tested on a small (2-3) group of animals followed by tight monitoring of their health status to make sure that the new substance does not induce any unexpected undesirable effects.</p>
<p>Which animal models and methods will you use during this project?</p>	<p>The majority of procedures outlined in this project will be under terminal anaesthesia, and therefore harm to the animals will be low. Mice that receive substances that alter platelet function or mice with uncharacterised genetic mutations will be closely monitored. The advice of the named veterinary surgeon and named animal care and welfare officers will be taken to ensure animal suffering is minimised where possible.</p>
<p>Why can't you use</p>	<p>We cannot use non-mammalian species for this work, as</p>

<p>animals that are less sentient?</p>	<p>mammals are the only animals to have platelets. In mice there is established and reliable genetic alteration technology, and established tests of platelet function. There are a large number of genetically modified mutant mice available and there is extensive amount of work that has already been performed and published using mouse models of thrombosis and haemostasis.</p> <p>The majority of procedures outlined in this project will be under terminal anaesthesia, and therefore harm to the animals will be low.</p>
<p>How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?</p>	<p>We will stay informed by advances in the 3Rs through attendance of seminars and conferences, as well as discussions with the NVS and NACWOs.</p> <p>We will review each experiment on completion to determine any refinements that can be applied to future experiments.</p> <p>Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially used to replace animal use.</p> <p>We will use SyRF the free online platform for researchers to perform a systematic review and meta-analysis of animal studies. https://www.nc3rs.org.uk/camarades-nc3rs-systematic-review-facility-syrf</p> <p>We will also stay up to date with guidance published by the International Society for Thrombosis and Haemostasis (ISTH) Scientific and Standardisation Committee on the most refined experimental methods for haemostasis and thrombosis research.</p>
<p>How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?</p>	<p>The majority of procedures outlined in this project will be under terminal anaesthesia, and therefore harm to the animals will be low. Mice that receive substances that alter platelet function or mice with uncharacterised genetic mutations will be closely monitored. The advice of the named veterinary surgeon and named animal care and welfare officers will be taken to ensure animal suffering is minimised where possible.</p>
<p>What published best practice guidance will</p>	<p>Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be</p>

<p>you follow to ensure experiments are conducted in the most refined way?</p>	<p>generated in the experiment.</p> <p>Experiments will be conducted in accordance with the guidelines published by the Laboratory Animal Science Association (LASA).</p> <p>The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE guidelines.</p>
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Project	Regulatory and Investigative Toxicology	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Understanding how safe a potential new medicine is before it is given to humans is an essential part of medicine development. Although some information on safety can be obtained without using animals, some tests must be carried out using animals to better understand how these medicines might affect the human body. The objectives of this project are as follows:</p> <ul style="list-style-type: none"> • Identify the right potential medicines for development which are safe to give to people and are most likely to be able to treat the target illness. • Identify any possible safety concerns and 	

	<p>understand how these might arise and whether they could cause harm to patients or human volunteers in clinical trials.</p> <p>Where possible, improve and refine our tests using animals to provide more relevant information to humans whilst minimising the use and impact on animals.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Achievement of the objectives will support development of safe, new medicines to improve health and quality of life of patients by generating high quality, regulatory acceptable data and will help to remove unsuitable candidates from the development pipeline at an early stage, thus minimising the use of animals and resources. The benefits gained by studies performed depends on the study purpose and type and include: Making decisions on whether potential new medicines are suitable for development as early as possible in the process to avoid wasting animals and money. We use the information generated during early studies to help to understand what we need to measure on future studies. To help us to decide the doses and endpoints to measure on early human studies to minimise the risk to human volunteers. To allow regulatory authorities to decide whether to allow the potential new medicine to be given to humans.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Over the 5 year period of the licence we expect to use approximately: 5500 rats 2000 mice 575 dogs 200 hamsters 200 rabbits 575 pigs</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Evaluation of safety to assess potential risk to humans requires the use high doses of a potential new medicine which can cause some adverse effects in animals. Adverse effects in animals are usually of mild or moderate severity. The most common effects will be loss of body weight or reduced weight gain, reduction in the amount of food the animals are eating and clinical signs such as reduced activity, postural changes, changes in faeces and in some species, vomiting. No animals will intentionally experience severe adverse effects but because early studies may be the first time that a potential new medicine is given to animals, effects may occasionally be more severe than expected. Animals are monitored closely and animals which</p>

	<p>show signs toward the limit of moderate severity are humanely killed. Most safety studies require examination of blood and tissues from animals to see whether the potential new medicine has caused any damage to organs or tissues, so the majority of animals are humanely killed at the end of a study and subjected to post mortem examination. Samples of tissues are then examined microscopically. On some early studies animals are not required to be killed and provided they have not shown adverse effects they may be used again in a subsequent study.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Whilst alternatives to in vivo animal models are being developed and are used where possible, there are currently no reliable models available for broad, primary toxicity screening and none that are acceptable to drug regulatory authorities, it is therefore necessary to screen for toxicity in animal models</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>For safety studies, guidelines require the number of groups, and animals per group to, be adequate to clearly demonstrate the presence or absence of an effect of the test substance. We have a track record of designing studies that provide us with the information we need to make decisions on the safety of our test substances (leading to continuing, or stopping, development).</p> <p>For preliminary studies, small groups are acceptable because of the endpoints used give a clear answer. Where group sizes are sufficient data from definitive toxicity studies are analysed statistically. Statistical input is sought, where necessary, to strengthen the overall scientific quality and relevance of the studies to be performed, with sample size calculations performed for specific studies to determine the group size. Group sizes in dog and pig studies are usually insufficient for valid statistical analysis. However, because toxicity is the result of changes in multiple parameters, assessment is made by examination of data from each animal and by correlation of in-life and post mortem findings within an individual.</p> <p>In order to minimise animal use, we will consider using animals on more than one study when this can</p>

	be justified on welfare and scientific grounds.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Regulatory guidelines state that toxicology studies in support of administration to man should be conducted in one rodent and one non-rodent species. Generally the rat is the rodent species of choice unless it is known to be an inappropriate model for man for the compound. The non-rodent species will be that likely to give the most satisfactory, reliable and regulatory acceptable results.</p> <p>The pig will be used as the preferred non-rodent species in this licence, unless it is shown to be unsuitable based on scientific information available, when the dog will be used. Where evaluation of all information indicates that both the pig and dog are a suitable non rodent species, the pig will be chosen.</p>

Project	Regulatory Ecotoxicology	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	X	Regulatory use and routine production
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>To allow the identification of hazards associated with the manufacture, transport and use of industrial chemicals, agrochemicals, pharmaceuticals, microbial pesticides and biocides such that their possible adverse effects on humans, wildlife and the natural environment can be determined. This will allow regulatory authorities to classify and label these substances, recommend safe handling procedures, and impose risk reduction measures if required such that the benefits provided by the substances can be safely achieved.</p> <p>Specifically this project will assess the ecotoxicological effects of these substances to fish following a single (acute) or series of</p>	

	exposures (chronic).
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The main benefit of this project is the development of data to support the risk assessment of chemicals such that any detrimental effects on the environment can be minimised.
What species and approximate numbers of animals do you expect to use over what period of time?	A variety of fish species including rainbow trout, fathead minnow, common carp, bluegill sunfish, sheepshead minnow, medaka and turbot are expected to be used. The total number of fish used over the 5-year licence period is expected to be approximately 125,000.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The individual studies undertaken involve exposure of groups of fish to varying concentrations of the chemical to assess the effect that the chemical may have on survival and/or growth of the fish and any interaction the chemical may have with the endocrine system. Adverse effects ranging from mild discomfort through to death are expected during the course of this project. However in the majority of exposed fish adverse effects will only be mild. The programme of work will be designed in accordance with the principles of the 3Rs in order to minimise animal use and severity of procedures. Tiered testing strategies will be implemented, so that the results of one study can be used to refine the remaining studies in the programme thus minimising the severity of any adverse effects. All fish that are exhibiting significant toxic effects, and those surviving to the end of each test, will be humanely killed as soon as possible to avoid unnecessary suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Current regulations e.g. REACH, require the use of fish to assess potential environmental effects. Non-animal alternatives have not yet been sufficiently validated for acceptance by various regulatory authorities and hence cannot be used to replace animal testing in this context.

<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of animals used in regulatory toxicology studies is specified in the relevant Test Guidelines and is the minimum that is sufficient to allow meaningful interpretation and submission to a range of regulatory authorities. The use of the specified numbers of animals ensures that the data generated will be acceptable to regulatory authorities and hence will minimise the need for subsequent duplication or supplementary testing.</p> <p>Where possible the results of QSAR predictions, physico-chemical testing and non-animal tests will be used to aid in the prediction of toxicity hence reducing the number of animals required to satisfy the regulatory requirement, e.g. by performing Threshold tests.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The fish species used have been selected in accordance with the relevant Test Guidelines and the age ranges of the fish are such that they are of the lowest neurophysiological sensitivity that will allow evaluation of the specific endpoints.</p> <p>The species selected are representative of wild species. The data generated is therefore designed to protect these representative species in the environment thereby minimising larger scale environmental effects of tested chemicals.</p> <p>Any fish that are showing a significant departure from the animal's normal state of health or well-being will be identified and humanely killed.</p>

Project	Regulatory Testing of Biological Toxins & Antitoxins	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>To undertake testing procedures to ensure the safety, efficacy, stability and overall quality of toxins and associated proteins used for medicinal products in accordance to registered marketing authorisations held with national and international regulators and in accordance with Good Manufacturing Practice.</p> <p>To provide testing services to assist with product development, improvement and clinical trials associated with toxins and associated proteins.</p>	

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The work detailed in this project will allow the continued safe development, production and use of botulinum toxins and their derived products for the treatment of a wide range of medical conditions.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Potency Assay for Biological Toxin: 500,000 mice Biological Toxin Antibody Assays: 10,000 mice Neutralisation Assay for Biological Toxins: 10,000 mice</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Occasionally mis-injection into the lumen of the intestine could potentially cause peritonitis and mis-injecting into the subcutaneous tissue can cause abscesses although the latter has never been seen in practice. Occasional injection into the bladder can occur. Careful injection by experienced technicians reduces these risks and any animal suspected of being mis-injected will be killed by a schedule 1 method. All animals except possibly those in the very low dose groups will show typical signs of the toxins to some degree; this includes difficulty with breathing (wasp waisting, deep gasping or abdominal breathing), cyanosis, ataxia, lethargy, ruffled coats, an inability to move and some limb paralysis. Some animals will recover from these signs over the course of the test so all need to be kept alive until they are showing severe clinical signs of toxicity at which point they are killed by a schedule 1 method. Despite frequent observations (hourly) some animals may die due to the potential rapid onset of the symptoms.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The mode of action of toxins is complex; this cannot be fully replicated in cell culture or other in-vitro techniques and currently requires an animal model. For example the neurotoxins are proteins that have similar molecular structure and molecular weights. They are usually associated with non-toxic protein both naturally and in-vitro. They have a di-chain structure consisting of a light chain which is the toxic portion of the molecule and the heavy chain</p>

	<p>which is responsible for targeting the cholinergic neurones. These neurotoxins act presynaptically by blocking the release of the neurotransmitter, acetylcholine, at the neuromuscular junction.</p> <p>Even with the development of non-animal assays there is still an ongoing need for the mouse potency assay. This is true for manufacturers who have yet to successfully developed an alternative as a replacement, for high potency products (where alternatives may not be sensitive enough) and as a "back up" to ensure product availability due to non-animal assay failure. There may also be a requirement to use the mouse assay for the qualification of reference standards and the comparability of both assays. Some manufacturers have developed toxin specific cell culture assays but these methods are not always available to other manufacturers and it may not be possible to validate other toxins, even those of the same serotype.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The animal numbers required for the potency assay has been reduced as experience has been gained at closely targeting the expected values. Careful design of the assays using a geometric progression of dilutions that results in a symmetric design about the known estimated potency ensures a robust assay with maximum precision from the number of mice used in the assay designed to be appropriate to meet the regulatory requirements to safely control the production and release of the product. The number of animals currently required per annum is based on the test history of these assays at this facility. The majority of samples received for potency assay are determined by the Routine Quality Control (QC) Assay.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise</p>	<p>Historically the majority of the work undertaken on biological toxins has been with mice as the animal model being the lowest neurophysiological model considered appropriate.</p> <p>The mouse lethality assay for the biological toxin test requires death as an end point;</p>

welfare costs (harms) to the animals.	<p>however, suffering can be reduced by killing, using a Schedule 1 method, any animals that it is predicted will die during the course of the test. Mice are observed at regular and frequent intervals, those showing severe symptoms will be killed. Approximate proportions of animals experiencing mild, moderate and severe severity are 7%, 32% and 61% respectively. However regular observations ensure that approximately 90% of mice are humanely killed before death from the effects of the toxin.</p> <p>The assay has also be refined for new clients in that wet mash is supplied to all animals. This allows for easier access to food and water and provides a more palatable system for animals that are affected by the toxin. It is not possible for all current tests as the benefit is outweighed by the number of animals required for comparative studies to introduce into validated assays.</p>
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Project	Remodelling and reverse remodelling in heart failure	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Heart failure is a massive health issue in the UK and the world. It is characterised by a degeneration of the heart known as myocardial remodelling. This project aims at investigating myocardial remodelling and studying new approaches that can lead to reversal of this phenomenon and ultimately to treatment.</p> <p>New therapies for heart failure that include manipulation of mechanical forces applied to the heart muscle., gene and cell therapy have given promising early results in reversing remodelling, but are still insufficient to induce significant changes. Left ventricular assist devices (LVAD) are mechanical pumps that are implanted in</p>	

patients to unload the heart from the excessive work load due to heart failure. This therapy induces improvement in the structure and function of the heart muscle in heart failure, yet the rate of clinical recovery in patients remains low. With this project, we aim to define additional combination therapies, including drugs, gene and cell therapies that may lead to an improvement in the rate of clinical recovery obtained with LVADs.

The project utilises animal models of disease to induce heart failure (by obstructing blood vessels of the heart in rats and mice) and animal models of mechanical unloading to simulate the LVAD action (by transplanting the failing heart in the abdomen of another animal). By unloading the failing hearts, we recreate the clinical scenario of heart failure patients treated with LVADs. The animals are then treated with pharmacological, gene or cell therapies, or the animals carry genetic mutations, so that we are able to identify therapeutic targets or strategies that may be beneficial in patients.

Given the complexity of the clinical condition and the interactions occurring in the patient, living animals are necessary and in vitro approaches are not suitable. Extreme care is taken to minimise suffering and to use the minimum number of animals. This is achieved by maximising the use of each single animal to produce samples from several experiments.

In addition, the use of animal with the same genes abolishes the risk that the body rejects the transplanted heart avoiding the use of toxic drugs employed to avoid this. The reason why rats will be used in this project is that no other species with identical genes are available.

The protocols involved are designed to produce models of disease and this would involve some degree of discomfort for the animals. However, we take extreme care to minimise this discomfort using painkillers and, in several years of experience with these procedures, we have noticed that the vast majority of treated animals only show signs of mild discomfort during the post-operative period (24 hours), with minimal problems thereafter. Performing the

	<p>studies described in this project will achieve a number of important goals:</p> <p>1) to determine the mechanisms underlying heart failure and its progression, 2) to assess a number of therapeutic approaches, including mechanical and pharmacological therapy, 3) to explore novel hypothesis and targets using gene manipulation technology, which is essential to open unexplored alternatives for treatment. Overall, this project will provide not only a profound advancement in the understanding of the mechanisms of heart failure and its treatment, but also indicate some useful strategies to manage thousands of patients suffering from heart failure and with poor prognosis.</p>
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project directly aims at understanding and testing new therapies for heart failure will potential to find a cure or alleviate symptoms for millions of people.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (4900 in 5 years) Rats (3000 in 5 years) Rabbits (250 in 5 years)
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Adverse effects are limited to normal complications occurring during surgery, such as bleeding and infections. Complications due to heart failure are possible and these include breathlessness, weight loss, and general deterioration. Given the large experience accumulated over several years of research, these effects occur at a low rate and are detected and dealt with very promptly. The expected level of severity is moderate because the animals will be culled humanely before the onset of significant clinical signs. The animals will be humanely killed at the end.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal</p>	The syndrome of heart failure is a complex disease and no alternative, non-animal platforms exist. Our laboratory is pioneering the use of human in vitro models using stem cells,

alternatives	but these systems are still inadequate.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Animals will be used in numbers sufficient to obtain meaningful results and calculated using well-established statistical techniques. Particular care will be taken to collect the maximum number of measurements from each animal and optimise the use of tissue obtained, in order to perform several series of experiments using the same group.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We will use rodents which provide solid and well characterised models of disease. The animals are housed in modern facilities, they are looked after by highly qualified and caring staff and are supported with professional veterinary advice at all times. Animals will be continuously monitored for pain and distress and, if these exceed acceptable, objectively-determined limits, they will be humanely sacrificed. We will use animals with the same genes to avoid the use of toxic drugs to prevent transplant rejection. This will also reduce the variability of the data helping reduce the number of animals.</p>

Project	Renal and intestinal transport in health and disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	It is recognised that eating a diet high in salt, fat and sugar can increase the risk of developing diabetes, cancer and heart disease. It is, however, becoming clear that an excess or deficiency in other ingredients in our food can also damage our health. For example, phosphate preservatives, which are commonly added to food to improve taste and increase shelf-life, are thought to cause damage to our heart and affect the strength of our bones. While dietary iron deficiency, which affects 2 billion individuals worldwide, can also negatively affect our heart and bone	

	<p>health.</p> <p>The aim of this project is to investigate how different nutrients are taken into our body from the diet and how the levels in the blood are then controlled to stop them from having damaging effects. The project will also determine if diseases such as kidney failure or diabetes alter these processes and further increase the risk to our health.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The study will increase our knowledge of the basic physiology of dietary nutrient absorption and the impact that this can have on heart and bone health. While it may not have an immediate measurable social and economic benefit the information generated will be of value to academics, clinicians and nutritionists interested in promoting healthy eating, both for lifelong health and for improvement of chronic disease. This basic science knowledge is necessary to underpin the long-term development of drugs to target intestinal transport processes for disease treatment.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Approximately 1450 rats and 1060 mice will be used over the 5 year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The techniques described in this proposal are of mild to moderate severity. The applicant or close collaborators have experience of all the surgical techniques and animal models described in this proposal. In addition, these have all been characterised in detail and are commonly used by the research community. The two surgical procedures that will be used in the project are done under general anaesthetic to cause kidney disease or for measurement of blood pressure. While the animals show clinical signs of mild kidney failure they display no significant adverse effect at the end-points proposed in this study. For blood pressure monitoring a small probe is inserted into one of the animal's arteries and a transmitter secured into their abdominal cavity or a space under their skin. They recover well from this short procedure with no adverse effects. For all surgical procedures</p>

	<p>the possibility of infection is minimised by using the same clean conditions used for human surgery, with the animals receive painkillers and post-operative care just like people recovering in hospital. Based on our previous studies animals undergoing any of the dietary changes, oral drug treatments or drug injections described in the proposal display no long-term adverse effects. Some animals may experience diarrhoea but recover within 48 hours. Metabolic cage experiments are known to lead to stress and weight loss, particularly in mice. To minimise this, the length of time animals are confined in the metabolic cages will be kept to a minimum and enrichment in the form of a nest box or crawl ball will be included in the cage. Body weights of the animals will be monitored as a guide to general health. If an animal undergoes weight loss approaching 20% of their starting weight, it will be killed by a Schedule 1 Method. If any of the procedures result in or induce evidence of suffering in an animal that is beyond those described in the licence, or in any way compromises normal behaviour, the animal will be humanely killed using a schedule 1 method unless, in the opinion of a veterinary surgeon, such complications can be remedied promptly and successfully using no more than minor interventions (such as providing wet mash, additional warmth or topical treatments).</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>An important aspect of the proposed work is to establish how the body as a whole responds to changes in dietary intake, what effect this has on the heart and bones, and whether diseases such as diabetes and kidney failure change these responses. Therefore, while cell culture experiments will be used to investigate how an individual cell responds to a specific nutrient, it cannot provide information on these whole body interactions. Where possible human tissue, or urine/blood samples will also be used to complement the proposed animal studies. I have access to samples from patients</p>

	<p>receiving some of the drugs outlined in the proposal and am developing new techniques using human urine to document how the kidney transports different nutrients.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The experimental approaches described above will allow a significant reduction in animal numbers required to achieve the objectives. Wherever possible, changes in kidney and intestinal transport processes will be established in one animal. This approach not only reduces animal usage but also allows more detailed and precise interpretation of data.</p> <p>When animals are required the numbers needed for each experiment will be determined using sample size calculators freely available on the internet. Statistical analysis will then be performed using tests appropriate for the data collected. All experiments will be performed according to the ARRIVE guidelines for animal research.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The protocols described have been designed to be the most refined possible, using the minimum number of animals, to provide statistically satisfactory results. These protocols have been planned to cause the least pain, suffering or distress whilst adequately addressing the scientific question they have been designed to answer.</p> <p>In most instances the rat is the animal of choice as surgical or chemical induction of chronic renal failure or diabetes is generally easier and more reproducible in rats. However, genetically modified mice, such as those lacking the gene for specific transporter proteins may also be used under this licence. For blood pressure monitoring although the animals undergo a surgical procedure, it is widely recognised that this technique provides more reliable results and is more humane than the previously used tail cuff procedure which involves significant stress due to repeated restraint.</p>

Project	Repair and regeneration of cardiac and skeletal muscle
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to understand the effects of stem/progenitor cells, exercise and ageing in the repair and regeneration of skeletal and cardiac muscle. As the majority of patients that will require reparative/regenerative therapies will be elderly, we are interested in ways to prevent or treat the effects of ageing to improve muscle repair and regeneration
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit)	My research is at the forefront of current molecular and cellular biology of stem/progenitor cells and muscle regeneration, and the findings are directly transferrable to the treatment of cardiovascular disease/failure, muscular

<p>from the project)?</p>	<p>dystrophy and age-related muscular diseases. The work has major societal, medical, technological and economic impact amenable to widespread use at a reasonable cost. My labs work has contributed towards the advancement of regenerative therapeutic cell approaches where the repair/regenerative mechanisms are activated in situ REDACTED. An effective therapy that could prevent a moderate proportion of patients from progressing to a chronic condition would lead to significant improvements in the social impact of the disease, and large reduction in healthcare costs. My research aims to address this unmet medical need.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice and rats will be used. Approx. 6500 will be used over 5 years. 62% of this number is for breeding and maintenance of genetically modified animals.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Some animals will be given ligation of an artery in the heart, to simulate a heart attack. This is considered severe. All animals will be anaesthetised so the level of pain and suffering should be zero during this time. Animals will receive stem/progenitor cells and/or perform exercise training to repair their heart so they should show improved cardiac function.</p> <p>The exercise training which is expected to be of enjoyment to the animals, who prefer an active lifestyle rather than a sedentary one.</p> <p>Some animals will receive drugs which target aged and damaged cells. These drugs have been shown to prevent or delay tissue dysfunction, physical dysfunction and extend health- and lifespan.</p> <p>At the end of the protocol the animals will be killed in a humane way and their tissues will be collected for us to analyse and determine the efficiency and effectiveness of the repair and regeneration process.</p>

Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Due to the types of procedure involved (i.e. damage to cardiac muscle due to ligation of a major artery) there is no alternative other than to use animals. Together with ethical constraints for removal of human heart tissue, this amount and sort of analysis cannot be obtained with human samples. We obtain human myocardial and skeletal muscle samples but these are small (~200mg) and limited to the atria and individual skeletal muscle. We use human cells to model ex vivo 3D culture systems and screen for relevant agents (i.e. senolytics) involved in cell survival and proliferation.</p> <p>However, due to the complexity of the interactions between different cell types present in each tissue and the body, we are unable to completely model what happens using cell culture or ex vivo systems.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We have undertaken statistical tests to estimate the number of animals needed to obtain significant results. We archive tissue samples.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>My lab strives for and is committed to a constant refinement in our protocols to improve the welfare of the animals. For example, we do not use electric shock grids to enforce exercise on animals. We encourage the animals to exercise by prodding with a paintbrush or tapping the side of the treadmill panel. We have invested in training in the microsurgical techniques and refinement of protocols and procedures. All personnel are trained and signed off as competent in each procedure. Animals are monitored closely during recovery from surgery to assure adequate analgesia. Our experiments are conducted under the supervision and advice of veterinary surgeons and named animal care</p>

	and welfare officers.
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Project	Repairing the damaged brain after hydrocephalus	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The objectives of this project are to determine changes that occur after induction of hydrocephalus, a condition where fluid builds up on the brain and causes damage to brain tissue by a build-up of pressure. We are particularly interested in learning how neurons deal with the injury that makes them vulnerable to death, the scar tissue that forms after injury and the pathways involved in pressure regulation.</p> <p>This will allow for a better understanding of the mechanisms of hydrocephalus and will help us to identify therapeutic drugs that will be used to protect nerve cells from death, dissolve scar</p>	

	tissue and reduce raised pressure.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The project will provide important data that will improve our understanding of the changes that occur after hydrocephalus and provide an insight into what is required to promote nerve cell survival, removal of scar tissue and promote nerve regeneration. This will underpin the discovery of novel therapeutic drugs that will be used to promote nerve cell survival, scar tissue removal and reduce raised pressure in the brain.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats: 2,050 Over a period of 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Potential harm results from hydrocephalus, which will be created under general anaesthesia. In the vast majority of cases, there is no adverse response to induction of hydrocephalus. However, hydrocephalic animals do show retarded weight gain, a dome-shaped head and gait instability. There are clear guidelines in place in our facility to ensure that suffering in animals is minimised by either administration of pain-killers or termination of experiments. Soft mash will be provided on the floor of cages as well as injections of fluids and extensive care within the first three days after induction of hydrocephalus. We will remain vigilant for any adverse effects and will promptly provide pain relief or treatment if appropriate, or humanely kill the animal. Animals will be killed by Schedule 1 methods or perfused with 4% paraformaldehyde under terminal anaesthesia for histological analyses.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no adequate substitute for using the <i>in vivo</i> models described in this application. Establishment of potential clinical relevance of regulatory molecules interacting in a dynamically changing hydrocephalic site can only be achieved in an animal model. A less sentient animal such as fish cannot be used since they spontaneously regenerate their damaged axons after injury and achieve complete recovery of function. Therefore,

	<p>rats and mice are our prototypic laboratory animals and have been rigorously characterised by ourselves for the hydrocephalus paradigm and shown to be representative of the human condition by us and others. The tools for the project have all been prepared in relation to the models described herein and continuity of the study in these species will be essential for significant progress to be made in a timely and efficient manner.</p> <p>However before embarking on each experiment, we will consider key issues, which include: is the research necessary?; what has already been done in this area/; what models have been used?; what are the best methods/procedures?; alternative consideration for potential pain and distress?. We will then search online databases such as Pubmed and Web of Science for alternatives to animal experiments and systematically review the number of hits using defined search terms that will seek out alternatives to animals. All of these strategies will be used to address possible alternatives to animals, prior to embarking on experiments in live rats</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Some of the end-point measurements (e.g. nerve regrowth, scar formation etc) may be essentially qualitative and for these we use 3-6 animals per treatment group. In most experiments with quantitative end-points, 6 animals are randomly assigned to each treatment group, a number calculated as the minimum required to provide statistically significant results. This has been determined on the basis of our previous experience with these procedures, the methods of analysis and after consultation with statisticians to calculate power.</p> <p>All experiments will be designed and appropriately powered using the NC3Rs experimental design tool to ensure compliance with ARRIVE guidelines.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having</p>	<p>The models selected closely resemble the features seen in humans after hydrocephalus.</p> <p>All therapeutic agents are evaluated and optimised <i>in vitro</i> prior to <i>in vivo</i> application. We</p>

<p>regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>keep our experimental time points in longitudinal studies to a minimum and use archival control results where possible. Multiple analyses are conducted on all harvested tissues. We use the minimum number of interventions and minimal volumes for drug delivery during experiments and continually seek methods to reduce these by studying alternative drug delivery strategies. These refinement steps significantly reduce animal usage and severity</p> <p>Before conducting each experiment, it is discussed with the NACWO and NVS routinely to ensure animal welfare is maintained throughout the experiment and that minimum suffering is caused to acquire the scientific endpoints. We will also review each experiment on completion to see what lessons can be learned from the study in terms of endpoints (scientific and humane) and any animal welfare issues that may have arisen during the experiment that could then guide the next experiment.</p> <p>The surgical technique for inducing hydrocephalus has been refined to minimise injury to the animal and aid with recovery. In addition analgesics are given post-operatively (following day) to minimise pain from the operation.</p>
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Project	Reproduction Safety Tests on Industrial and Agricultural Chemicals
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Basic research
	X Translational and applied research
	X Regulatory use and routine production
	Protection of the natural environment in the interests of the health or welfare of humans or animals
	Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The purpose of this project licence is to establish toxicological and safety data in animals following exposure to industrial and/or agricultural chemicals that Man may be exposed to. These studies performed are a regulatory requirement for successful market authorisation.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Chemicals play an important role in daily life. Therefore, their safety for Man, other animals and the environment has to be considered carefully. By establishing sufficient toxicological and other safety data in animals, safe handling precautions may be determined thus protecting the health and welfare of hundreds (e.g. for a site limited industrial chemical intermediate with limited potential for human exposure)

	<p>to millions (e.g. industrial or agricultural product with world-wide market) of humans and animal species which may contact the materials concerned and facilitate the world-wide marketing and safe use of products. The projects performed under this licence provide safety data to facilitate sound regulatory decisions worldwide that protect the public and the environment from possible hazards. The regulated products have the potential to improve and enhance the health, well-being and quality of life of people and animals. For example, improved crop-protection increases food security, while development of safer chemicals and chemicals with reduced environmental impact is clearly beneficial for human and animal health and in environmental protection. The projects undertaken use methodologies that are well established and known to produce accurate and reliable results that can be used in regulatory risk assessment. Furthermore, the studies can rapidly identify any overt toxicity which would cease the development of the test item and therefore enable the Sponsor to make a decision at the earliest opportunity to cease production: reducing the risk of possible human exposure and avoiding unnecessary expenditure and use of resources. The work performed under this licence will be undertaken in a GLP compliant laboratory thereby ensuring data integrity and accuracy.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The studies performed will typically use the rat and rabbit, Where the rat is deemed not to be the most appropriate species the mouse will be considered. It is expected that approximately 10000 mice, 17000 rats and 2700 rabbits will be used over a five year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>All animals will be dosed with the test chemical via a route that would mimic accidental exposure in man, typically in food, water or contact with skin at normally three dose levels and monitored closely for signs of toxicity. The majority of animals will have no clinical effect, with approximately 25% expected to lose weight or not gain weight at the expected rate, with only a minority (<5 %) exhibiting clinical signs of toxicity such as prolonged diarrhoea, tremors etc which necessitate immediate killing to protect animal welfare. The administration of the test chemicals to a pregnant animal may cause unborn foetuses to be affected which may include foetal abnormalities which result in death in the uterus. Some study designs require</p>

	<p>assessments on the development and behaviour of the adults. Assessments include the monitoring of activity and behaviour and testing of an animals grip strength, these test cause minimal discomfort only. All procedures performed on the animals are fully validated and established within the industry to cause the minimum distress and will only be undertaken by trained staff. On completion of each study the animals will be humanely killed and a post-mortem performed in order to establish effects on organs and tissues which aid the evaluation of the toxicity of the chemicals.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>It is generally accepted that the way in which a material is metabolised and distributed within a living body has a significant effect on how it works and its potential toxicity. In addition, effects on complex interacting biological systems cannot yet be replicated in in-vitro or ex-vivo tests. Consequently, for the majority of chemicals it is imperative they are tested on living animals in order to assess for toxicity to tissue, organs and systems e.g. the cardiovascular, respiratory and reproductive systems following repeated exposure.</p> <p>As the use of alternative methods, including the use of dead animals cannot, at this moment in time generate relevant data which supports the submission of safety data to international regulators, alternative methods such as in-vitro techniques will be used as much as practicable to supplement the work involving protected animals.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of animals used will comply with the requirements of the relevant regulatory guidelines and will be the minimum practicable to achieve the objectives of the study and allow meaningful interpretation of the data. The definitive number of animals required depends on whether or not the group is expected to demonstrate an effect. For a high frequency effect fewer animals are required, to presume the absence of an effect the number required varies according to the endpoint being considered, its prevalence in control populations or dispersion around the central tendency.</p> <p>For all but the rarest events such as malformations, and total litter loss, evaluation of between 16 and 20 litters per group for rodents and rabbits tend to provide</p>

	<p>a degree of consistency between studies. Where there is a steep dose response, or blood samples are needed for toxicokinetic purposes for example, then additional groups may be necessary.</p> <p>REDACTED has significant experience running reproduction toxicology studies and the knowledge gained will be used to design studies capable of achieving their objectives.</p> <p>Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of the studies to be performed, with power-sample size calculations performed for specific studies if necessary to determine group size. For preliminary studies, small groups are acceptable because of the use of overt toxicological endpoints. Where group sizes are sufficient (rodent studies), data from definitive toxicity studies are analysed statistically.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>It is generally desirable to use the same species and strain as in other toxicological studies. Reasons for using rats as the predominant rodent species are practicality, comparability with other results obtained in this species and the large amount of background knowledge accumulated.</p> <p>In the majority of cases outbred rodents and the New Zealand White Rabbit will be used as these strains have good fertility and provide sufficient foetues/litter sizes for the assessment of test item related findings.</p> <p>In embryo-foetal toxicity studies only, a second mammalian species traditionally has been required; the rabbit being the preferred choice as a non-rodent species. Reasons for using rabbits in embryo-foetal toxicity studies include the extensive background knowledge that has accumulated, as well as availability and practicality. Where the rabbit is unsuitable for example they do not show exposure, a second rodent species,forexample themouse may be acceptable and should be considered on a case by case basis.</p> <p>Regulatory authorities require characterisation of toxicity at the Maximum Tolerated Dose (MTD) to ensure robust evaluation of safety before potential human exposure; it is therefore, necessary to perform toxicity studies at high doses that produce overt toxicity, usually in terms of clinical signs or body weight loss or depression of weight gain against age-matched controls. Response to observed effects will depend on</p>

the objective of the study; on preliminary studies where the objective is to determine the MTD then doses will be increased until effects are evident; once clinical signs or extent of weight loss indicates that a dose is unsuitable for use on a definitive study then action would be taken to alleviate the clinical signs, usually this would involve termination of the sex/group or may involve reduction of the dose.

Definitive studies should also show evidence of toxicity and typically clinical signs or reduced body weight gain would be seen in the high dose animals following dosing: some clinical signs may be present for up to 8 hours after dosing but would be expected to show clear signs of recovery at this time. Generally clinical signs would be expected to be absent prior to dosing on the subsequent day although some animals may still show some moderate clinical signs. Clinical signs will generally be expected to be up to moderate in severity. However, because toxicity can become worse with increased duration of dosing or the exposure of animals to the test substance may increase over time, sometimes high dose animals and occasionally lower dose animals may show significant adverse effects and in this case action will be taken to alleviate the clinical signs such as withdrawal of the animal from dose, reducing the dose (if appropriate) or termination. In some cases, effects seen on definitive studies can differ from those seen on preliminary studies for no apparent reason. A single clinical sign is generally unlikely to be sufficient to warrant termination unless the presentation is severe and the combined effects of multiple signs will always be assessed for cumulative harm to the animal. Preliminary studies when performed will allow the delimitation of appropriate intervention and humane end points. Definitive studies will be performed within a moderate severity limit, as the use of humane endpoints, careful monitoring and rapid response to observed effects will negate the need for a severe limit.

Where little is known about a substance, or a class of substances, before commencing preliminary studies consideration will be given to using staggered starts where the effects in one group are assessed before commencement of dosing groups at higher doses. In all cases, dose selection will be based on all available information; data from preliminary studies or in some cases, and data from published literature.

Project	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project is being performed to detect test substance related impaired mating performance/fertility, damaged reproductive organs or, impaired survival, development or growth of fetuses/offspring, as required by the regulatory authorities.</p> <p>Authorities can then make informed risk based decision concerning the known reproductive/developmental toxicity hazards associated with a drug and whether the drug should be approved for use on clinical or veterinary trials, or approved for marketing, and to prescribe</p>	

	<p>conditions for its safe use.</p> <p>For chemical and agrochemicals, the project will enable regulatory authorities to make an informed risk based decision concerning the known reproductive/developmental toxicity hazards associated with the substance and whether the substance should be approved for marketing, and to prescribe conditions of safe use and handling of the substance].</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Safer human or veterinary drugs will be approved for clinical trials and marketing. The regulatory authorities will thus be able to better prescribe conditions for the safe use of drugs. Critically, on the basis of risk assessments completed, based on information made available through animal testing, those drugs that are deemed unsafe will be precluded from further development/marketing. Safer chemicals and agrochemicals will be approved for marketing, and regulatory authorities will thus be able to better prescribe conditions for the safe use and handling of these substances.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>124000 Rats 6000 Mice 8500 Rabbits Over the 5 year life of the project licence. A small proportion of these animals may be genetically altered.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Rats, mice or rabbits are dosed (e.g. by oral gavage, injection or inhalation) with test substances. The majority of animals are expected to show little or no reaction to treatment. A few animals may show transient body weight loss and reduced appetite following the start of treatment with the test substance, but are expected to recover quickly. Rats and rabbits may have minor surgery to implant a cannula into a vein or a device under the skin that can release a medicine slowly. They are expected to recover quickly and will be given painkillers and post-operative care just like people recovering in hospital. Animals may be subject to restraint or restriction of free movement during exposure to test substances (e.g. held in inhalation chambers, in jackets or tethered to a drug delivery system or may be housed in a metabolism cage for short periods of time to enable collection of urine and /or faeces for analysis); none of these procedures are expected to affect the clinical</p>

	condition of the animals or elicit more than transient body weight loss and reduced appetite. Animals will be killed at the end of all regulatory studies for reproductive/pathological evaluations to achieve defined regulatory endpoints.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals because non-protected animal alternatives cannot replace the extremely complex series of events involved in reproduction and/or subsequent development of young and reproductive organs: these processes cannot be effectively modelled in the laboratory in test tubes/dishes or by the use of sub-mammalian animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	<p>The regulatory guidelines usually indicate the number of animals in a study; otherwise, the number used is the minimum to achieve the aims of the study.</p> <p>When testing several closely related test items in several different preliminary studies, use of a common control group/less control animals can decrease numbers of animals used per study. So can the experimental design which is optimised, being mindful of regulatory requirement. If a study is cancelled, in some circumstances animals may be reused on a different study rather than using an entirely new batch of animals. Animals which have only had restraint training and have received no treatment may be used on another study. Re use is subject to satisfactory veterinary assessment and is such that scientific outcomes can be reached with the most efficient use of animals and without data or welfare compromise. Methods used to measure the level of a drug in blood which are compatible with small blood volumes (less than 0.1 ml) will be used where available, and this can lead to a reduction in animal use.</p>
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general	Rats (Rodents) and rabbits (non rodents) have been chosen for use of these studies because rats and rabbits are the species preferred by the regulatory authorities for testing. Mice are rarely used when either the rat or rabbit are demonstrated to be unsuitable e.g. due to higher levels of drug being

<p>measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>present in the blood of mice or, the drug target responds strongly to the test item in mice but only weakly in rats or rabbits.</p> <p>Studies are performed in a stepwise manner, starting with preliminary studies using small numbers of animals where there is limited information, and building on the information obtained from these initial studies doses to be used, group sizes and procedures are optimised in the next phase/definitive regulatory studies. This gives the highest prospect of refining and optimising the programme to achieve the desired scientific outcomes (data) whilst minimising any pain, suffering, distress or lasting harm experienced by the animals on study.</p> <p>The clinical condition, body weight and appetite of all animals are regularly monitored for signs of any adverse effects on their health or wellbeing to prevent unnecessary suffering, early humane end-points are applied under appropriate veterinary guidance (e.g. modification/withdrawal of treatment with the test item, or humane killing of affected animals).</p> <p>Other examples of refinement are exposing the animals to test item in water or diet rather than dosing. Dosing of a drug via an infusion system rather than repeated injections can despite initial surgery involved to place catheter/delivery device be more refined as it avoids frequent repeat injection over the study period which may be up to 7 weeks.</p>
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Project	Research into infectious fish disease
Key Words (max. 5 words)	
Expected duration of the project (yrs)	3 Years 3 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>To better understand, diagnose, control and prevent fish diseases, thus improving production and welfare of farmed fish and protecting wild aquatic life.</p> <p>There are two main sub-objectives:</p> <p>a) To improve understanding of aquatic animal disease (host susceptibility, infectivity, and pathogenicity);</p> <p>b) To develop and apply methods examining the efficacy of substances for therapeutic and/or diagnostic use</p>
What are the potential benefits likely	With the ongoing depletion of wild fish stocks,

<p>to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>fish farming is increasingly a critical sector for aquatic food security. Despite major advances over the last 40 years, infectious diseases continue to be a major constraint, reducing productivity, fish welfare and resource use efficiency. Fish farms are usually in contact with the surrounding river/sea environment, meaning infections can move easily between farmed and wild stocks. Research to understand diseases and develop control methods (e.g. prophylactic treatments, vaccines, disease resistant strains) is integral to assuring the future sustainability of aquaculture. The UK has a high aquatic health status, which is under constant threat from emerging and introduced diseases. Government policy is to eradicate any notifiable diseases disease by slaughter and disinfection if possible. This policy requires reliable validated diagnostic methods and an understanding of disease risks to wild fish populations. Maintenance of the UK's aquatic biosecurity and compliance with national and EU legislation on aquatic disease requires knowledge of aquatic disease supported by long-term programmes of diagnostic tool development, disease monitoring, disease control and prophylaxis. These form the statutory and scientific basis of the aquatic animal disease research programmes for wild and farmed fish covered by this licence.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We seek authority to work with any fish species because fish from different environments and continents contribute to food security. In terms of wild fish disease research, we are likely to use endemic species. We seek authority to use a maximum number of 99,000 fish over a 5-year period; however, this number is expected to be much lower as it includes a large contingency in case a fish disease outbreak occurs requiring additional investigations</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at</p>	<p>One main procedure is used in four of the six protocols in this licence; this involves pathogen challenge, i.e. controlled exposure to pathogens. By nature of the serious pathogens of interest, the potential adverse</p>

the end?	effects are generally severe. The actual adverse effects are managed by defined humane endpoints implemented by intensive monitoring which involves both direct visual checks and desktop observations of live videos from in-tank underwater cameras. To our knowledge, we are the only research facility employing such remote monitoring to manage fish welfare.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	The development of disease or resistance is difficult to study without a whole animal model as it involves multiple tissues and organs. The early parts of our investigations are conducted in nonanimal models; however, the infection, pathogenesis, host immune response, treatment and vaccination responses require complex metabolic, anatomical and immunological mechanisms that cannot yet be modelled in vitro or in surrogate invertebrate species.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	Every effort is made to minimise the numbers of animals used in studies: statisticians advise on the numbers required to achieve meaningful results, animal husbandry experts advise on fish social needs and the ethics committee (AWERB) scrutinises each study plan. Members of the AWERB include scientists, veterinary surgeons, animal husbandry experts and lay people; they all have the power to veto study plans.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We want to have the ability to work with any fish species, of interest to both conservation and food production. The main tool we will employ to refine prospective severe procedures and minimise suffering is close monitoring.</p> <p>Fish are typically sourced from our own breeding establishment to ensure disease-free, high quality animals acclimated to experimental tank conditions. Externally sourced fish are health screened on arrival and quarantined to ensure a good health</p>

	<p>status and acclimation before use. We have a dedicated, high-tech aquarium facility, with monitoring and call-out alarms (water temperature, flow, depth). Named persons oversee staff training and performance, care of fish, and dissemination of information. Close links with the international fish research community ensures we are aware of any developments in fish care and biosecurity. Consideration is given to all aspects of the environment (including enrichment) e.g. space, water quality and current, conspecific density, lighting, shading, refuges and diet. We have a dedicated team of specialist aquarists complemented by long-standing experience in fish husbandry. Stock and experimental fish are closely monitored and interventions (including veterinary treatments) are implemented wherever possible. We believe we have a strong institutional culture of care and have review processes to identify where improvements in care can be made.</p>
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Project	Research on Bacterial Products used in Medicine	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aims and objectives of the project are to be able to provide assurance that bacteriological products used in medicine, many of them paediatric vaccines, are safe and likely to be effective and also to allow the production of antisera necessary for the evaluation of these bacteriological products.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit)	The principal benefit from this research is that the Division will have an improved understanding of whether a new vaccine or therapeutic product is likely to be safe and how	

<p>from the project)?</p>	<p>it works. The knowledge gained will be used to inform regulatory process in the UK (MHRA), in Europe (EMA and EDQM) and worldwide (WHO). In addition, the data will be used for the development of an appropriate package of batch release tests. Typically the output from such research would be published in peer reviewed journals.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The project involves use of small animals only (mice, rats, hamsters, guinea pigs and rabbits). For the duration of the project (5 years) it is expected that up to 21,335 animals will be used (18020 mice, 1050 rats, 500 hamsters, 1,700 guinea pigs and 65 rabbits).</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The project includes 16 protocols, of which one is unclassified procedure, one is mild procedure, 10 are moderate procedures and 3 are severe procedures. The majority of animals are not expected to experience more than moderate adverse effects (for example irritation or inflammation at the site of injection, or signs of general toxicity such as loss of appetite, weight loss and reduced activity). Frequent monitoring and supportive husbandry measures are used to minimise the impact of these adverse effects. For the 3 protocols with a severe severity limit, 2 involving mice and one involving rats, adverse effects may include shock, convulsion, weight loss, trauma (as a result of brain injection), and loss of consciousness. In some cases death occurs for some of the test animals (up to 20% across these three protocols). At the end of all tests animals are humanely killed using a schedule 1 method. The impact of these adverse effects is mitigated as far as possible by rigorous observation of the animals by experienced staff and the application of recognised humane end points where necessary.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal</p>	<p>Protective potency and safety of biological medicines cannot be examined without the use of animal procedures, as multiple factors contribute to a protective immune response and</p>

<p>alternatives</p>	<p>the immune system itself is too complex to be modelled in vitro. Also in vitro biochemical models for safety are often product specific and in vivo assays are still required for new products or formulation as well as for product specific validation. The purpose of much of the research described in this licence application is to facilitate further reductions in the use of animals by validation of alternative procedures. However, multiple factors contribute to a protective immune response thus, in many instances there is no alternative but to generate data on vaccine safety and efficacy using existing animal models. The aim of this objective is to provide data to validate modifications, refinements and in vitro alternatives to widely accepted pharmacopoeial or other regulatory procedures. This can only be achieved by comparing the existing animal procedure with the novel assay designed to replace it.</p> <p>Production of polyclonal antibodies by immunising animals with the required antigens is necessary to produce reagents needed for the evaluation of biologicals medicines and we continue to investigate the possibility of using of monoclonal antibodies produced in vitro (e.g. phage display) for suitability to replace the use of polyclonal antibodies.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of doses and group sizes used in the protocols described in this license are based on well established and validated methods that have been laid down in reference documents issued or endorsed by scientific advisory bodies such as the WHO, licensing authorities and national or European Pharmacopoeias.</p> <p>In the absence of regulatory endorsed procedures, assays are designed based on the ARRIVE guidelines taking advice from biostatisticians, with an aim to use the minimum number of animals that is expected to provide information of required accuracy, precision and reproducibility . Normally an experiment will consist of a sufficient number of experimental and control groups, each containing an appropriate number of animals, to obtain</p>

	statistically significant results.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>This work will employ animal species chosen from information published in literature or as required from regulatory guidance, documents or monographs on the basis of generating relevant effect and suitable dose responses. Such information will often be supported by collaborative studies taking into account suitability per product, generating information on multiple antigens and with the least severe end points where possible. Extensive validation studies have contributed a significant body of information on the use particular species or end point.</p> <p>Most of the protocols are based on regulatory requirements and that some of these are severe due to the systemic nature of the infection/disease to be modelled. Efforts continue to refine these models and improve the welfare and monitoring of the animals during the studies.</p> <p>In all probability the replacement or modification of an existing bioassay will ultimately result in the use of a less severe procedure for testing the potency or safety of bacteriological products. Thus, the existing pharmacopoeial protocol represents the “worst case” in terms of the severity of adverse reactions. To obtain the necessary comparative data for the validation of the new assay, it is necessary to perform the existing pharmacopoeial assay exactly as specified to obtain the definitive data. Prior to validation however, most, if not all, the development work leading to the establishment of the new assay can be carried out as standalone experiments without the need to include the existing assay for comparison.</p> <p>Efforts continue to refine procedures with substantial or moderate severity limits. Challenge assays will only be used when there is no alternative. Protective animal models are considered more severe than serological models for the evaluation of vaccine potency. Where serological correlates of protection provide reliable indicators, serology is</p>

performed on a routine basis and protection models are used only occasionally to confirm potency of a new vaccine or therapy or to validate the serological assays, as protection is not a simple measure of antibody response but also depends on the affinity and the specificity of the induced response which could not be determined solely from the serological assay, unless it has been validated for the particular product under testing. The introduction of serology as alternative method to challenge means that the severity of the procedure is mild compared to the moderate limit assigned to the challenge models. The infant rat model is only used when a new vaccine/therapy is evaluated to demonstrate the correlation between in vitro and in vivo protection.

As part of routing ongoing animal welfare measures, animals are housed in groups in cages suitable for the species used, with a range of varied and appropriate enrichment to allow natural behaviour.

Project	Research on products used in prevention and therapy of tuberculosis and malaria	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The broad objective is to provide quality assurance that vaccine and therapeutic products used in medicine for tuberculosis and in some cases, malaria, are safe and likely to be effective. This also includes studies of how these medicines work in a living body.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The principal benefit is to provide assurance that vaccine and therapeutic products used in medicine for tuberculosis and in some cases, malaria, are safe and likely to be effective. A high level of assurance is particularly important for vaccines given to healthy people. If public	

	confidence in vaccine safety/ effectiveness falls, vaccine uptake and coverage may be reduced and diseases that were previously well controlled by vaccine can re-emerge. Other potential benefit includes improvement of understanding whether a new vaccine or therapeutic product is likely to be safe, how it works and how its quality to be regulated. The knowledge gained will be used to inform regulatory process in the UK and worldwide.
What species and approximate numbers of animals do you expect to use over what period of time?	The project involves use of small animals only (mice, guinea pigs and rabbits). For the duration of the project (5 years) it is expected that up to 5460 animals will be used (maximum of 5000 mice, 460 guinea pigs).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The project including 8 protocols consist of procedures of moderate severity level. The animals may receive substances such as vaccines by standard routes (for example subcutaneous and oral). Animals may be challenged with malaria or TB including malaria by mosquito bites which is carried out under anaesthesia. Blood samples may be taken. Non-invasive imaging may be carried out under short duration anaesthesia. The majority of animals are not expected to experience more than mild to moderate side effects (for example skin irritation or swelling at injection sites). In rare occasions, uncontrolled infection would result in gradual loss of body weight and reduced physical activity. Frequent monitoring and supportive husbandry measures are used to reduce the impact of these side effects. At the end of all tests, animals are humanely terminated. The impact of these side effects is reduced as far as possible by frequent observations of the animals by experienced staff and the application of recognised humane end points where necessary.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal	Some protocols used in this project are performed according to methods described in regulatory guidance. In some cases, no suitable non-animal alternatives are currently available.

<p>alternatives</p>	<p>In particular, protection and safety of a biological medicine cannot be fully examined without the use of animal procedures, as multiple factors contribute to protective response and the immune system itself is too complex to be modelled in cell culture. Efforts are continuing to develop suitable alternatives to some of the methods that are retained in this project licence.</p> <p>The detection of biomarkers of protection could in the long-term result in progression towards suitable <i>in vitro</i> detection systems to replace some of the <i>in vivo</i> work.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>For some protocols used in this project, the number of animal groups and size of each group are based on validated methods that are described in regulatory guidance. The number of groups and/ or group size of the other protocols are based on previous experience on small pilot studies, or on information from published literature or collaborators gained with the similar procedure and the advice of experienced biostatisticians. Many tests require the use of one or more control groups and where possible, many test samples will be included together in a single experiment to maximise the use of these control groups and therefore minimise the total number of animals used during the project. New whole body imaging method is also introduced for monitoring distribution of biological medicine within a sedated live animal in order to reduce number of animals sacrificed at various time points.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The protocols involving infection with tuberculosis and malaria are designed to reduce signs of disease and severity. Humane end points are determined such that animals can be euthanized at the earliest stage of unexpected adverse events to minimize the impact of infection on animals. Frequent monitoring by experienced staff, including out-of-hours checks for some procedures help to ensure that animal welfare is maintained wherever possible. As part of routine and ongoing animal welfare measures, animals are housed in caging</p>

	suitable for the species used, with a range of varied and appropriate environmental enrichment to allow natural behaviours.
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Project	Research to improve the treatment of envenoming
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To protect the health of human patients, this project will use mouse models to preclinically test the effectiveness and safety of antibody-based anti-toxins and other toxin-neutralising therapies.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits of this project will be the demonstration of the preclinical efficacy and safety of new therapies designed to treat various aspects of toxin-induced pathologies, that affect >500,000 people each year across the world. These new therapies are designed explicitly to provide superior efficacy and safety than existing conventional anti-toxin therapies,

	<p>which have many limitations that result in poor patient outcomes in victims. Our findings will therefore underpin the selection of highly promising therapies for future use in early clinical trials in humans, following the demonstration of prerequisite safe and effective use in a proven animal model. This data will also be used to establish the initial dose of new therapies for those human trials, and thus be utilised for regulatory requirements during product translation. The relevant UK/EU Pharmacopoeias and WHO guidelines stipulate that the effectiveness of anti-toxin therapies should be preclinically tested in mice before use in human patients. Thus, in line with regulatory and public health policy, outputs from our previous project licenses relating to this work resulted in: 1) Provision of 37,000 vials of effective treatments to help save lives in Africa 2) Cessation of manufacture of a poorly efficacious anti-toxin product destined for Africa before it reached the market. 3) Validation of the efficacy and stability of products to be used by the NHS and in European countries for treating intoxications. It is important that human victims of intoxication receive appropriate treatment: the inappropriate distribution of untested products in numerous African countries resulted in an increase in mortality of treated patients from 0.5% to 12%. This example demonstrates that while the cost to mice of these preclinical tests are high, that benefit to humans is greater. The cost/benefit ratio of preclinical testing is greatly skewed in favour of human health benefit.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We anticipate requiring a total of 3,950 mice and 18 rabbits over a five year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Many of the proposed experiments are classified as severe due to the likely adverse effects the natural toxins being used to challenge the mice with are likely to cause. Such toxins cause a variety of severe pathologies, summarised broadly as cardiovascular, neurological and/or local disturbances. Thus, specific adverse reactions relating to this toxin research could present as:</p>

	<p>neuromuscular paralysis, laboured respiration, seizure, anaemia, haemorrhage, oedema, weight loss, and/or local inflammatory, haemorrhagic and/or dermonecrotic lesions. Previously defined humane endpoints (see refinement) will be implemented as indicators for euthanasia, in order to minimise animal suffering. We do not anticipate a high incidence of adverse effects for experiments relating to animal immunisation and the toxicity testing of new anti-toxin therapeutics. These experiments do not use high doses of natural toxins, and thus experiments relating to the safety testing of new therapeutics have a moderate severity designation, with potential adverse events relating predominately to the longer-term maintenance of mice (e.g. weight loss, hypothermia, immobility). Immunisation experiments have a higher risk (although still relatively low) of observing adverse events, including the potential for ulceration at the injection site, along with longer term health issues as the result of mice ageing (e.g. tumour development, inner ear infections, skin lesions and weight loss). At the end of the experiments all animals will be euthanised using a schedule 1 method.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Currently there is no <i>in vitro</i> alternative method for raising polyclonal antibodies - animals must be used.</p> <p>The mouse model of toxicity has proved a satisfactorily accurate representation of the effects caused by these natural toxins in humans. There is no <i>in vitro</i> alternative assay yet devised to supplant these animal tests (see below) because natural toxins can cause a variety of different physiological effects simultaneously, and intoxications are the result of complex mixtures of these toxins at the same time. Although we have investigated if <i>in vitro</i> techniques could be used in lieu of <i>in vivo</i> testing, we have found that they are insufficiently accurate to accurately predict the outcomes of <i>in vivo</i> tests. We continue to actively investigate methods that may provide</p>

	accurate assessments of therapeutic efficacy <i>in vitro</i> .
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The literature and results of previous experiments are closely examined to reduce the range of toxin doses, and therefore the numbers of mice, needed to establish the statistical validity of the assays. To further substantially reduce the numbers of mice required to achieve the objectives, we use (i) preliminary range finding studies and (ii) dose-staging methods to accurately determine the lethality of the toxin/s and/or potency of the antibody or inhibitor therapies.</p> <p>Statistical analysis is performed on all the results, and the minimum number of mice required for statistical validity is used throughout.</p> <p>During this project we will continue our vigilance to identify methods that show promise in reducing the numbers of mice required for these assays.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the physiologically least advanced rodent species that could be used for the preclinical assays. For the immunisation experiments we will also use a low number of rabbits following optimisation experiments conducted in mice. Rabbits are the most appropriate model for these experiments as their blood volume will permit collection of sufficient antibodies for later preclinical testing of new anti-toxin therapies.</p> <p>All of the previous preclinical tests on the efficacy of antibody-based therapies have been performed on mice. It would therefore be illogical to change the animal model species. The physiology of mice has been well characterised and the effects of toxin/s can therefore be accurately determined. The consistent use of mouse genetic strains (e.g. CD1) reduces independent variability and therefore (i) reduces the number of animals required for statistical validity and (ii) increases the validity of comparing results from different experiments.</p>

	<p>To refine protocols, we will:</p> <ul style="list-style-type: none">● Undertake <i>in vitro</i> tests to reduce the number of candidate therapies requiring <i>in vivo</i> testing● Undertake <i>in vitro</i> tests to reduce the number of candidate therapies requiring <i>in vivo</i> testing● Undertake <i>in vitro</i> tests to reduce the number of candidate therapies requiring <i>in vivo</i> testing● Undertake <i>in vitro</i> tests to reduce the number of candidate therapies requiring <i>in vivo</i> testing● Undertake <i>in vitro</i> tests to reduce the number of candidate therapies requiring <i>in vivo</i> testing● Use tests of the shortest possible duration● Maximally implement analgesia● Use terminal anaesthesia for certain procedures to reduce pain and suffering● Use range-finding and dose-staging protocols to reduce the animal numbers required for the experimental outcomes● Use existing and develop new less-severe humane end points to reduce pain, harm, and suffering and distress to the experimental animals● Facilitate the implementation of these endpoints, and in turn further reduce animal suffering, by implementing rigorous and frequent animal observations.
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Project	Respiratory tract regeneration and tumorigenesis	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this licence is to establish the cells and the molecules that are responsible for maintaining the respiratory tract (lungs, airways, ear, nose and throat) in healthy individuals and the processes and factors that drive respiratory disease and tumour development.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We will increase our understanding of respiratory tract biology in health and disease and in the development of cancer. This will enable us to identify and develop new therapeutic agents that could potentially be used to treat human respiratory diseases and/or to halt or reverse the progression of cancers. In addition, increasing	

	our knowledge of the cells and processes that maintain the respiratory tract will enable us to bioengineer segments of the respiratory tract for tissue engineering applications, which could eventually be used to treat patients with irreparable damage and poor quality of life.
What species and approximate numbers of animals do you expect to use over what period of time?	9,500 mice over a 5-year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of animals will be used in models of lung disease, tumour development or airway tissue engineering. These animals may develop tumours but these are generally well-tolerated. Some animals may experience reduced mobility, reduced lung function and some short periods of respiratory distress, but are not expected to show prolonged signs of breathing difficulties. In addition, some animals used in this project will experience weight loss but this will be closely monitored. A small proportion of animals will undergo a surgical procedure but these procedures are expected to pose minimal risk to animal welfare. Furthermore, some animals used in this project will be immune-compromised but these animals will be carefully looked after to minimise chances of infection. Studies will be designed to ensure only the minimum number of animals required are used. Throughout studies, animals will be regularly monitored; if any animal causes concern, action will be immediately taken to alleviate this and if this is not possible the animal will be humanely euthanised. At the end of each experiment, all animals will be culled using humane methods and tissues will be taken for further analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The respiratory tract is extremely complex, with interactions between various different cell types and processes. This cannot be adequately mimicked using <i>in vitro</i> studies and, although <i>in vitro</i> and <i>ex vivo</i> experiments will be used to establish whether <i>in vivo</i> experiments are necessary, studying the respiratory tract in health

	and disease in a whole animal is crucial.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will plan our studies so that only the minimum number of animals required is used. We will also make use of minimally invasive imaging techniques to enable us to monitor changes in a process longitudinally in a single animal, which will eliminate the need to use more animals at different time points. Finally, wherever possible we will make use of alternative methods to genetically modify mice rather than by doing this through mouse crosses, which will reduce the number of animals generated unnecessarily.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse models we intend to use have been chosen because they are widely accepted to be the most appropriate and relevant to the human condition they mimic. We have extensive experience in each of the chosen models, which allows us to reduce the number of animals required, to limit the invasive procedures carried out and to limit the discomfort experienced by the animals. Throughout this programme of work we will continue to monitor our own practices and the literature to look for ways to refine our procedures; these will be incorporated into our protocols wherever possible.</p>

Project	Restoration of function after nervous system injury																
Key Words (max. 5 words)																	
Expected duration of the project (yrs)	5 Years 0 Months																
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<table border="1"> <tr> <td data-bbox="722 622 746 712">X</td> <td data-bbox="746 622 1402 712">Basic research</td> </tr> <tr> <td data-bbox="722 712 746 801">X</td> <td data-bbox="746 712 1402 801">Translational and applied research</td> </tr> <tr> <td data-bbox="722 801 746 891">X</td> <td data-bbox="746 801 1402 891">Regulatory use and routine production</td> </tr> <tr> <td data-bbox="722 891 746 1059"></td> <td data-bbox="746 891 1402 1059">Protection of the natural environment in the interests of the health or welfare of humans or animals</td> </tr> <tr> <td data-bbox="722 1059 746 1149"></td> <td data-bbox="746 1059 1402 1149">Preservation of species</td> </tr> <tr> <td data-bbox="722 1149 746 1238"></td> <td data-bbox="746 1149 1402 1238">Higher education or training</td> </tr> <tr> <td data-bbox="722 1238 746 1328"></td> <td data-bbox="746 1238 1402 1328">Forensic enquiries</td> </tr> <tr> <td data-bbox="722 1328 746 1462"></td> <td data-bbox="746 1328 1402 1462">Maintenance of colonies of genetically altered animals</td> </tr> </table>	X	Basic research	X	Translational and applied research	X	Regulatory use and routine production		Protection of the natural environment in the interests of the health or welfare of humans or animals		Preservation of species		Higher education or training		Forensic enquiries		Maintenance of colonies of genetically altered animals
X	Basic research																
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	Preservation of species																
	Higher education or training																
	Forensic enquiries																
	Maintenance of colonies of genetically altered animals																
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project studies the consequences of different types of nervous system injury. It will develop new tools for manipulating gene expression in the nervous system. It will also test potential therapies for restoring function lost after nervous system injury. This is important because there are no fully restorative therapies for disabilities resulting from stroke, spinal cord injury or peripheral nervous system injury.</p> <p>Stroke: Cerebral ischemia (stroke) kills about 4.5 million people every year and there are over 9 million survivors left afflicted. In the UK and USA, stroke is the third greatest killer and</p>																

	<p>the leading cause of disability. In the UK, stroke services are estimated to account for up to 6% of the NHS budget, not including costs to social services and carers. Treatment for stroke after the acute phase is currently limited to rehabilitation. There is a particular need for stroke research using aged animal model systems: stroke is most prevalent and disabling in elderly humans yet few preclinical studies use aged model systems. This may explain in part why treatments that showed pre-clinical promise failed in clinical trials: it is now recommended that aged animals be used in some preclinical studies. There is a need for therapies that work when initiated hours or days after stroke because of delays to hospital admission and diagnosis: this is especially the case for existing survivors of stroke. We have established a model of stroke in aged rats and will now test therapies for improving limb function at different times after injury.</p> <p>Spinal cord injury: Human spinal cord injury (SCI) is often incomplete, leaving some sensory and motor circuits partially functional. Optimising the function of surviving neurons and nerve fibres is desired because it can lead to improvements in function. We will use models of partial SCI together with manipulation of gene expression in spared (uninjured) tracts using new gene therapy techniques. We will develop and use viral vectors to increase or decrease expression of potentially therapeutic molecules in neurons to determine whether limb function improves.</p> <p>Peripheral nervous system injury: Peripheral nerve cells can sprout new processes after injury but the mechanism is not well understood. A better understanding could lead to the development of novel therapeutic strategies after nervous system injury. We aim to identify the transcripts and proteins involved. The eventual aim is to develop therapies that restore function lost after nervous system injury.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or</p>	<p>The work of my laboratory aims to advance science and quality of life across two fronts: 1. Provision of new research tools: Our viral</p>

<p>humans or animals could benefit from the project)?</p>	<p>vectors could be used to modulate gene expression in other cell types with a view to improving outcome after different types of nervous system injury. Naturally, we will make these tools available to other researchers. 2. Testing new therapeutic targets: We aim to determine whether anatomical and functional recovery can be obtained following different types of nervous system injury when novel therapeutic strategies are employed and tested. With this program of work we hope to identify methods for improving function after nervous system injury.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Per annum, we expect to use 100 adult rats and 100 aged rats, although this will depend on the success of each study. Per annum, we expect to use twenty breeding pairs of adult rats to provide pups for cell culture studies. Per annum, we expect to use 100 normal adult mice and 100 genetically modified mice. Breeding and stock control can lead to larger numbers of animals being used. For example, when breeding genetically altered mice, not all pups in a litter will possess the desired genetic alteration: one therefore has to breed a larger number of mice to ensure sufficient numbers of the correct type are obtained.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>1. Cell culture work involves removal of tissues after humane killing; no additional adverse effects are expected. Severity level is mild. 2. Stroke and spinal cord injury work involves surgeries performed with anaesthesia and analgesia. We use models that only cause clumsiness in the animals rather than severe disability. They are not paralysed permanently, for example. However, for the first two or three days after brain or spinal cord injury (or up to two weeks after "contusion" (a controlled bruise to the spinal cord), rodents require special care because they may be transiently weak or paralysed, may feed and drink less, and appear unkempt. We provide them special intensive care including soft bedding, with additional fluids (by injection), with pain relief medication, with additional food (feeding by hand if necessary). Because of this additional temporary suffering, two of our procedures are</p>

	<p>classified as “severe”. However since 2015 we use a more refined model of stroke. We will continue to strive to improve the welfare of our animals and to reduce their suffering. Animals that do not recover with this additional special care will be humanely killed. However, we have considerable experience caring for animals of this kind and the majority recover from the temporary weakness such that they walk and feed and drink normally again within a few days. Animals will be humanely killed at the end using an approved method.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Animals need to be used in all these studies because many techniques are not possible or feasible at present in humans. Cell culture and computer simulation techniques are also not sufficiently advanced that they can model the integrated actions of the nervous system. This is largely because our understanding of mechanisms within the nervous system is insufficient to allow effective modelling. Thus, we will undertake some of our work in animals. Mostly we are interested in animal models of human disease or pathology, and so some of our experiments will make use of such models. Some of these models are short onset and short duration and can therefore be studied acutely in animals. Other models, like their human counterparts, develop and change over time and so some of these experiments may last weeks or even months. The prolonged time course of some experiments, and the fact that one of the most important outcome measures in our work is behavioural assessment of the animal, means that only some work can be done on animals under terminal anaesthesia: the remainder will require the use of recovery protocols.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We aim to measure many different variables in each rodent, thereby reducing the number of animals used, and improving the power of the study. As a generalisation, each rodent will receive a nervous system injury under anaesthesia. A potential therapy will be administered and tract tracing performed to</p>

	<p>identify whether nerve cells change their patterns of wiring. Analgesia will be given during the acute recovery period. Weekly testing will be performed to determine whether functional recovery occurs. Histology will also be performed on each rodent to see whether predicted or unexpected changes occur.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We will use rats or mice in our experiments.</p> <p>We require a mammalian model as the studies need to correlate to the human brain and these species represent the lowest sentient mammalian species. Furthermore, rats and mice are the lowest species commonly used in experiments of this kind over the last 50 years meaning a great deal is known about their anatomy, neurophysiology, genetics and behaviour.</p> <p>Mice are being used in some of these studies because a powerful approach to studying the role of genes is to use genetically altered animals. Animals with an inducible or a tissue specific mutation are likely to be of particular value. Rats are being used in other experiments because they are more easily assessed after nervous system injury using behavioural techniques.</p> <p>Recovery experiments will be conducted under general anaesthesia and the animals' physiology will be closely monitored. We have experience of all the techniques detailed in this project and the experiments we conduct on animals are expected to cause minimal stress. All animals are subject to regular inspections by the scientists, NACWO and veterinary surgeon and mild health problems are dealt with accordingly. In the event of any unexpected adverse reaction during experiments the animal will be humanely killed.</p>

Project	Retinal ganglion cells: when, how much and how do they contribute to the design and function of the visual system?	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project aims to improve our understanding of the way in which the visual system develops in early life, particularly the development of the way in which details of the image formed on the retina at the back of the eye are transmitted to the brain.</p> <p>The project will focus on retinal ganglion cells (RGCs), the only cells connecting the eye to the brain. Our visual world is compressed into</p>	

	<p>electrical impulses generated in the RGCs, and these signals are sent to the brain via the optic nerve. RGCs come in an incredible functional variety. There are 1 million RGCs in the human retina (45,000 in mouse), grouped into different functional classes. Each class conveys information about a different feature of the image (e.g. how bright it is, contrast, sensitivity to movement and direction and/or orientation of the stimulus, colour), forming thousands of parallel information channels. This is what we call “the retinal code”, allowing us to recognize and interpret the complex images that form our visual world.</p> <p>During early life, around the time of birth, RGCs also play a crucial role in guiding the formation of connectivity throughout the visual system, both at the level of the retina as well as in brain visual areas that receive a direct input from RGCs. Immature RGCs have unique features: (1) they are spontaneously active, generating bursts of impulses that occur simultaneously in neighbouring RGCs, resulting in waves of activity sweeping across the RGC layer in the perinatal retina; (2) RGCs undergo massive naturally occurring perinatal cell death (PCD), or apoptosis (a very common phenomenon in various parts of the developing central nervous system), resulting in over 70% of RGCs dying before reaching adulthood.</p> <p>In this project, we will investigate the role of retinal waves and PCD in determining how the RGC population impacts on the maturation of visual function. In addition, we will also investigate how different functional types of RGCs (some responding when the light is turned on, or when it is turned off, or when visual stimuli move in a particular direction, or are presented at a certain orientation) contribute to the retinal code.</p> <p>Our work will use a combination of pharmacogenetics, <i>in vitro</i> and <i>in vivo</i> electrophysiology, neuroanatomy, behaviour and mathematical models to address these important questions.</p>
What are the potential benefits likely	This project will offer unique opportunities to

<p>to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>investigate fundamental open questions about how the strategy these cells use to communicate determines how vision develops in the mammalian visual system. It will advance our understanding of RGC functional diversity in the mature retina, and how early-life potential for changes (plasticity) impacts on RGC functional development. The importance of retinal waves in guiding the development of visual connectivity is well established, but we will go an important step further by manipulating RGC activity levels in a reversible manner during distinct periods of development after birth, without the complications of permanent plastic changes normally arising from genetic manipulations. This will enable us to draw firm conclusions about how critical it is for RGCs to be spontaneously active and generate waves of activity during specific periods in early life in order to allow healthy maturation of the visual system. These experiments will clarify which aspects of visual development are more prone to irreversible modifications in adulthood (leading to disorders like amblyopia, or blurred vision, a common visual disorder known to have its roots in early development). Our project will also clarify for the very first time to what extent the size/density of the overall RGC population determines our visual performance (e.g. our ability to discriminate details) in adulthood. These experiments will provide important new knowledge for regenerative medicine (design of retinal prosthetic devices, stem cell therapy), by determining the optimal cell/electrode density required to restore vision, and more generally, by paving the way to design more efficient therapies for neurological disorders linked to changes in cell number (e.g. Down syndrome, autism) based on stem cell repair or neural prosthetics. Finally, our studies on understanding how different RGC classes contribute to the retinal code will provide a useful novel, global method to study cell diversity and classification and could be applied to other systems where cell diversity is key to understanding functional complexity.</p>
<p>What species and approximate numbers of animals do you expect</p>	<p>The project will be done entirely on genetically modified mouse lines. Most mice we will use will have conditional gene expression in specific</p>

<p>to use over what period of time?</p>	<p>retinal cell classes, which means that the mutation will not be expressed in any other cell in the organism except in these specific cell subclasses. We expect to breed about 5000 mice in total over a period of 5 years, enabling us to have sufficient animals with the appropriate combination of gene expression (1000-1200 animals).</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We expect our experiments to cause very minor adverse effects. None of the mouse lines we will generate are expected to look or behave any differently to normal mice, or to experience any adverse effects as a result of the genetic manipulation. Most of the procedures are classified as mild. The only procedure classified as “moderate” is intraocular injections of drugs or anatomical tracers. These will be done under general anaesthesia, and animals will be given analgesics to ensure they do not suffer pain and discomfort. They will be monitored during their recovery from anaesthesia and checked on at least a daily basis by the animal facility technicians for possible complications such as post-operative infections, in which case they will be given appropriate antibiotic treatment. Some other animals will be killed by Schedule 1 to isolate the retina for in vitro electrophysiology. Some experiments will end in procedures under anaesthesia with no recovery. These include animals used for in vivo electrophysiology and animals that will be humanely killed following intraocular injections of anatomical tracers to retrieve the brain and retina for anatomical studies.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>To understand an organ as complex as the brain or the eye, how it develops and functions in health and disease, there is no alternative to studying that organ in a living animal. Therefore, the use of intact animals is essential, particularly in the investigation of the senses such as vision. Moreover, we are particularly interested in understanding basic principles of neural wiring during development, and the best approach to reach this goal is to modify normal development, and this can only be done in</p>

	<p>intact animals. Alternatives such as cell culture do not allow access to the working sensory system.</p> <p>Computational approaches are useful to work in synergy with experimental work, but they cannot replace it because we do not know enough about biological processes to build realistic computer models. Models can at most help us to refine our knowledge and understanding in an iterative manner. We have established collaborative work with several theoretical neuroscientists and we will put all our experimental data at their disposal to develop biologically realistic models.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use electrophysiological approaches to record electrical activity from RGCs (<i>in vitro</i>) and from neurons in the brain visual areas (<i>in vivo</i>). For <i>in vitro</i> experiments, we will remove the retina from freshly killed mice and place it on an array of electrodes that will record the activity from RGCs. For <i>in vivo</i> experiments, we will insert electrodes into specific areas of the brain that receive input from the retina. This will be done in anaesthetised mice. Our electrophysiological approaches, both <i>in vitro</i> and <i>in vivo</i>, provide a very high data yield per experiment. Indeed, we use large arrays of electrodes, allowing us to record from thousands of cells in each experiment. This approach allows us to drastically reduce the number of experiments required to achieve results with robust statistical significance.</p> <p>Wherever possible, we will reduce the number of animals by performing behavioural testing before we use them for <i>in vitro</i> or <i>in vivo</i> recordings or anatomical tracing.</p> <p>We routinely analyse data soon after each experiment. This allows us to see trends emerging from our results, helping us to reduce and refine our experimental work, and to stop gathering data once our results reach statistical significance.</p>
<p>3. Refinement</p> <p>Explain the choice of species and</p>	<p>The reason for choosing mouse lines in this project is first of all because it is a mammalian species, hence closer to humans than</p>

<p>why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>amphibians, reptiles or birds. It is widely used in vision research, and especially in developmental studies. Indeed, pups are very immature in terms of visual function at birth (they are completely blind), and the most important steps towards maturation occur during the first 2-3 postnatal weeks. There is tremendous potential plasticity during that relatively short period, allowing us to undertake many experimental permutations that can potentially result in permanent modification of visual connectivity and function in the adult, which is precisely the goal of this project.</p> <p>Except for some control experiments using wild type mice, all the models we have chosen are genetically modified, most of them with conditional gene expression in specific cell types. This is one of the great strengths of our programme. Indeed, the models we have chosen will allow us to specifically silence early spontaneous activity (waves) at will (during distinct postnatal periods controlled by us) in most RGCs (but not in other cell types), or to modify the amount of PCD in the RGC population, yielding adult animals with modified visual connectivity and/or with either more or fewer RGCs. These manipulations will shed new light on the role of important early developmental events that are known to determine both structure and function in the mature visual system.</p> <p>Animal suffering is minimised by using anaesthetics for protocols whenever necessary. Surgery for recovery procedures will be carried out in consultation with a veterinary surgeon. Any animal that exhibits untreatable signs of pain or distress during recovery will be humanely killed.</p>
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Project	Rodent brain activity map	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Electrical signals are important for communication between brain cells. To understand how the brain works, we need to observe these signals in the brain under normal operation.</p> <p>Additionally, different brain areas far apart often work together, and within each region there are different types of brain cells. Current methods provide a fuzzy image of these electrical activities, and it's not possible to look at large brain areas all at once.</p> <p>To overcome these limitations, we both develop and use innovative optical methods and molecular tools that allow us to use light to both control and monitor electrical activity from</p>	

	<p>specific types of brain cells that are located far apart. We can use this approach to understand how electrical activity in the brain forms different behaviour, both under normal and diseased conditions.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Many of the devastating brain-based pathologies known in medical practice have neither cures nor effective treatments, in large part because it is difficult to provide a treatment for a dysfunctional organ when one does not know how it works. Brain-based pathologies include changes in brain circuits that can manifest as neuropsychiatric diseases such as autism and schizophrenia. Understanding of our brain's circuits will facilitate development of advanced cures and effective treatments of diseases that have been associated with dysfunctions of neuronal networks. Moreover, the fundamental importance of generating a fruitful theory of brain function lies in the fact that as humans, more than any other species, we are defined by the higher cognitive abilities generated by our brains. Thus, scientific understanding of our brains will enable deeper knowledge of ourselves and of our minds, all of which would be highly beneficial. More specifically, cells in our brains belong to different groups (and express different set of genes), and these groups have different roles in the functional brain network. When the interplay between these groups are not optimal, neurophysiological disorders occur, including but not limited to autism, schizophrenia, ADHD, etc. Our project will develop advanced molecular tools in the laboratory. By genetically expressing our molecular tools in specific cell groups in the brain, we can optically control and monitor the specific activities of these cell groups during behaviour. In this way we can tease apart the roles of these different groups during behaviours (like decision-making and using of memories) in a normal functional brain, and subsequently in model organisms of brain disorders towards better and more useful clinical interventions.</p>
<p>What species and approximate numbers of animals do you expect</p>	<p>The current project will last 5 years. For the experimental work, we will need to use rodents (mice or rats). For the creation, breeding and</p>

<p>to use over what period of time?</p>	<p>maintenance of GA rats and mice, we anticipate to use less than 800 mice/year and less than 40 rats/year. For behavioural and imaging studies, we anticipate to use less than 100 mice/year, and less than 50 rats/year.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We like to treat animals in a way that they don't feel unnecessary pain or distress. However, surgery under general anaesthesia will be necessary to observe brain activity. Since recovery from surgery is likely to have an adverse effect to the animal, we rate the level of severity as up to moderate. Typical case scenario: An animal will be born and expresses the transgene(s) needed. The animal will be handled and behaviourally trained to perform tasks like licking water upon seeing/hearing/feeling a non-aversive stimulus. The animal will have an implant to allow optical access to its brain. It will sit under a microscope and its brain activity will be optically monitored as it performs the task. The animal may receive brain state influencing agents, and its behavioural performance under the brain state influencing agents will be tested. The animal will be humanely killed at the end of the study and its brain may be used for post-mortem tissue examination.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>All experiments that can be done in cultured cells will be done in those, using methods to minimize the number of animals. We also use computer simulation to add value to our experimental data and to replace animal use where possible.</p> <p>Currently, we can reliably study higher brain functions such as perception, motor control and learned behaviour only in living animals. Rodents are likely to offer the simplest models in which brain processes that underlie human cognition can be studied with confidence.</p> <p>Mice are currently the species of choice in most areas of biomedical research where genetic alterations have already been well refined.</p>

	<p>However, some behaviours are more experimentally established and better defined in rats so, where appropriate, rats will be used instead of mice.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Although scientifically driven, our approach that combines several methods (e.g. imaging, anatomy, and behaviour) in single animals enables far more data to be obtained from single animals than would be obtained from a much larger number. We also use systemic viral approach to reduce transgenic breeding and therefore reduce the number of animals used.</p> <p>We will use advanced statistical techniques that ensures the minimum number of animals to be used to generate the maximum amount of statistically significant (and hence useful) information.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rodents are among the simplest mammals in which to study functions that depend on the cerebral cortex.</p> <p>Mice (and to a lesser extent rats) are currently the species of choice in most areas of biomedical research, where genetic alterations have already been well refined.</p> <p>All surgical procedures will be conducted with state-of-the art techniques for analgesia and anaesthesia.</p> <p>All procedures are based on protocol steps that have been refined over many years. However, we will strive to find additional refinement, if possible at all. Water reward as a positive reinforcement during behavioural tasks has been shown to be an effective motivation technique in head-fixed animals (Guo et al 2014 Neuron, Guo et al 2014 Plos One). Other motivational paradigms involving food restriction may be less well tolerated as shown by behavioural and biochemical measures (Tucci et al 2006 Behav Brain Res).</p>

Project	Rodent Imaging for Translational Research	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	When researchers test out new drugs in humans (clinical trials), they often want to use different types of specialised imaging to see what the drug is doing to the patient. If the new drug is unlike anything used before, the researchers may not be quite sure what type of specialist scan to use, or how to interpret any changes in the scan after the drug is given. We will use similar types of scan to look at the new drug in mice or other rodents. We will use our scanning results to advise medical researchers how best to scan the human patients. Our scanning results will also help the researchers understand the changes they see in the scans of human	

	<p>patients. This is called “translational research”.</p> <p>We will use our specialist rodent scanners to understand how such substances distribute around the body, and we will try to improve the scanning techniques.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<ul style="list-style-type: none"> • Our imaging studies will play a part in making new medicines available for doctors to prescribe • We will come up with ways to make it more convenient and comfortable when patients have to undergo a scan as part of a clinical trial • Sometimes the ideas for new medicines that come out of the lab are just not good enough to make a worthwhile new medicine. We will help stop those projects as soon as we can • We have a lot of experience with rodent scanning, and how to avoid pain and distress. We will work hard to share our methods and ideas with others, so they make the very best use of animal scanning.
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use about ~25 rodents, mainly mice, per week, which adds up to 5000 over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Sometimes the animals will get the new medicine. They will be scanned, sometimes on several occasions. To stop them moving during the scan, we give them an anaesthetic (just as doctors sometimes do when scanning babies). We have to kill the animals at the end, because we need to know how what we see in the scans when the animals get the new medicine relates to what’s going on in the body. For cancer medicines we may inject some cells under the skin which grow to form a lump (tumour) under the skin, which we can scan. Although this looks unpleasant it doesn’t cause the mice much distress. We don’t let the tumour spread to form secondary’s (metastases), and we know from humans that cancers are seldom painful until they spread.</p>
<p>Application of the 3Rs</p>	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>When a completely new type of medicine arrives in the body, it can change the ways the scans look in surprising ways. Because of all the processes that occur in the cell, together with blood flow and motion, we simply can't calculate these without doing an experiment.</p> <p>So, although it is impossible at the moment to achieve the scientific data necessary to inform human clinical trials without the use of animals we do constantly review the literature for potential non-animal models as well.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We have a lot of experience in designing clever experiments that give a lot of information with small numbers of animals. We work with statisticians to calculate how few we can get away with and still get reliable answers.</p> <p>Wherever possible we publish in journals which support the ARRIVE guidelines which in turn underpin good experimental design and all the benefits that brings to the reduction in the use of animals</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We mainly use mice and rats since they have been used a lot in the past by other researchers, and we can build on what they know. It can be unpleasant for the rodents when they come around from anaesthetic and we need to watch them carefully and keep them warm. Some of the potential new medicines might be unpleasant or even harmful and we follow detailed guidelines written by vets to ensure we can pick up if an animal is suffering and step in quickly.</p> <p>After every experiment we will critically appraise what we do to seek out any ways to improve our models to reduce harm to animals.</p>

Project	Rodent Regulatory Genotoxicity	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
	<input checked="" type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The aim of this rodent regulatory genotoxicity project is to evaluate the potential of pharmaceutical (human or veterinary/animal health) or non-pharmaceutical (agrochemicals, food additives and industrial chemicals) compounds to cause genetic damage in rodents, principally the rat and mouse. Genotoxicity is a term used to describe the propensity of a compound to to damage the genetic information within the cell causing mutations which may lead to cancer in the future. Thus, genotoxicity studies are part of an overall work package as part of the safety evaluation process.</p> <p>Initially if insufficient data is available for the project</p>	

	<p>a preliminary test is performed to determine a top dose level and additional suitable dose levels for the main test. The highest dose of a test substance that can be administered either on one occasion or repeatedly to an animal, but which does not cause a degree of pain, suffering or lasting harm ('limiting' signs or effects) that would prevent the completion of the treatment period, thereby compromising the scientific objective of the study, is usually referred to as the 'maximum tolerated dose' (MTD).</p> <p>Studies are undertaken with the intention of producing data that will be used to gain regulatory approval to initiate or support ongoing clinical trial programs and/or obtain a product licence to market the substance.</p> <p>The project can also generate biological samples to enable or support identified programmes of work and allow for the development of new or refined methodologies/technologies prior to integration onto tests for determining genetic damage.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The data produced in this project allows the support of ongoing clinical trial programs and aids in the ability obtain a product licence to market the substance. The development of new medicines, veterinary/animal health products, food additives, agrochemicals and industrial chemicals is necessary for the continued success of efforts to combat disease, maintain food supplies and achieve improvements in the quality of life. It is a fundamental expectation that such substances should not pose an unacceptable risk to the health and well-being of the human population or target animal populations, or to the environment. This project contributes directly to that expectation, and facilitates the development of products that will have minimal adverse impact. The principal benefit is that the work performed will facilitate sound regulatory decisions for the purposes of gaining authorisation for clinical trials or product registration, which otherwise could not be done with as high a degree of assurance. These studies also contribute to minimising the number of animals used, as an adverse response identified in these studies may halt the development of the test substance.</p>

<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Rats – 3250 Mice - 2250</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Animals will be exposed to test substances (as detailed earlier in the NTS) via routes including inhalation, application to the skin, orally, in food or water and injection either into the skin, muscle or bloodstream. This may require anaesthesia on a number of occasions. Administration/infusions of substances can be performed using delivery devices such as catheters in blood vessels. Animals may be blood sampled to confirm exposure, or restrained to enable exposure. Animals will be humanely killed at the end of the study and tissues harvested for examination. Administration of test substances may result, usually at the highest dose level in mild to moderate signs of toxicity. The rats and mice used in these studies may show effects at all dose levels, but they are expected to be transient. Experience shows that (~60%) of animals are not expected to show any clinical signs of suffering. A percentage (~20%) may show transient subtle to mild clinical signs such as reduced weight gain or weight loss, subdued behaviour and fur ruffling. Moderate signs (hunched posture and abnormal breathing) of adverse effects may be seen in some animals (~20%), usually in the higher dose groups. Despite the close monitoring some animals may sometimes inadvertently experience severe toxicological adverse effects such as repeated convulsions, persistent laboured breathing or indeed be found dead. Lethality and/or severe effects are not the desired outcome and animals will be closely monitored and promptly humanely killed at predetermined humane endpoints to minimise the likelihood of unexpected death as far as possible. Most of the dosing techniques, manipulations or investigations do not cause any lasting adverse effects, but a small number of animals may show temporary moderate transitory distress due to, for example, withdrawal of blood.</p>
<p>Application of the 3Rs</p>	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>There is currently no regulatory acceptable alternative to the use of animals in these studies. It is mandatory for animal genotoxicity studies, usually involving rodent and non-rodent species, to be undertaken before regulatory approval is given prior to allowing a new drug to be tested in human or veterinary trials or for an agrochemical, food additive, or industrial chemical, medical device/article to be marketed and used safely.</p> <p>We will however remain vigilant to the possibility of the development and emerging use of any non animal regulatory acceptable alternatives should they become available in future.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The guidance usually indicates the design and number of animals included in a study therefore, there is lesser scope in regulatory toxicology for reduction than in other fields of work. Attention is paid to good study design to use minimum number of animals in the most refined way to achieve the aims of the study.</p> <p>Some study design decisions may have to be made based on data from bacteria or cell based assays or from preliminary data in rodents, from which a progressive approach to the accumulation of information is adopted. This orderly sequence of data collection reduces the number of animals used and restricts the procedures to which they are subjected. For studies that are being performed at a later stage, where studies in the rodent (and other species) may have already been performed, decisions on study design can usually be made with a higher degree of confidence leading to lower animal use. The involvement of Scientists and Statisticians will be obtained at an early stage as required, so that advice can be given on implementation of the 3Rs and study plans developed which minimise severity of procedures applied as far as possible.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures</p>	<p>Rodents (either rats or mice) are used because their use is mandated by regulatory bodies who carry out the relevant risk assessments/safety evaluations for the studies in this project. There is considerable experience and background data for the species and studies in this project and the most</p>

<p>you will take to minimise welfare costs (harms) to the animals.</p>	<p>refined methods will be used.</p> <p>Studies are performed in a stepwise manner, starting with preliminary studies using small numbers of animals where there is limited information a so called 'pilot study'. This gives the highest prospect of refining and optimising the programme eg by optimising specific doses of substances given to achieve the desired scientific endpoints in the main study and also in consequence minimising the pain, suffering, distress or lasting harm for the animals used on study.</p> <p>All animals are regularly monitored for signs of any adverse effects on their health or wellbeing, and to prevent unnecessary suffering, early pre-determined humane end-points are applied under appropriate veterinary guidance (e.g. modification/withdrawal of treatment with the test item, or humane killing of affected animals).</p>
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Project	Role of AMPA receptors in synaptic plasticity	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>We know that memories are stored in the brain as changes in the strength of connections ('synapses') between the brain cells ('neurons') that are part of a given experience. In this project, we aim to shed new light on this question and to visualise these changes. To achieve this, we will develop new techniques allowing us to track individual molecules at synapses that are centrally involved in this process.</p> <p>Specifically, when brain cells communicate with each other, the sender neuron releases messenger molecules (such as 'glutamate'), which are detected by the receiver neuron by</p>	

	<p>specific receptors. When a memory is formed, 'glutamate' receptors change so they become more effective at receiving the message. We plan to use genetic tools to tag glutamate receptors so they can be tracked in a microscope. In this way, we will be able to see the trafficking of receptors at individual synapses and measure how their numbers and composition change over time. We will do this in mouse brain tissue, as this is the only experimentally amenable access to neuronal synapses. By developing and using these new tools, we hope that our research approach will shed new light on how memories are encoded and stored at synapses at the molecular and cellular levels.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The process of memory-formation remains one of the most fundamental, open questions in modern neuroscience and goes awry in dementias, such as Alzheimer's disease. The decline of memory function with age and an ever increasing ageing population have important implications for life-long health. Hence, an understanding of learning and memory mechanisms will be essential for devising new strategies to promote life-long health and well-being.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use a maximum of 15,000 mice over 5 years, the majority of which will be genetically altered.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Mice will be housed in a social environment and will have regular access to food and water. Surgery will involve administration of substances or altered genes into the brain; mice will be anaesthetised prior to surgery and will be given pain relief prior to recovery from anaesthesia. Mice will also be killed under terminal anaesthesia for collection of tissue or perfusion purposes. Only a minor proportion of animals used will undergo surgery, with the majority used for breeding purposes. Substances may be administered to mice through standard routes, such as subcutaneous injection, with volumes and frequencies to published guidelines. No injected substances are expected to cause any more than transient</p>

	harm.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Mice will be used throughout this project as this species is evolutionary sufficiently close to humans to make our findings relevant for mechanisms in the human brain. Mice are widely used to study synaptic memory processes, allowing us to build upon results from numerous previous experiments.</p> <p>It is well established that mice are capable of declarative memory (spatial memory), which requires synaptic plasticity in a brain region termed 'hippocampus', a region first recognised in the 1950 as the seat for episodic memories in humans. Mice are therefore the ideal model system to address the question of memory formation at synapses under physiological and pathological conditions.</p> <p>Our experiments also involve cell lines, to inform us about the function of altered glutamate receptors before testing them in neuronal settings.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use an absolute minimum number of mice, sufficient to give statistically significant results. Breeding will be strictly monitored to ensure that no surplus is generated throughout this project. Further, cryopreservation will be used to preserve essential mouse lines, allowing us to only maintain lines needed for specified experiments.</p> <p>We have optimised a number of experimental procedures, such as surgical injection of neonatal animals, improving targeting of brain regions, so that fewer animals are required for each experimental result to be obtained.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to</p>	<p>The brain structure of mammals is overall similar and mice are best suited for this project as multiple mouse models have been developed to study memory mechanisms. For our purpose lines will be used from which glutamate receptors can be genetically excised and replaced with altered versions. Genotyping of</p>

<p>minimise welfare costs (harms) to the animals.</p>	<p>our lines will be done in the most non-invasive way, using ear punches. Animal technicians are well informed about our lines which have no avert phenotypes and are healthy and overall normal even after excision of glutamate receptors.</p> <p>Surgeries are commonly carried out to minimum standards for asepsis and ‘Guiding principles for preparing for and undertaking aseptic surgery’ (2010) will be followed closely. All animal experimentation carried out under this project licence will comply with the document ‘Animal Usage Guidelines’</p> <p>The duration of surgical procedures will be kept to a minimum to keep the occurrence of any possible adverse effects. We have optimised the procedure for injection of neonatal animals through design of a custom 3D-printed pup immobilisation mould, which improves both the specific targeting of brain regions that we are interested in, and improves pup recovery after procedures.</p>
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Project	Role of Arp2/3 isoforms in mouse development and tissue homeostasis	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The actin cytoskeleton, part of the cells skeleton, provides driving force and structural support for the physical integrity of cells and a wide range of essential cellular processes such as membrane trafficking, cell adhesion and cell migration. The correct regulation of the actin cytoskeleton in space and time is essential during development and throughout the lifetime of multi-cellular organisms. Moreover, deregulation or malfunction of the actin cytoskeleton results in a variety of developmental syndromes and diseases as well as tumour cell metastasis. The investigation of	

	<p>the molecular operation, regulation and organization of the actin cytoskeleton is thus essential to understand human development, physiology and disease. The Arp2/3 complex, consisting of seven proteins (Arp2, Arp3, ArpC1-5), induces the formation of branched actin filament networks that perform an essential function in a wide variety of cellular processes. In humans and mice, the Arp3, ARPC1 and ARPC5 protein subunits are encoded by two different isoforms. The overall aim of this project is to investigate the physiological function of these different Arp2/3 complex subunit isoforms during development and tissue homeostasis.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The project will provide advances in fundamental knowledge by allowing us to understand which physiological and developmental processes are controlled by the different Arp2/3 isoforms. Given recent observations with patients with mutations in the ARPC1B subunit of the Arp2/3 complex, it is likely that our analyses will also uncover and provide insights into a variety of human conditions and diseases including tumour metastasis, immunodeficiencies and muscle myopathies. The insights we will obtain will provide new disease markers and opportunities for therapeutic intervention.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use mice. We will use approximately 3000 per year. However, most animals will only be used for breeding of new mouse strains.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Genetically altered mice may develop moderate or more adverse outcomes, which will depend on which Arp2/3 isoform is being altered and in which tissue the manipulation is performed. Many of these adverse effects will present at embryonic and foetal stages, and are not usually compatible with continued life. The maximum expected level of severity for any procedure conducted within this project is mild and follows strict guidelines in accordance with the Home Office. At the end of procedures, animals will be killed by an approved method.</p>

Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The study of Arp2/3 dependent actin polymerization in biochemical assays in the test tube and in cell culture models can provide mechanistic insights into different cellular processes. Such analysis may guide, but will not fully uncover the roles of Arp2/3 isoforms during mouse development and tissue homeostasis. This is because these highly complex processes involve the coordinated interaction of multiple proteins and cellular systems, as well as, their higher level organization in a wide variety of different cell types over time. Currently, no biochemical assay or cell based model can fully recapitulate or take into account all of these factors. Therefore, to understand the importance of Arp2/3 isoforms, for example which cell types and physiological processes depend on them most critically, and at which stages of life they are most important, we need to study the consequences of their loss in whole, living animals.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use state of the art genome editing to generate mice carrying mutations to reduce the need for extensive breeding programs. Where possible, we will perform in vitro experiments in cell culture using tissues or cells derived from knockout mice to reduce the need performing experiments on living animals. In turn, hypotheses generated from in vitro experiments will also allow us to perform more focused animal experiments, reducing animal usage. Experimental designs will use the minimal number of mice required to obtain statistically significant data. We will maximise the amount of data obtained from each mouse by studying multiple tissues, by analysing them using several different methods. We will review our breeding strategies regularly and cryopreserve any strains which are not under current investigation.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will</p>	<p>Mice share similar genetics and physiology with humans and so are an appropriate mammal for providing insights into human development and disease. They are not a primate or an</p>

<p>use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>endangered species. Mice are the lowest mammalian organism that expresses all Arp2/3 isoforms that is suitable for efficient genetic modifications. Advanced genome editing works very efficiently in mice and will help limit animal numbers. Mice also have well-established laboratory procedures and advanced genetics, which both help to expedite research progress. In all cases, animal suffering will be minimised by following strict guidelines in accordance with the Home Office and by regularly monitoring animals in consultation with an animal care and welfare officer and a veterinary surgeon.</p>
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Project	Role of blood-derived growth factors in brain health																
Key Words (max. 5 words)																	
Expected duration of the project (yrs)	5 Years 0 Months																
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<table border="1"> <tr> <td data-bbox="718 622 746 712">X</td> <td data-bbox="746 622 1402 712">Basic research</td> </tr> <tr> <td data-bbox="718 712 746 801">X</td> <td data-bbox="746 712 1402 801">Translational and applied research</td> </tr> <tr> <td data-bbox="718 801 746 891"></td> <td data-bbox="746 801 1402 891">Regulatory use and routine production</td> </tr> <tr> <td data-bbox="718 891 746 1059"></td> <td data-bbox="746 891 1402 1059">Protection of the natural environment in the interests of the health or welfare of humans or animals</td> </tr> <tr> <td data-bbox="718 1059 746 1149"></td> <td data-bbox="746 1059 1402 1149">Preservation of species</td> </tr> <tr> <td data-bbox="718 1149 746 1238"></td> <td data-bbox="746 1149 1402 1238">Higher education or training</td> </tr> <tr> <td data-bbox="718 1238 746 1328"></td> <td data-bbox="746 1238 1402 1328">Forensic enquiries</td> </tr> <tr> <td data-bbox="718 1328 746 1462"></td> <td data-bbox="746 1328 1402 1462">Maintenance of colonies of genetically altered animals</td> </tr> </table>	X	Basic research	X	Translational and applied research		Regulatory use and routine production		Protection of the natural environment in the interests of the health or welfare of humans or animals		Preservation of species		Higher education or training		Forensic enquiries		Maintenance of colonies of genetically altered animals
X	Basic research																
X	Translational and applied research																
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	Forensic enquiries																
	Maintenance of colonies of genetically altered animals																
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The blood contains not only the familiar red and white blood cells but also small cellular fragments designated platelets. They originate from large cells located in the bone marrow called megakaryocytes. These cells express a number of genes related to the main function of platelets which is to prevent bleeding. Beyond gene products related to blood coagulation, megakaryocytes also express a number of genes encoding growth factors thought to help tissue repair and wound closure. In primates including humans, megakaryocytes also express the gene encoding brain-derived neurotrophic factor (BDNF), a growth factor best known for its activity on brain cells,																

	<p>including a role in memory and in the prevention of the death of nerve cells. Whilst the activity of BDNF in the brain seems to be similar in mice and humans, mouse blood platelets do not contain detectable levels of BDNF. As a result of a lack of experimental system allowing hypotheses to be rigorously tested, the role of BDNF in human platelets is still entirely unclear. Yet the levels of BDNF in human blood have been measured in a number of conditions and these levels are decreased in a number of conditions including depression and neurodegeneration including Alzheimer's disease. Conversely, BDNF levels increase after physical exercise. A few years ago we discovered that human, but not mouse megakaryocytes express the <i>BDNF</i> gene. This made it possible to generate a new mouse model whereby the mouse genome has been engineered to force the expression of BDNF in megakaryocytes and platelets</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The lack of BDNF in mouse platelets offers a unique possibility to test the potential benefit of platelet-derived material to brain health. Platelets are not only very small but they also represent the main source of even smaller particles in the circulating blood called microvesicles. It is conceivable, but not proven yet, that these growth factor-containing microvesicles may reach the brain. It is this hypothesis that is being tested with the experimental animals in this project. If functionally relevant quantities of BDNF, used here as an example as so much is known about the function of this growth factor, blood-derived microvesicles may be used in the future to facilitate the diffusion of other biologically relevant molecules such as for example drugs ameliorating brain function or preventing the growth of tumours in the brain.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The work is based on the use of mice as the existence of well characterised mutants is a key aspect of the project. We estimate that the project will use a total of approximately 2500 animals during a period of 5 years. Only 560 animals will undergo additional procedures</p>

	(outside breeding).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The adverse effects to be expected with the GA lines are quite limited in view of the design of the experiments which is to ameliorate the functional deficits of animals with well-defined, compromised functions of the nervous system. The behavioural tests planned will allow us to re-use the GA animals at the end of experiments to maintain the colonies. Most animals under this licence will only experience transient discomfort. There is a possibility that some of the mutant strains will experience stress related to behavioural experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The entire project necessitate live animals to test the exceedingly complex nature of the interactions between blood vessels and the nervous system, in particular the diffusion of molecules such as growth factors embedded in platelets or in microvesicles derived from platelets.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Appropriate statistical methods will be used to design effective animal experiments to produce valid scientific results. Colony sizes will be kept to a minimum and only expanded to reach the appropriate numbers of animals needed for specific experiments. Typically pilot studies will be performed on small numbers first and calculations made regarding how many animals will be needed for further study. Standard statistical evaluation methods will be used to evaluate the results following procedures. When a strain is no longer needed they will be cryopreserved.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard	The interpretation of mutations and polymorphisms subsequently discovered in humans have greatly benefited from the results obtained with mouse mutants.

<p>to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The adverse effects experienced by the animals will be minimised at all times during the duration of the project. The vast majority of animals show no adverse effects or low levels of adverse effects. Genetically altered animals that do show adverse effects show a deterioration of movement. Behavioural testing will also result in only low levels of adverse effects except where genetically altered animals of a moderate type are used. The unilateral sectioning of the facial nerve does not cause adverse effects as the animals are so young that peripheral sensory nerves are not yet functionally connected with the cerebral cortex and hence unable to sense pain. There will also be a non-recovery procedure performed for the collection of fluids, which does not cause any pain or suffering as animals are deeply anaesthetised throughout and do not recover.</p>
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Project	Role of cellular senescence in normal physiology and disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Research for the last 10 years has demonstrated that certain cells, called senescent cells accumulate in the organs as animals age and contribute to the development of many diseases including cancer. The overall aim of this application is to gain new insights into the function of these cells using advanced research approaches and mice. Our interest is mostly on brain, pituitary and lung cancer. Brain and pituitary tumours are clinically very relevant, mostly in children, and associated with high mortality and poor quality of life due to the damage caused by the tumour and the current	

	<p>treatments (e.g. radiotherapy is toxic for the developing brain of a child). Lung cancer is a major cause of death in adults. The ultimate goal of this project is to identify novel treatments against these clinically relevant human cancers by performing research in mice.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The proposed project will help understand how tumours develop and will speed up the development of novel anti-cancer treatments by testing drugs in mouse models of human tumours/cancers. We will generate new mouse models of human cancer for the benefit of the research community. We have an excellent track record in generation of mouse models for biomedical research. These mouse models will be made available to other researchers so that they can also benefit from the research outlined in this proposal. We will investigate in this proposal how cancer develops at the initial stages, aiming to facilitate cancer prevention and improve current treatments. The findings of our research will be published and given access to the research community, medical doctors and the public in general. We expect that through the mouse research in this proposal, we will initiate human studies to help cancer patients in the next 5 years.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>4,000 mice per annum.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Adverse effects will not exceed the moderate banding. Adverse effects include unexpected events such as infection following surgery (e.g. in embryo recipients or manipulations of embryos in utero). These complications are rare and are minimised by using aseptic techniques and antibiotics. Pain resulting from surgery is also minimised by using pain killers. Harmful defects can also result from crossing of genetically altered mice. In this licence, the most common harmful defects will be related to the growth of tumours. Mice tolerate brain and lung tumours well and do not show symptoms of ill-health for weeks of months. It is normally after a long latency period that symptoms develop. Tumours may cause neurological</p>

	<p>defects (brain/pituitary tumours) or breathing difficulties (lung tumours), which usually result in abnormal behaviour, lack of grooming, weight loss or inactivity/lethargy. Tumour bearing mice will be monitored daily to prevent the development of defects beyond the authorised severity banding of the procedures and minimise welfare costs. In the case of scientifically informative and important strains that would be classified "moderate" but have been generated under a "mild" Protocol, the mouse facility management and veterinary surgeon will be consulted and the Home Office Inspector informed. We anticipate that these cases will be rare. All other mice will be humanely killed at the end of the experiments.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>It is well established that cancer formation is linked to embryonic development. This is particularly true in the case of paediatric cancers, which normally are initiated in the developing fetus. Paediatric cancers are developmental diseases and as such, their study within the developing embryo is warranted if we aim to understand the mechanisms underlying tumour initiation and growth. For example, brain and pituitary can develop in a sustainable way only within the context of the developing embryo and no in vitro system can mimic these conditions. Hence, an understanding of normal development, and of disturbed development that leads to birth defects (including cancer), requires analysis of whole animal embryos.</p> <p>Alternatives that have been considered, and which will be used where possible, include:</p> <p>Tissue/cell culture: In the last few years we have developed in vitro systems to test the efficacy of specific inhibitors to suppress tumour growth in cell culture. For example, we can maintain small pieces of mouse and human tumours in culture for 1-3 days, which allows to assess the effects of specific drugs. This has a direct impact in the numbers of drugs that are subsequently tested in mice, and so reducing mouse usage, as we test in vivo only those that</p>

	<p>show the highest efficacy/lower toxicity in vitro. We will use tissue culture for cellular studies, where analysis of whole embryos or mice is not essential.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>In vitro experiments using tumour cells will reduce the number of mice to use in in vivo experiments, e.g. by identifying the most efficacious/less toxic compounds.</p> <p>In this licence we will only use mice. Where suitable lines already exist, mice will be obtained from the relevant supplier. Otherwise, we will make the required mouse lines ourselves. We routinely use a modern technology called CRISPR/Cas9, which is much more efficient than conventional methods and requires fewer mice.</p> <p>Efficient colony management, i.e. calculating the minimum number of mice required for the research, ensures that only colonies that are actively being used are mated to produce mice. Those that are no longer required are 'frozen as embryos' to reduce numbers of live mice in the facility. We use good practice in experimental planning, including statistics and follow national and international guidelines.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Genetically altered mice offer the most incisive approach to the analysis of birth defects mechanisms and cancer because:</p> <p>Mouse genetics is understood almost as well as in humans, offering the best possible means for genetic analysis in a mammal.</p> <p>Birth defects and cancer in genetically-predisposed mice closely resemble those in humans, providing excellent models for analysis.</p> <p>Modern technologies to genetically alter the mice offer a sophisticated route towards studying the effects of genes in particular tissues, or at specific stages.</p> <p>Most of procedures are moderate in severity. If unexpected adverse effects are observed (e.g. neurological signs for brain pituitary tumours and breathing defects in lung tumours), mice</p>

	<p>will be humanely killed immediately. Mice likely to develop tumours and show signs of ill-health are labelled appropriately and personnel looking after the mice are informed of the expected adverse effects. A clear communication strategy is implemented to ensure that the staff looking after the mice are able to contact welfare and scientific support so as to be able to act swiftly if the occasion requires to do so. The researchers, veterinary surgeon, managers of the laboratory and the Home Office Inspector have worked together to elaborate a scoring system to assess mouse's welfare and minimise welfare costs</p> <p>Some types of mouse models will be generated through manipulations under anaesthesia (as appropriate for the technique) and analgesia. This is done in designated areas using aseptic technique to prevent infections or any complications.</p>
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Project	Role of D1R-D3R heteromers in L-DOPA-induced dyskinesia	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	A part of the brain that is essential for all manifestations of Parkinson's disease is called striatum. It is controlled by inflow of impulses that release a substance called dopamine. Not all nerve cells in striatum are the same: there are two subtypes with contrasting properties, particularly how they react to dopamine. The main problem of Parkinson's disease is that the source of dopamine is lost. Nerve cells communicate and transmit information across structures called synapses. The sending nerve cell (presynaptic) relays the information by releasing chemical transmitters. The receiving cell (postsynaptic) detects that signal by specialized receptor proteins present on its body or fine extensions called dendrites. At the points of contact, dendrites have bud-like protrusions called dendritic spines that possess molecular machinery necessary to process the signal. Different types of receptors in striatal spines	

	<p>respond to dopamine. Normally they stand apart, but can also aggregate into combined, higher-order structures called heteromers. Almost nothing is, however, known whether the dopamine receptors behave differently when they form heteromers, or about the consequences that heteromers may have in Parkinson's disease. This is important because it could tell us why patients' brains make wrong calculations and send wrong signals that result in unwanted movements. To answer all these questions, we will use mice with altered genes that allow us to tell between subtypes of nerve cells in striatum, even allowing us to see when heteromers are present in them. Using special methods, we will be able to track dopamine receptor heteromers and see if they come close to other receptors in spines. Then, we will record activity of striatal nerve cells. To achieve all this, we will combine a powerful confocal microscopy to see tiny details within nerve cells and topnotch electrophysiology to record synaptic currents in them.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Many people with Parkinson's disease do not respond well to therapy with a widely-used drug called L-DOPA, having uncontrollable body movements that make them feel ashamed or even fall and get injured. We and other doctors think this occurs because proteins called dopamine receptors associate in unusual structures called heteromers in nerve cells. However, no one knows how and where heteromers are formed, whether they affect other receptors and if it actually makes people feel bad. We are the first to show that there really are many more heteromers in brains of animals with experimental Parkinson's disease put on human therapy. Understanding what heteromers do will help us try to find the way to prevent them from overtaking control over striatum. This will help us devise a new strategy in fight against Parkinson's disease and its devastating consequences.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mouse, 600</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of</p>	<p>The animals will be genetically modified, but this should cause no adverse effect by itself. They will undergo injections and behavioural testing before being killed humanely. Their tissues will be used after death for the imaging and electrophysiological studies. Anaesthesia and analgesia will be used as necessary and any animal</p>

severity? What will happen to the animals at the end?	experiencing an unexpected adverse effect will be treated as advised by the NVS or will be killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	<p>Currently, there is no satisfactory alternative model for investigation of the mechanisms of synaptic changes in Parkinson's disease models that does not require the use of brain tissue acutely removed from animals.</p> <p>The project is intended to result in development of the new transgenic mouse strains. I will make extensive use of the transgenic mouse strains engineered to evaluate the role of dopamine receptors in striatal synaptic transmission. Therefore, this requires maintaining viable breeding colonies.</p>
2. Reduction Explain how you will assure the use of minimum numbers of animals	Using the preliminary data, we have used validated the statistical procedures to calculate the minimal number of animals necessary to produce meaningful data, without compromising the scientific validity of the study. In addition, I will share the tissues with other groups to ensure that neuronal and non-neuronal tissue from the animals is used to the fullest extent possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	<p>We chose mice as the species widely used in transgenic animal design, while also simultaneously validated as the species of choice by current scientific literature in procedures directed at evoking experimental Parkinsonism and abnormal involuntary movements (AIMs). Further, there is a wealth of correlative studies between mouse and human which indicate that the results gained by the animal use are translatable.</p> <p>All of the procedures I propose a) are validated in current scientific literature b) will be performed according to the relevant legislature and c) will be performed by trained staff. Animal suffering will be minimised by the use of anaesthesia and analgesia and by implementing a clinical score chart that measures the well-being of the animals. Mice will be monitored on a daily basis and any animal that shows signs of adverse or unexpected responses, depending on the severity, either advice will be sought from the local NACWO and/or NVS or the mouse will be culled immediately to limit any additional discomfort.</p>

Project	Role of Gut-Brain axis on brain and behaviour	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<ol style="list-style-type: none"> 1. To determine the mechanism underlining the negative impact of prolonged exposure of milk casein at early developmental age on emotional behaviour (mood). More specifically we aim to determine if gut microbiota and hence gut-brain axis regulation are involved in the underlining mechanism. 2. To specifically determine the influence of bioactive breakdown products of casein, b-casomorphines, exposure at early developmental age on emotional behaviour (mood) 3. To investigate the effect of early-life 	

	<p>protein malnutrition on emotional behaviour (mood)</p> <p>4. To determine the involvement of the endocannabinoid system on emotional behavioural change induced by early life protein malnutrition</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The planned work in this project is 2 fold: 1) It will help re-examine the question in humans “is post-weaning consumption of milk actually beneficial for emotional regulation?” and 2) It will examine the question “Is the endocannabinoid system involved in the mechanism underlining the effect of the early life protein malnutrition on emotional behaviour”. We expect the results from the proposed work will be of interest and of benefit to the nutrition, neuroscience, psychiatry, microbiology, pediatric and agricultural community. In particular it will be of interest to the dairy industry. It may also drive policy in term of advising on nutritional content and length of breast feeding and formula milk exposure. It may also advise relevant bodies on the detrimental impact that protein malnutrition has at early age on emotional regulation. Given that early life exposure to nutrients is known to have long term effects on health, we believe that this proposed work would be highly beneficial and impactful to the community on the whole.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use rats and mice. We expect to use 380 rats and 500 mice</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We have found that milk casein when consumed by weaned infant rats induces depression and alter brain receptors and gut bacteria. We believe that gut bacteria may be responsible for causing this depression. We plan to see if the effect of milk casein remains or not if we knockout the gut microbiota by treating weaned rats with antibiotics. If gut microbiota are involved, then we would expect to see a reversal of this depression state following milk casein exposure. We are also planning to investigate if exposure of A2 milk,</p>

	<p>which does not produce the bioactive molecule which we believe to cause depression, in weaned infant rats induces less “depression” than rats exposed to A1 milk which can produce the bioactive molecule b-casomorphins. We are also planning to investigate if exposure of low protein diet (i.e. protein malnutrition) in weaned infant mice induces depression and changes in the endocannabinoid system of the brain which is known to modulate mood. To measure depression the movement of rats as well as their social interaction and urine of the opposite gender sniffing behaviour will be assessed. This causes a slight distress to the rats which is why we used “moderate” as level of severity. Adverse effects of low protein diet is weight loss and of antibiotic treatment is diarrhoea. Animals will be killed at the end of the treatment but brain, gut content and urine will be extracted for analysis of brain, gut bacteria content and metabolic profile of the urine.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The behaviours proposed cannot be studied in <i>in vitro</i> assays and we are not aware of any computer programme which could simulate them. Epidemiological studies in offspring of breastfeeding mothers are planned as part of the PhD project, however, due to ethical reasons it is impossible to directly explore the mechanism underlining the effect of prolonged maternal milk exposure on psychological state in humans.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>The between subject variability for the behavioural outcomes on the animal model proposed is low, which results in the study being highly powered with small sample size. From each animal, we will obtain behavioural, neurochemical, metabolomic, endocrinological, histological and microbiological data. Correlation between these factors will be analysed by factorial design in order to maximise the amount of data obtained with a high degree of precision, without the need to</p>

	use more animals.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Due to the severity of the forced swim test for rodents, we have removed this test from all out protocols. Instead we are using locomotor, social interaction and urine sniffing tests to assess depression. These test are less severe and much much less distressing that the forced swim test.</p>

Project	Role of innate lymphoid cells in cancer.	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Despite recent advances in cancer immunotherapy, there are still many unknowns that limit our ability to harness the power of the immune system in the fight against cancer. The tumour environment can use different mechanisms to promote their growth and evade anti-cancer immune cells. We need to understand these complex interactions in more detail to design better cancer therapies.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could	We will explore the function of immune-regulating immune cells on the development and spread of cancer. Our research will specifically investigate the function of immune-regulatory cells, which are	

benefit from the project)?	critical for controlling a type of inflammation that promotes cancer. More specifically, the proposed research will investigate the role of specific immune cells in different stages of cancer, with the ultimate aim of developing new therapies to combat or control this deadly disease. We will use several physiological models of cancer induction, including surgical administration of cancer cells or cancer-inducing reagents. We will also investigate the effect of radiotherapy, commonly used in humans, on how local radiation-induced inflammation influences the immune response in cancer. Furthermore, we will perform intra-vital live-imaging studies to visualize these actual interactions. Importantly, we will aim to translate these results to human disease. Already there are safe treatments in the clinic that target these immune cells for different diseases, and our work may lead to the “repurposing” of these available therapies for cancer treatment.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse. Maximum 5,240 per year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Adverse effects relating to tumour establishment, development and the assessment of tumours and the administration of substances and sampling procedures are mild. Our severity limit is 'Moderate'. The majority of mice will experience mild to moderate symptoms. All tumour-bearing animals will be closely monitored and will be killed should clinical indications develop, such as loss of condition, a greater than 20-25% loss in normal body weight, significant abdominal distension, dyspnoea, digestive disturbances or neurological/behavioural abnormalities. Animals will also be killed if the tumour ulcerates or if tumour burden impedes any vital function (such as locomotion, vision, eating or excretion). In all cases, knowledge of the models will be used to guide health observations and to inform decisions on killing of animals before they become severely ill. Animals will also be observed to best ensure the detection of tumour development at unexpected sites. At the end of experiment, all animals will be killed

Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>My research dictates the use of animals, as the process of cancer development and spread is currently most accurately and efficiently modeled in mice. Specifically, the role of the immune system for carcinogenesis is best studied in mice for several reasons: 1) We can answer detailed questions about cancer immunology by genetically modulating immune cells in mice. This is still impossible to achieve in humans, or in a petri dish. 2) The complex interactions in immunology and cancer are impossible to model accurately outside of the body. 3) Mice still represent the best model system for studying cancer.</p> <p>Nevertheless, I have previously developed techniques to model very specific aspects of the immune system in a petri dish. I will employ this philosophy to my future studies, with the aims of substituting animal experiments and/or reducing the number of animals in experiments whenever appropriate.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Power calculations will be used to determine how many mice are required for studies to show statistical and biological significance.</p> <p>As mentioned above, we plan to employ (and develop) techniques that reduce the number of mice. These techniques include 'organ in a dish' cultures.</p> <p>Furthermore, I will collaborate with imaging experts to accurately monitor tumour development over time. This allows for the longitudinal analysis of single animals, leading to more robust control parameters and statistics that will ultimately reduce the number of animals required.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take</p>	<p>Mice are the best animal species for my research. As specified before, the many parallels between mice and humans are exploited in normal and transgenic animals. Moreover, as mentioned before, I will employ some of the best established and characterised murine cancer models with state-of-the-art immunology reagents to address</p>

<p>to minimise welfare costs (harms) to the animals.</p>	<p>questions with important implications in human disease. We will continuously refine these models to more accurately address relevant questions in human cancer research. For example, we amended our PPL to allow targeted radiotherapy treatment, which is known to involve immune responses in the cancer. Also, to accurately study the development of cancer, we will surgically inject tumour cells or cancer inducing reagents locally, which is critical for mimicking how humans develop disease.</p> <p>We have optimised the procedures to minimise potential pain, suffering or distress, and enhance animal welfare. For example, new types of soft bedding material will be used for recovery from some procedures where the animal will experience pain. Also, we have developed new more refined genetic mouse models, which avoid the previous need for more harmful procedures such as cell-transfusions and irradiation. We continue to strive to develop new refinements that help us address important scientific questions with more refined (and therefore fewer and more humane) animal experiments.</p>
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Project	Role of intracellular effector mechanisms in immunity to pathogens	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>We are investigating natural immune responses to viral infection and other pathogens and designing antivirals, antimicrobials and vaccines to augment these processes.</p> <p>Another area of interest is neurodegenerative diseases and potential role of TRIM21 in preventing spread of misfolded tau. Finally, we are interested in immune response against gene therapy vectors used to treat cancers.</p>	
What are the potential benefits likely to derive from this project (how	Infectious disease is the biggest killer of mankind worldwide. Viruses alone kill more	

<p>science could be advanced or humans or animals could benefit from the project)?</p>	<p>than twice as many people each year than cancer. There is therefore a desperate need to understand how pathogens cause disease and the strategies used by our immune system to combat them. We are identifying new anti-pathogen mechanisms and investigating how they work and how they are antagonized by some pathogens. Our ultimate aim is to develop anti-pathogen drugs that will be efficacious in people. Our work will help to understand the biology of AdV vector interaction with immune system and design strategies that potentially open up the use of viral vectors in gene therapy against cancers much more widely than at present.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice can be used in the development of antiviral strategies and therapeutics. Research programmes are undertaken over 5 years with the expectation to use 17000 mice.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The mice will be infected with pathogens such as viruses and bacteria. Only about 10% of the mice will get sick enough to need culling prior to the end of the experiment. Signs such as listlessness, standing in a hunched way, and occasional abnormal breathing will indicate that the mice need to be culled. The remaining 90% of mice show no signs of ill health except weight loss. Weight loss of 20% from the start of the experiment is the endpoint for our experiments. In case of stereotaxic surgery animals are expected to experience only a transient discomfort without lasting harm and should make a rapid recovery from anaesthetic. In the uncommon event that animals failed to do so or exhibit signs of pain, distress or significant ill health they will be culled by Schedule 1 method. In case of external tumour model the majority of the animals are expected to develop external tumours, however mice are not expected to exceed moderate severity. Animals will be culled immediately if the tumour size reaches 12 mm in any dimension or the total tumour mass reaches 10% of the body weight.</p>
<p>Application of the 3Rs</p>	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Due to the nature of our research, which is concerned with the response of whole animal immunity to infection, there are no alternatives to an animal model. Where possible we will carry out analogous experiments <i>in vitro</i> in culture cells. However, this is not always possible because we cannot recreate the multi-cellular organisational structure that comprises the immune system, <i>in vitro</i>.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Breeding programmes are optimised to ensure as little over-breeding as possible. Power analysis will be applied to ensure that only the minimal numbers of animals are used per experiment. Where new protocols are being undertaken pilot studies will be used to inform on the optimum protocol format.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are known to share many similarities with the immune response in people, particularly the key anti-viral responses we are studying. We have undertaken <i>in vitro</i> experiments to show that the proteins we work with are highly conserved in mammals.</p> <p>Genotyping is undertaken almost exclusively from ear biopsies rather than tail biopsy.</p> <p>Models of disease are chosen to allow the lowest dose of pathogen that allows immune responses in animals to be studied.</p>

Project	Role of nitric oxide and reactive oxygen species in cardiac function	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The main objective of this project is to study how nitric oxide (NO) and reactive oxygen species (ROS) regulate cardiac function.</p> <p>Our previous work and that of others have already identified changes in the production of these messenger molecules in situations where adverse left ventricular remodelling takes place leading to heart failure or heart rhythm disturbances.</p> <p>Based on these results we will:</p> <ol style="list-style-type: none"> 1. Test the beneficial effects of modifying NO 	

	<p>and ROS signalling pathways on hypertrophy, inflammation, atrial fibrillation and diabetes.</p> <p>2. Study other genes that will serve to identify novel molecular targets for future therapeutic interventions.</p> <p>As these conditions are common (1 in 4 life-time risk of development) and often requiring long, and often acute hospital stays, the cost of caring for patients with heart failure (HF) and/or atrial fibrillation (AF- most common heart rhythm disorder) has been estimated to consume ca. 4% of the annual NHS budget in the UK. These data underscore the major public health burden posed by HF and AF in our society and the need for a better understanding of the mechanisms that control the evolution from myocardial remodelling to pump failure and rhythm disturbances.</p> <p>The Framingham Heart Study was the first to demonstrate that diabetes (DM) and obesity are independent risk factors for developing AF. AF is associated with considerable morbidity, decreased quality of life, and increased mortality as a consequence of heart failure and thromboembolic events. AF accounts for 25-33% of all ischemic strokes. Diabetes is also a common condition and growing healthcare burden, particularly in developing countries. The total number of people with diabetes is estimated to rise from 366 million in 2011 to 552 million by 2030. A high proportion of patients (50-75%) will develop diabetic cardiomyopathy. An important need remains to further delineate the basic mechanisms of diabetic cardiomyopathy and to translate promising therapies in preclinical models to humans.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>By elucidating the mechanisms leading to an increased production/bioavailability of reactive oxygen species in the diseased myocardium, our reaserch will increase our understanding of myocardial NO-redox biology, identify new biomarkers of disease evolution and enable us to test whether specific interventions targeted to restore a normal myocardial NO-redox balance will prevent or retard the evolution towards HF and AF in chronically stressed hearts.</p>

<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We have estimated that a maximum of 10,900 mice will be used over a period of 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We expect that the level of severity will be moderate for the majority of mice. The experiments we are proposing include phenotyping new genetically modified mouse models as well as generating mouse models of human disease, notably diabetes and cardiac hypertrophy secondary to pressure overload (e.g., a situation that can be observed in patients with high arterial pressure or aortic valve stenosis) on which we could also test new therapeutic strategies. Characterisation of these models would typically include non-invasive (e.g., echocardiography and/or magnetic resonance imaging) under anaesthesia and/or invasive (e.g. Left Ventricular haemodynamics or transoesophageal pacing) evaluation of cardiac function under terminal anaesthesia and ex-vivo investigations in myocardial tissue and cells. Type 1 Diabetes will be induced by the administration of streptozotocin that is toxic to pancreatic beta cells, type 2 Diabetes by dietary modification or the use of transgenic models. These mice will experience high blood glucose, which will be monitored, and polyuria/polydipsia, therefore, they will have free access to water and absorbent bedding will be added to the cage whenever necessary. Cardiac hypertrophy will be induced by aortic banding or by administration of angiotensin II or isoproterenol via an osmotic minipump implanted subcutaneously. We routinely apply pre- and post-operative care to the mice that undergo procedures (anaesthesia/analgesia). After they recover from surgery most mice will be free from symptoms for the duration of the study. Occasionally animals will suddenly develop deep abdominal breathing, which is the first sign that they have developed heart failure. Suffering is minimised by using this as an immediate humane end-point. These animals are likely to experience a severity level that is severe. However, the majority of animals are killed humanely at the scientific endpoint without exhibiting any symptoms of heart failure. At the end of these experiments the animals will be humanely killed and tissues collect for</p>

	biochemical and histological analysis.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Although we are actively engaged in the process of reduction, we recognise that at present, it would be impossible to model the behaviour of such a complex system <i>in silico</i> or using cell lines. We are carrying out a number of complementary experiments in humans and surplus human tissue from patients undergoing cardiac surgery as well as developing a computational model of the healthy and diseased myocardium to guide our experiments and minimise the use of animals and <i>in vivo</i> experimentation.</p> <p>In order to characterise the function of a specific gene on NO-redox balance we will initially make use of isolated cardiomyocytes and perfused heart preparations from mice, as these experiments are the least severe. To complete these first investigations and gain insights of the function of these genes in disease. We will need to use models of human disease (diabetes, hypertrophy).</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The minimum numbers of animals required have been carefully reviewed by the funding agencies and have been based on power calculations.</p> <p>Numbers have been reduced in single cell based experiments (cell shortening, calcium measurements and patch clamp, immunohistochemistry and molecular studies) due to the availability of three different cellular electrophysiology set ups as well as other equipment (e.g. confocal microscope/FRET system) on site, allowing us to have several scientists working on cardiomyocytes isolated from one heart.</p> <p>To maximise the use of animals, we share our surplus tissue with other groups with non-cardiac research interests.</p> <p>As part of the strategy to reduce the number of animals we are developing the optical mapping of the atria. This technique will allow us to assess cardiac electrical properties in perfused hearts</p>

	<p>instead of using live animals, thereby reducing the number of <i>in vivo</i> experiments.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Surgical and imaging techniques are constantly refined and used by all the groups in our Department. Any step forward on the refinement of these procedures that leads to minimise welfare cost to the animals is actively sought and shared in our regular animal welfare meetings.</p> <p>We have modified the protocol to induce type 1 diabetes in mice. This allows us to generate a less severe model where we can study early changes in the myocardium. The severity and variability of previous protocols have been significantly reduced. Diabetic mice are subject to regular screening (blood glucose measurements and body weight monitoring). Careful attention is given to excess consumption of water or frequent wetting of bedding material. Absorbent bedding is added whenever necessary.</p> <p>Refinements have also reached other techniques such as the reduction in volume of blood required for glucose measurements or the delivery of drugs by using implants instead of multiple injections.</p> <p>Strict humane end-points, evaluated by Vets, will be applied to minimise suffering of the animals.</p> <p>We routinely apply pre- and post-operative care to the mice that undergo procedures, ie, analgesia, heat support, access to water-softened chow, subcutaneous fluids and oxygen. Mice are allowed to recover in a heated chamber and checked after recovery. Recovery surgery is performed earlier in the day to allow sufficient monitoring within normal working hours.</p>

Project	Role of oxidative stress in angiogenesis pathophysiology including pregnancy disorders	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>We are interested in how new blood vessels form, a process known as angiogenesis. Too much or too little angiogenesis can cause disease. Increased angiogenesis can lead to blindness or tumour growth, whereas low angiogenesis prevents wounds from healing and can also contribute to the development of cardiovascular disease, heart failure or pre-eclampsia. Age, diabetes, obesity and pregnancy are all</p>	

factors that can lead to faulty regulation of angiogenesis.

The aim of this study is to develop a better understanding of how angiogenesis affects different vascular diseases. Of particular interest is i) peripheral artery disease which is common in diabetes and lead to amputation of lower limbs, and pre-eclampsia (where the blood pressure rises to dangerous levels in pregnancy). We also wish to develop an understanding of how pre-eclampsia can pre-dispose people to heart failure later in life.

My research focuses on redox signalling in cardiovascular system. Antioxidants are commonly mentioned when talking about the benefits of red wine, dark chocolate and vitamin C, these protect against oxidants. All cells within the body have enzymes which produces oxidants and antioxidants. Redox signalling refers to the fine balance between these factors and how they modify proteins to switch them on and off. Upsetting the balance in redox signalling causes diseases such a peripheral artery disease and heart failure. In preeclampsia, oxidants are known to be high, yet little has been done to fully understand how this effects the disease.

We believe that redox signalling may contribute to the severity of pre-eclampsia and other disorders, a hypothesis which we now wish to test. We also wish to learn more about the mechanisms involved in these disorders.

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Preeclampsia is a life threatening condition for both mother and child that occurs during pregnancy causing maternal high blood pressure and kidney dysfunction. The symptoms can rapidly develop and the only cure is delivery of the baby and placenta. The current treatment, aspirin, is only effective if given early (12-14 weeks), but our ability to detect which women will develop preeclampsia is limited.. Pregnancy complications are rising in line with obesity and diabetes epidemics, with a five-fold increase in risk of pre-eclampsia in woman with diabetes. Women who have suffered from pre-eclampsia during pregnancy have increased risk of cardiovascular disease later on in life, for reasons that are unclear at present. This project sets out to better understand vascular complications such as pre-eclampsia at a molecular level. Gaining a better understanding may pave the way to identifying targets for new treatments. We also expect to integrate data from human clinical samples and animal models to develop a genetic/molecular “signature” of disease, which could be used for prediction and earlier detection and treatment. We also wish to study the molecular connections that must underpin the link between pregnancy disorders and heart failure later in life. Finally, this project is part of a larger programme to improve modelling of placental disorders, possibly via a “placenta on a chip” and via sophisticated computer models of this organ. The data from this project in animals will be used to assess and validate these new models, perhaps leading to a reduced use of animals in the future.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The main species studied will be mouse. A small number of rats will also be used. The maximum total number of animals used in experimental procedures over the duration of the project will be around 2100, with another approximately 2000 being used for the necessary breeding of informative lines of genetically altered animals. These species were chosen because of the existing literature describing relevant methods and techniques in the field of cardiovascular disease and because of the availability of mice bearing highly informative genetic modifications. We also plan to use imaging to assess endothelial function, which has direct clinical significance as similar</p>

	techniques can potentially be used in detection and assessment of disease in patients.
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The basic design of all the experiments performed in this program will be to induce signs of pre-eclampsia by surgical manipulation of the vascular system or by using genetically modified mice in which vascular development is perturbed. We will follow the processes of angiogenesis by measurements made on the blood vessels in the skin (for instance using laser doppler). Some animals will be fed an altered diet intended to result in features of obesity and/or diabetes. These diets should not cause overt adverse effects in themselves. Animals may receive potentially protective agents in water or the diet or by injection; these agents are not expected to have any toxic side effects. Occasionally animals will be given the drugs over a period of time, in which case it may be necessary to implant a device to release the drug at a precise rate. Occasionally animals may be implanted with a device that can measure body temperature and/or blood pressure and broadcast the data wirelessly (telemetry). The genetically altered mice are not expected to show significant adverse effects in normal maintenance and breeding. Specific matings will be set up in which to study pre-eclampsia but the mother and the foetuses will be killed humanely (and the placenta harvested for detailed analysis) before more than a moderate rise in blood pressure is detected. Surgical procedures will be carried out using appropriate anesthetic and analgesia to minimize distress and discomfort. Animals will be killed humanely at the end of the studies, so that tissues can be collected for further analysis.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Alternatives to animals cannot be used to meet the aims and objectives of this work at present, because <i>in vitro</i> models of angiogenesis differ fundamentally from the <i>in vivo</i> process. Many of the characteristics of growing blood vessels in tissue culture systems, the influence from surrounding cell types and extra-cellular matrix molecules is missing, and the time course of adaptations cannot be mimicked realistically.</p>

	<p>However, we are also continuing to refine our <i>in vitro</i> methods and, if successful, these will lead to a degree of replacement of animals in future research.</p> <p>Notably, data collected from experiments outlined in this protocol will be used to develop both a novel 3D <i>in vitro</i> model (placenta-on-a-chip) and an <i>in silico</i> model REDACTED. These developments may help in the future replace some of the experiments that currently require the use of animals in some of these studies by providing a different way to model the placenta function in disease.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Breeding will be optimised to produce the genotype(s) required, with the programme subject to regular review to optimise numbers to experimental demand. The numbers bred will be managed carefully to avoid over-supply. The number of animals will be minimised by careful experimental design and appropriate statistical analysis.</p> <p>By using appropriate <i>in-vitro</i> systems throughout we will reduce animal usage significantly.</p> <p>We share tissues and data with others where possible.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	

Project	Role of Pattern Recognition Receptors in Immunity	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Our objective is to understand how cell based molecules, called pathogen recognition receptors (PRRs), enable the functioning of our immune system during infection with medically relevant pathogens and during the development of autoimmune diseases of the joints (arthritis), lung (asthma), eye and kidneys). We also need to develop tools such as antibodies and transgenic T-cells to study PRRs in these systems. These tools are made widely available to the scientific community following appropriate material transfer agreements, to achieve maximum impact.</p> <p>Experimentally, in order to determine the role of</p>	

	<p>a particular PRR, we compare immune responses of normal mice to those of mice genetically deficient in that PRR. In this way we can determine which PRRs are important in the control of particular infections or in the prevention of certain autoimmune diseases. This strategy will help us to understand the disease process and the way in which the body attempts to control it. Understanding these mechanisms is the foundation for being able to identify avenues for new and better treatments in humans.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The direct benefit of our research is the furthering of scientific knowledge of the underlying molecular and cellular mechanisms of immunity. This advancement of knowledge will identify novel avenues for future therapies to improve public health in the longer term. Our work has already led to substantial academic advances, as evidenced by publication in high impact journals, and has also directly led to an understanding of disease susceptibility and to novel therapies in man.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>20,000 mice and 100 rats during the 5 year PPL. These numbers represent the theoretical maximum, and in practice will likely be less. Estimates are based on the required group sizes for experiments, experience on how many experiments are required to complete the studies of the number of researchers operating under this PPL.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Mice are injected with natural or synthetic substances which cause immune responses that can induce diseases which we need to study, including inflammation, eye disease, arthritis, asthma and renal disease. We also retain a small number of mice for aging studies. While the majority (approximately 80%) of mice undergoing these procedures will have no significant adverse effects, in some studies the mice can become moderately unwell for a few days. These animals may show weight loss, become less active, have ruffled fur and appear hunched. During models of arthritis, their limb joints may temporarily become slightly red and swollen, and the mice may limp when walking</p>

	<p>on solid surfaces. For models of infectious disease, mice are infected with medically relevant infectious microbes. In most studies the mice can become very unwell and therefore this protocol is listed as severe. Animals will show up to 30% weight loss, become less active and isolated, have ruffled fur and appear hunched. In all our experiments, mice which become ill/lame are humanely culled as soon as the scientific outcomes have been achieved within defined clinical endpoints, and are closely monitored during the course of the experiment. We have already established such a monitoring system for our experimental models in consultation with the senior animal technicians and the veterinary surgeon. All animals are killed at the end of the study and tissues analysed.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Our mouse models (<i>in vivo</i>) are for studying clinical diseases for which there are no other laboratory (<i>in vitro</i>) alternatives. Experiments with cultured cells in dishes cannot recreate the complex cellular interactions required to fight disease <i>in vivo</i>. Wherever possible we use cell lines, human cells, or other <i>in vitro</i> methodologies (as they are developed), particularly for understanding the molecular mechanisms of immune cells. Approximately 20% of our work is carried out <i>in vitro</i>.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>Reduction in animal use will be achieved by:</p> <ul style="list-style-type: none"> -multiple readouts from animals <i>post mortem</i>. -use of appropriate group sizes to obtain statistical significant results. -use of inbred strains of age/gender matched mice which reduces intra-group variation. -optimisation of procedures and protocols to minimise experimental variability. -freezing mouse embryos/sperm for strains not required for some time.

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Since mice are the worldwide standard laboratory animal model for immunological study. Most reagents available for this species, as are genetically altered animals. We use rats to generate rat anti-mouse monoclonal antibody reagents. The use of non-mammalian species will not provide the crucial insights required to understand human resistance to disease.</p> <p>We optimise and refine our techniques to ensure maximum output from the minimal number of animals, in part through literature searches and discussions with other investigators to ensure the latest practices are employed. We make use of small pilot experiments in new areas to gain insight into potential adverse effects, and define humane endpoints. The experimental data that we generate feeds back into planning for future refinement.</p> <p>Where there is potential suffering for the animals, this is minimised by ensuring that all personal licence holders are appropriately skilled and trained in our clinical monitoring systems to identify humane end points, and through the use of techniques for the alleviation of pain and distress (e.g. analgesia).</p> <p>We are continuously refining our clinical monitoring systems. This is being achieved through observations made by the experimenters and animal care staff and discussions with the senior animal care technicians and the veterinary surgeons.</p> <p>Our studies make use of killed pathogens, where possible, to minimise the effects on the wellbeing of the animals.</p>
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Project	Role of the cytoskeleton in cardiovascular diseases
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
What's the aim of this project?	The aim of this project is to understand the roles of specific proteins in the regulation of the function of blood platelets, and the implications for diseases related to platelet malfunctioning.
Why is it important to undertake this work?	Cardiovascular diseases are one the leading cause of death worldwide. In the UK cardiovascular disease causes 26% of all deaths, accounting for more than 129,000 people each year and goes with a financial burden (premature death, lost productivity, hospital treatment and prescriptions) that is estimated at £18 billion. Thrombosis is a major component of cardiovascular disease. Thrombosis is the formation of a blood clot within the blood vessel that can lead to occlusion of the vessel and starve the tissues and organs of oxygen and nutrients and result in myocardial

	<p>infarction (heart attack) and brain infarction (stroke).</p> <p>The role of platelets in thrombosis is exemplified by the success of anti-platelet drugs like aspirin in reducing adverse cardiovascular events in high-risk patients. However, current therapies can also have significant side effects, most notably increased bleeding, and therefore more effective and specific anti-thrombotic therapies are required to treat and prevent thrombosis. It is crucial to identify the specific roles of the proteins that regulate platelet function and to evaluate their potential as targets for the development of new antiplatelet drugs that could reduce/prevent heart attacks and strokes.</p>
What outputs do you think you will see at the end of this project?	We will endeavour to publish our findings in international peer review journals. Our research may also inform the development of new antiplatelet drugs that could reduce/prevent heart attacks and strokes.
Who or what will benefit from these outputs, and how?	Our studies will expand our knowledge of the area of research dealing with platelet function and cardiovascular disease and will help other researchers, both in the UK and internationally, who are focussed on solving problems associated with thrombosis and other aspects of platelet function. Patients suffering such conditions may ultimately benefit.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	The outputs will be disseminated through participation in scientific conferences and publication in peer review journals. The use of genetically modified mouse models is crucial to understand the role of platelet proteins due to the impossibility of genetically manipulate platelets and therefore use in vitro models. For this reason negative results in this are provide valuable information and are regularly published.
Explain why you are using these types of animals and your choice of life stages.	Because platelets lack a nucleus (the part of the cell that contains the genetic information), they cannot be manipulated to introduce mutations in the genes of interest. For the same reason platelets cannot be cultivated. The only source of sufficient amounts of platelets is from an animal model. The use of genetically altered mice has become an established method to

	address the function of a particular protein in platelets.
Typically, what will be done to an animal used in your project?	The animal is killed using one of the approved humane methods and immediately bled with a syringe and needle aimed at the heart or a large blood vessel. The blood is then used to isolate platelets by separating them from other blood components and the platelets are used in a series of tests that analyse the platelet function.
What are the expected impacts and/or adverse effects for the animals during your project?	The animals are not expected to undergo any adverse effect other than mild pain during collection of a tissue biopsy (ear punch) for determining their genetic status.
What are the expected severities and the proportion of animals in each category (per animal type)?	Mild (all) as explained above.
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	Platelets are not amenable to genetic manipulation because they lack a nucleus. Transfection studies commonly used by many other researchers as an alternative to animal experiments work only with nucleate cell systems and are therefore not possible. For the same reason platelets cannot be cultivated. The only source of sufficient amounts of platelets is from an animal model. A common approach is to use chemicals that can act as inhibitors or activators of particular proteins. These chemicals usually target proteins with enzymatic activity. Structural proteins and many signalling components usually cannot be targeted using chemicals; this applies particularly to cytoskeleton components. In light of this information the use of genetically altered mice has become an established method to address the function of a particular protein in platelets.
Which non-animal alternatives did you consider for use in this	1. Use of chemicals that can act as inhibitors or activators of particular proteins.

project?	<p>2. Non-sentient animals</p> <p>4. Production of genetically modified platelets using gene silencing in vitro from bone marrow culture or stem cells.</p>
Why were they not suitable?	<p>Inhibitors usually target proteins with enzymatic activity. Structural proteins and many signalling components usually cannot be targeted using chemicals; this applies particularly to cytoskeleton components.</p> <p>Non-sentient animals cannot be used for our studies since they lack recognisable platelets. Previous and on-going studies make use of genetically modified mice to investigate the role of particular genes in platelet function.</p> <p>Although progress is being made with techniques of in vitro production of platelets, currently the yield of in vitro thrombopoiesis is too low for most biochemical and functional assays.</p> <p>In light of this information the use of genetically altered mice has become an established method to address the function of a particular protein in platelets.</p>
Enter the estimated number of animals of each type used in this project.	mice: 300
How have you estimated the numbers of animals you will use?	We use power calculations to ascertain the minimal number of animals required to use in a single experiment. Our calculations are based on previous studies in the area of research and account for biological variability between individuals.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	Power calculations have been made with the support of expert statistical advice from REDACTED to ensure that experiments are neither over- nor under-powered.
What measures, apart from good experimental design, will you use to optimise the number of animals you	We closely monitor breeding to ensure we obtain an optimal number of animals for experiments. Experiments are planned so as to maximise the usage of animal material. Surplus animals and tissue that is not of interest for our projects is routinely shared with other

plan to use in your project?	investigators
Which animal models and methods will you use during this project?	<p>Mice are the lowest vertebrate group amenable to genetic modifications on which well characterised models for the study of platelet function are established.</p> <p>In line with developments in the establishment of improved methodology for the maintenance of genetically modified mouse colonies, we are mindful that tissue biopsy by ear punching is considered less stressful and painful than tail tipping.</p> <p>Power calculations have been performed to enable the minimal use of animals to obtain statistically meaningful data.</p>
Why can't you use animals that are less sentient?	Non-sentient animals cannot be used for our studies since they lack recognisable platelets.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	We are monitoring closely studies in other laboratories that have begun to examine ways to produce genetically modified platelets using gene silencing in vitro from bone marrow culture or stem cells. Although progress is being made with those techniques, currently the yield of in vitro thrombopoiesis is too low for most biochemical and functional assays. Should this technology become available will look to adapting it for our own studies.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	Breeding will be conducted using standard procedures.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	

Project	Role of the immune system in deep vein thrombosis	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Deep vein thrombosis (DVT) is formation of blood clot in vessels called veins that bring blood back to the heart. The veins with DVT clots are usually (but not always) located in the legs. More than 60,000 people in the UK develop DVT every year. Clots in the leg, being painful and impairing the quality of life by themselves, can get detached and travel to lungs, where they occlude vessels important for oxygen delivery to the tissues as we breathe. This causes an emergency state called pulmonary embolism (PE) that causes breathing difficulties and frequently leads to death (sometimes sudden death).</p> <p>Despite high prevalence and acute nature of</p>	

	<p>the problem, mechanisms that lead to the development of a DVT remain elusive. Current methods of DVT prevention are largely based on targeting blood clotting mechanisms, but this approach is far from ideal because the same mechanisms are also involved in normal bleeding control and therefore many patients receiving such treatment experience dangerous bleeding as a side effect. In addition to creating severe health problems, modern methods of DVT prophylaxis lead to the need to monitor many patients in hospitals putting a heavy financial burden on the NHS. All this shows why studies into mechanisms of DVT are needed to find fundamentally new ways to efficiently and safely fight this devastating disease.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>During recent years, we and others have demonstrated that the immune system, which normally protects us from microbes, plays an unexpected role in DVT initiation. Different types of cells, usually participating in such deleterious process as inflammation, get together to create and maintain several vicious circles triggering the development of venous clots. The main goal of this project is to delineate new cells and/or molecules in the immune system that can be successfully targeted to prevent formation of blood clots in veins. This will provide a fundamental basis and give rise to development of new drugs for this purpose. Importantly, the immune system and specifically those cells and processes that we plan to investigate are not involved in normal bleeding stoppage and therefore new approaches that we are going to develop will be safe and will not lead to bleeding complications.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We plan to use up to 6400 mice over a five-year time period. This number of animals represents statistically calculated and predicted maximum and it is highly likely that lower amount of mice will be needed. The mice have been chosen because: a) the system of blood clot development in mice is reasonably similar to this system in people; b) mutations in different molecules responsible for blood clot</p>

	<p>development can be much easier achieved in mice than in other animals (in most of which this cannot be achieved at all). Such mutations represent an important if not a unique tool to understand the role of each molecule in blood clotting; c) mice can easily be administered different drugs and most of the drugs that work in humans work also in mice. This makes it highly likely that new drugs developed in mice will do the job also in patients; d) mice are easy in maintenance and results obtained on them are highly reproducible, which makes it possible to use a small number of them to get convincing unambiguous results.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We plan to model DVT on strong discomfort mice by creating blood flow disturbance in a large vein. This is a major driving force of DVT in humans at risk, for example, people in bed-ridden position, after surgery or even in long-haul flights. This manipulation is performed under general anaesthesia, which is carefully controlled so that mice don't feel any pain or discomfort. After flow disturbance application, animals usually quickly recover, move and eat almost normally and don't lose weight. To avoid any suffering, mice will receive a strong pain killer regularly until the end of experiment. During recovery process and after that mice will be tightly monitored and if signs of strong discomfort are detected (which may happen rarely) this mouse will be immediately withdrawn from the experiment and humanely killed. Thus, the expected and likely level of severity is defined as moderate. At the end of experiment, mice will be humanely killed by one of the officially approved methods avoiding any excessive suffering, pain or discomfort.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Several separate aspects of DVT can be modelled in the test-tube and we plan to do as many experiments as possible using this method to avoid using animals. For example, in collaboration with colleagues from the Chemical Engineering department we will extensively use a specially designed device called "microfluidics" as well as computer</p>

	<p>simulations to explore blood flow geometry in DVT. Prior to moving to work with mice, we will carefully select the most promising targets, either cells or molecules, that are most likely to protect from blood clots in veins. However, final verification of selected targets can be performed only using animal models because it is impossible at the current stage of research techniques to reproduce in vitro all the complexity of factors influencing thrombus formation in a real organism.</p> <p>The mice (and not non-protected animal alternatives) have been chosen because: a) the system of blood clot development in mice is reasonably similar to this system in people; b) mutations in different molecules responsible for blood clot development can be much easier achieved in mice than in other animals (in most of which this cannot be achieved at all). Such mutations represent an important if not a unique tool to understand the role of each molecule in blood clotting; c) mice can easily be administered different drugs and most of the drugs that work in humans work also in mice. This makes it highly likely that new drugs developed in mice will do the job also in patients; d) mice are easy in maintenance and results obtained on them are highly reproducible, which makes it possible to use a small number of them to get convincing unambiguous results</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will undertake several steps to ensure that we will use minimal number of mice required to obtain scientifically solid unambiguous results. First, a special statistical prediction called "power calculation" for the experiments involving animals has been performed by a professional statistician . The calculation was based on our extensive experiments with the models as well as literature data and consultations with research groups dealing with similar scientific directions worldwide. The variability in results in the DVT models is reasonably small and differences between experimental groups that we and others obtained so far were quite striking and did not require more animals than the power calculation predicted. As an additional control</p>

	<p>for the sources of variability (and therefore a measure to minimize the number of animals) we will ensure that similar experiment are always performed by the same person and that conditions of mice maintenance prior and after the surgery are identical. If the difference between the experimental groups is achieved on a smaller group of animals than predicted by the power calculation, the experiment will be stopped, and no additional animals will be used. In the course of the research, we will continue to consult with world-renowned experts in the DVT models regarding the experimental design and validity of the obtained results to make sure that the smallest possible groups of animals are used.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We have chosen mice because the essence of the work makes it necessary to utilize genetically altered animals (and this is achievable only in mice. Most of the genetically altered mice that we intend to use, already exist and therefore there is no need to develop them from the beginning. The technique of DVT surgery in mice is well-established and my lab has a long-standing expertise in it so no additional training for this is required. The results of DVT experiments in mice are reasonably stable and reproducible when the sources of variability mentioned above are considered and controlled.</p> <p>To minimize suffering all the animals will be administered a pain killer prior to surgery and then on daily basis throughout the course of the experiment. Mice will be monitored until full recovery after anaesthesia and then checked at least twice a day to ensure that any abnormalities in their well-being are immediately noticed and a required action is taken. Mice demonstrating signs of severe distress or bleeding, will be immediately withdrawn from the experiment and humanely killed</p> <p>After every experiment, its course and the model, including any complications if they happen, will be critically re-evaluated and any potential measures that could make refinement more efficient specifically for these models will</p>

	<p>be taken in the following experiment. We will also follow the literature and communicate with other groups worldwide who use these models to make sure that we have complete updated information about modern refinement approaches and enforce in our practice any novel methods of refinement that might appear in the future.</p>
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Project	Safety and efficacy of anti-parasitics in food producing animals																
Key Words (max. 5 words)																	
Expected duration of the project (yrs)	5 Years 0 Months																
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<table border="1"> <tr> <td data-bbox="724 638 751 734"></td> <td data-bbox="751 638 1401 734">Basic research</td> </tr> <tr> <td data-bbox="724 734 751 824"></td> <td data-bbox="751 734 1401 824">Translational and applied research</td> </tr> <tr> <td data-bbox="724 824 751 913">X</td> <td data-bbox="751 824 1401 913">Regulatory use and routine production</td> </tr> <tr> <td data-bbox="724 913 751 1081"></td> <td data-bbox="751 913 1401 1081">Protection of the natural environment in the interests of the health or welfare of humans or animals</td> </tr> <tr> <td data-bbox="724 1081 751 1171"></td> <td data-bbox="751 1081 1401 1171">Preservation of species</td> </tr> <tr> <td data-bbox="724 1171 751 1261"></td> <td data-bbox="751 1171 1401 1261">Higher education or training</td> </tr> <tr> <td data-bbox="724 1261 751 1350"></td> <td data-bbox="751 1261 1401 1350">Forensic enquiries</td> </tr> <tr> <td data-bbox="724 1350 751 1480"></td> <td data-bbox="751 1350 1401 1480">Maintenance of colonies of genetically altered animals</td> </tr> </table>		Basic research		Translational and applied research	X	Regulatory use and routine production		Protection of the natural environment in the interests of the health or welfare of humans or animals		Preservation of species		Higher education or training		Forensic enquiries		Maintenance of colonies of genetically altered animals
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	Forensic enquiries																
	Maintenance of colonies of genetically altered animals																
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	All food producing animals are exposed to challenge from parasites, which may compromise their health and wellbeing. In livestock, they can also have a major impact on productivity and profitability. Common parasites of cattle, sheep and pigs include gastrointestinal nematodes, tapeworms, lungworm, liver fluke, protozoa, lice, mites, ticks and flies. Parasites of poultry include intestinal nematodes, caecal worms, thread worms, tapeworms, gapeworms, mites, lice and ticks. Symptoms of parasitic diseases can vary and range from weight loss, inappetance, anaemia, bloat, colic, diarrhoea, blood in																

	<p>faeces, coughing, pain and discomfort observed from behavioural changes, excessive scratching and licking. As well as direct clinical effects, infections with parasites can compromise the immune system and lead to secondary infections with bacteria and viruses. The diversity of parasitic organisms has led to the development of an accordingly broad range of products for their control in various species. Anti-parasitics include many different product types, which may be effective in one or more of the parasite classes such as protozoa, nematodes, cestodes, trematodes and arthropods. However, parasite resistance has compromised the efficacy of several long-established classes in both the insecticide and anthelmintic sectors. This development of resistance has encouraged the search for new groups of novel mechanisms or action that will not suffer from cross-resistance problems. Since immunological approaches to parasite control would overcome the resistance problems associated with pharmaceutical approaches, the development of effective vaccines against parasites is a subject of high interest. In addition to research into new actives and products, there is also further research into improving current products and generics which have improved application techniques which can improve the accuracy of application to prevent further resistance and improve animal welfare, e.g. pour-ons instead of injectables.</p> <p>The overall aim of the programme of work is to provide efficacy and safety data for products for the control and prevention of parasites in farm animals. Veterinary anti-parasitics are continually being developed, but it is a legal requirement for these to be fully tested for safety and efficacy prior to them being marketed. This licence will enable studies to be carried out on behalf of pharmaceutical companies to satisfy these legal requirements. Some work of a supportive nature may also be conducted, for example validating challenge models in advance of an efficacy study being conducted.</p>
<p>What are the potential benefits likely</p>	<p>The overall aim of the programme of work is to</p>

<p>to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>develop safe and effective means of controlling parasites in farm animal species. Disease and ill health caused by parasites in farm animal species continues to be a worldwide welfare concern. This problem is being exacerbated by the rising levels of resistance to various anti-parasitics. The results of studies conducted under this license will be incorporated into dossiers submitted to the regulatory authorities to allow new anti-parasitics to be marketed. This will allow more effective and safe anti-parasitics to be available to veterinary surgeons and farmers. This will result in more effective and safe means of controlling / preventing ill health caused by parasites in farm animals. Animals should benefit through the availability of safe and more effective (including less resistance) products on the market. The availability of safe and more effective products is likely to be within five years of studies being conducted.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Cattle 2000 Pigs 1900 Sheep / goats 2000 Chicken 2600 Turkey 700 Rabbit 700</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>For a typical safety study, animals are administered the anti-parasitic and observations made. Adverse effects are generally mild and can include injection site reactions, increase in temperature and reduced activity. For an efficacy study with challenge, animals are usually challenged with a typical form of the disease experienced in the commercial environment to determine the efficacy of the treatment. Symptoms of the disease are monitored closely, and continuously (including overnight) for some of the more extreme challenges. Symptoms are only allowed to progress to a point that allows proof of efficacy to be determined, and this is very often dictated by European guidelines. In the majority of cases the adverse effects are likely to be minimal or mild. Where at all possible, animals will be returned to local farms following certification by a veterinary surgeon that they are fully recovered. Alternatively, animals may be sent directly for humane slaughter in the same manner as other farm</p>

	<p>animals. Where this cannot occur (an unregistered product for example), animals will be humanely euthanised and the carcasses will be incinerated.</p> <p>For a typical efficacy study, animals may be sampled or have external parasites counted on a number of occasions before, during and after test product administration. Challenge with the disease may be by finding naturally infected animals on commercial farms or challenging animals artificially with a material of known potency. Animals are then monitored closely for signs of disease and treated / euthanised to ensure symptoms remain within pre-determined limits.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>In order to get a marketing authorisation for an anti-parasitic, efficacy and safety data for that anti-parasitic must be provided to the regulator. European guidance documents stipulate that the target species of animal is used to produce this efficacy and safety data. Legal requirements and the use of non-animal alternatives will be kept under constant review.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Where there is a European guidance document detailing the requirements, we will comply with these. Guidance documents usually stipulate the design to be used and the minimum number of animals. Where there is no guidance document, we will take the advice of a statistician on the design and the minimum number of animals required to maximise the chance of achieving the desired result. The principles of good experimental technique will be implemented, and will include sourcing even / representative animals, allocating treatments randomly, blinding of study staff to treatments administered, accurate data collection and prompt checking / processing / analysis of data.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use</p>	<p>The animal species we propose to use are as dictated by European guidance documents. In most cases the adverse effects are likely to be</p>

<p>are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>minimal or mild. Where adverse effects are anticipated, animals will be monitored regularly to ensure that severity limits are not exceeded. Where severity limits might be exceeded, we will intervene to treat the animal.</p> <p>Animal husbandry is well above commercial standards, with animals kept in smaller numbers, monitored very closely by experienced stock people and with frequent inspection by veterinary surgeons. Animal accommodation is substantially better than Defra code of recommendations for the welfare of livestock and is compliant with A(SP)A codes of practice. Each individual study is reviewed ethically before commencement, paying regard to the methods proposed.</p> <p>Where possible animals will be penned in groups of similar animals. Where individual penning is required, animals will be within sight and sound of compatriots. Environmental enrichments will be provided where possible and these are tailored to the requirements of the species. Dust baths, perches, nest boxes, toys and auditory stimuli (radio) will be provided for poultry. Toys will be provided for pigs and nesting material for farrowing pigs. A variety of enrichments will be provided for rabbits, including multi-levels, tunnels, toys, chews, nest boxes and offset barriers.</p> <p>Animals are usually examined by a veterinary surgeon or experienced stock person on their farm of origin. There is usually an acclimatisation period to allow animals to become accustomed to their new surroundings prior to the commencement of procedures. Following acclimatisation, and before the start of regulated procedures, there is usually a further examination by a veterinary surgeon or experienced stock person to ensure that the animals are healthy and fit for study. Samples may be taken before the commencement of a study to check that the animals are healthy and within normal reference ranges i.e. blood chemistry and haematology.</p> <p>Animal husbandry is to a very high standard, by experienced staff following up to date guidelines and regulations. Prompt veterinary</p>
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	<p>attention is provided to ill animals, which are observed closely until resolved. For parasite challenge models, observations are at a frequency to try to ensure that animals do not progress beyond their severity limit. Interventions, treatment or euthanasia, will be as soon as study requirements are achieved and well in advance of severity limits.</p>
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Project	Safety and efficacy testing of veterinary products	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim of this licence is to conduct safety and efficacy testing in support of marketing authorisation applications for new veterinary products, or for revisions to authorisations for licensed products.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The successful licensing of new veterinary products will provide additional tools for veterinarians and animal care workers in the fight against pathogens on commercial livestock facilities and will result in reduced suffering for animals and reduced financial burden for farmers.	
What species and approximate	Over the 5 years of the licence we expect to	

<p>numbers of animals do you expect to use over what period of time?</p>	<p>carry out procedures in cattle, sheep, pigs, poultry, mice and possibly horses. The maximum number of animals over the five years of the licence is expected to be around 16,000 but may be considerably lower than this.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The products being tested under this licence are in the final stage of licensing and as such any adverse effects following use are likely to be minor (such as transient injection site reactions or pyrexia, a common side effect of vaccination). On occasions more severe adverse reactions may occur but this is extremely rare for products at this stage of testing (less than one animal in a 1000 would be expected to have a more extreme reaction to any product). For animals included on efficacy studies, untreated / challenged animals are expected to develop clinical disease during the study. The severity of the disease models used is such that only mild to moderate levels of disease are expected. The majority of the animals used on studies will be euthanased at the end (often they are euthanased to provide additional samples for testing as part of the protocol) since animals which are administered unlicensed products or pathogens, cannot be returned to the food chain. On some occasions it is however possible to return the animals to stock.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>At this time the regulations for approval of most veterinary products require the conduct of in-vivo studies to provide safety and efficacy data for inclusion in the regulatory submission. While in-vitro models are available for some procedures required in the licensing documentation (such as minimum inhibitory concentration - MIC, to provide information relating to efficacy of an antibiotic against pathogens), in-vivo studies are required to generate the vast majority of the information required for the portfolio. In some cases, in-vitro product data does not always correlate with in-vivo data, possibly due to complicating factors that are present in the host animal but</p>

	<p>not in the in-vitro system. Every effort will however be made to identify in-vitro testing that would replace in-vivo testing where acceptable to the regulatory authorities.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>During the design of studies, reduction in the number of animals used is always included in discussions to ensure that the minimum viable number of animals, sufficient to fulfil the objectives of the study, are selected. For safety studies, the number of animals to be used is generally fixed by the guidelines and there is little scope for revision. For efficacy studies, more flexibility is available and the number of animals to be used is generally confirmed by statisticians following review of the data, in order to produce a suitably powered experiment.</p> <p>Where possible a reduction in the number of control animals used is included in the study design (where statistical comparison with treated animals is not required and therefore matched numbers are not appropriate).</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The animals used in studies are of the age, breed, sex and species required for the product under test (and in line with regulations).</p> <p>The animal models are continually refined with a review of the outcome of all disease model studies being carried out on completion. This will include a statistical review of the outcome (where relevant) in order to determine whether in the future, it may be feasible for a reduced number of animals to be included in the study.</p> <p>A review of any issues raised during the study with regard to animal welfare and any changes made during the study to correct the issues are also carried out to ensure that the lessons learned are taken into consideration in future studies. This may require a further validation of the challenge model if clinical parameters do not meet specifications which may mean that further refinement may be necessary.</p>

Project	Safety Evaluation in Dogs and Pigs	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
	<input checked="" type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project contributes to the safety evaluation of new medicines, agrochemicals or other chemicals to which humans are likely to be exposed, by investigating their toxicology and metabolism in two non-rodent species (dogs and pigs).	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	New medicines have the potential to provide new or improved disease treatments; new chemicals/agrochemicals have the potential to increase or protect food production while minimising safety risks to consumers and/or adverse effects on the environment. Before potential new medicines are administered to humans, or new chemicals/agrochemicals used in	

	<p>the environment, their safety must be evaluated. This testing is a mandatory legal requirement and provides information on risks to patients and consumers. At present there are no alternatives to animal use that are scientifically, ethically or legally acceptable as replacements for systemic toxicity assessment.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Dogs 5400, Pigs 4700 over 5 years. These estimates are based on historical usage under previous projects with the same overall aims, and on anticipated trends in regulatory and scientific requirements for safety/toxicity data in non-rodents.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Animals are dosed by the intended/likely route of human exposure (for example oral administration, injection, infusion or inhalation), and observed regularly to monitor appearance, behaviour and clinical health. Some animals may undergo a surgical procedure under general anaesthesia, eg placement of a deep vein catheter for intravenous infusion, or implantation of a monitoring device or minipump. Investigative procedures carried out in these studies are similar to diagnostic procedures that might be used medically to monitor progress of a human patient and include, for example, collection of blood and urine samples for laboratory investigations, or ECG monitoring to assess heart rate/function, or examination of the eyes using an instrument similar to those used by opticians. Other more unusual tests might include assessment of retinal function, taking small samples of tissue under general anaesthesia, collection/examination of body fluids such as tear fluid or semen, collection under general anaesthesia and examination of lung washings or spinal fluid, body temperature by rectal thermometer. A minimal degree of restraint or confinement may be required for some procedures. Where appropriate, positive reinforcement training (using treat rewards) is used to encourage co-operation in (and minimise any stress of) handling/procedures. Environmental enrichments appropriate to the species, including provision of suitable bedding materials, toys, exercise and socialisation periods, are used within the animal facilities. Some animals may be used on procedure on more than</p>

	<p>one occasion (re-use); such re-use is limited and strict criteria are applied, eg veterinary examination indicates that it is appropriate to do so. Some animals (dogs only) may be re-homed via the establishment's rehoming scheme if it is in their best interests, but most animals are humanely killed at the end of the study to allow detailed examination of the organs. Most animals are expected to experience no, or only mild, adverse effects during the course of the study such as slight weight loss. A small percentage of animals may show more significant adverse effects, such as more marked weight loss, reduced activity, vomiting or tremors. No animals would be expected to die or to suffer prolonged adverse effects as a result of the procedures, and where necessary early humane end-points are applied, under veterinary guidance as necessary, to prevent this; such end-points might include interventions to discontinue dosing, or to provide supportive treatments, or if necessary to humanely kill the animal.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Testing pharmaceuticals, agrochemicals and chemicals to which humans are likely to be exposed is a mandatory legal requirement and provides information on the risks to people of such exposure. At present there are no alternatives to animal use that are scientifically, ethically or legally acceptable as replacements for systemic toxicity assessment.</p> <p>Non-animal testing methods and computer modelling are used in combination with animal studies to inform study designs and assist in understanding of potential toxicity but cannot yet replace studies in animals.</p> <p>We maintain a constant awareness of regulatory guidance and ensure that where non-animal methods exist which fulfil the regulatory requirement they are used in preference to animal studies.</p>
<p>2. Reduction</p> <p>Explain how you will assure the</p>	<p>Each study is designed to use the minimum number of animals necessary to achieve its objectives, drawing on scientific knowledge of the</p>

<p>use of minimum numbers of animals</p>	<p>test item to be evaluated and of the animal model, on statistical considerations and on recognised guidelines on regulatory study design. Where appropriate, additional investigations may be included in a study in order to avoid the need to conduct a separate study with more animals. In some cases, re-using animals in a second study instead of using a new batch of animals is possible, and may reduce the overall welfare cost as well as the number of animals used.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>There is a scientific and regulatory need for safety/toxicity data in non-rodent species such as dogs or pigs to supplement rodent data and enable a complete risk assessment. We use pigs in preference to dogs wherever possible; dogs are only used where necessary to achieve the study objectives, ie when the pig is unsuitable (for example due to species-specific differences from humans, confounding pharmacology or toxicological responses, or practical limitations due to anatomy or physiology).</p> <p>All procedures are subject to ongoing assessment and technique improvement and we participate in cross-company working parties on best practice. Animals are regularly reviewed for general health and veterinary staff are on call at all times to assess any adverse events and provide supportive care and treatment as appropriate.</p>

Project	Safety Pharmacology and Pharmacokinetics/Toxicokinetics in the Non-Human Primate	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project will allow for safety pharmacology and pharmacokinetic tests to be conducted in non-human primates (monkeys) on pharmaceuticals, bio-pharmaceuticals and biologic drugs for use in humans. It will also be required for associated research and development studies to, for example, gain comparative data and critically evaluate new test methods, equipment and techniques which may give better data. The project aims to make sure that drugs going into clinical trials in patients dont have any side effects on the heart or the circulation, and there is enough of the drug in a patients system to allow it to have its effect. These tests will be run to satisfy the International Committee for harmonisation (ICH) S7A and S7B guidelines (Cardiovascular/Respiratory safety and Potential effects on	

	<p>QT prolongation) and the S3A & S3B guidelines (Pharmacokinetics and Toxicokinetics). Both of these types of tests are required by the Governmental regulatory authorities around the World before drugs can be tested in humans.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The overall benefit of this project is that it supports the development of safe, new medicines to improve the health and quality of life of patients by generating high quality data that is acceptable to regulatory authorities and enables internal decision making (i.e. whether drugs are safe to be tested in humans). This can include data that actually prevents a drug actually ever reaching patients because its not safe. Drugs that have the potential to affect the rhythm of the heart are potentially fatal. Drugs that don't reach a patients system so that it can have its effect are also unlikely ever to become drugs. The tests carried out under this licence will enable safe candidate medicines to progress to the next stage of pharmaceutical development.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Expected usage would be in the region of 200 macaques It is difficult to accurately predict the number of animals that will be used, as this depends upon many factors. But we expect that these numbers will be enough based on previous work we have done. The licence will last for 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>On these studies, animals will be dosed with test materials (potential human pharmaceuticals) by a variety of different routes (including by injection and via inhalation by a special fitted mask) and undergo various tests to look at the effects of these drugs in the animals' system. This will include taking blood samples from the animals from a vein (as a patient would give a blood sample to test their blood) to see how much of these test materials are present for example. Or sometimes in animals that have been implanted with a device to allow the measurement of blood pressure, heart rate and ECG, to look at the effects of drugs on those parameters. These tests are key to help determine if potential new drugs are safe for first use in man. Most animals are expected to experience no adverse effects, or only mild effects such as slight weight loss. The doses used in these studies will be based on data gained in preliminary studies using small numbers of animals. A small percentage of animals may show more significant adverse effects indicating moderate severity, e.g. more marked weight loss or reduced activity. Animals will be carefully monitored for clinical signs or other effects on their health and well being, and in order to prevent unnecessary suffering, humane end-points are applied under appropriate veterinary guidance (e.g. treatment with the test substance will be stopped or supportive or therapeutic</p>

	<p>treatments will be given to help the animals recover). Animals in surgical studies may, as a result of the surgical procedure, experience some adverse effects similar to those that might be experienced by human patients; for example, in the case of cardiovascular telemetry studies, animals may experience post-operative pain/distress and possible infection. However, supportive treatments are given to eliminate or minimise these adverse effects, and all surgical procedures are performed under anaesthesia, with analgesia (i.e. pain-relieving medication) and antibiotics given both before and after surgery under the supervision of a vet. The care these animals will receive would be just like a patient would receive in hospital after a similar operation. Animals may be re-used if approved after a thorough examination by a veterinary clinician. If the animals are not approved for re-use by a veterinary clinician, they may be humanely killed at the end of a study. Using animals again is a good way of reducing the numbers we have to use in the first place.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>There are many validated non-animal or <i>in vitro</i> tests available to examine the effects of test substances on specific cellular processes. There are, however, as yet no non-animal tests or <i>in vitro</i> test systems available that can fully replicate the effects of substances on the complex biological interactions that occur between the various cells, tissues and organs that constitute a living organism. We would only use primates in these studies because the regulators demand we use more than one type of animal species (other than say a mouse or rat) to confirm that drugs are safe. Normally researchers would use something like a dog or a pig for this purpose, but sometimes this is not possible due to the specific type of drug that is being investigated, and the target it hits, which may not be present in dogs or pigs. Its only then we'd use primates, and only after there had been specific justification to do so.</p> <p>These tests are also mandatory for all drugs prior to use in humans (clinical trials and in clinical use in patients).</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>All experiments will be designed in order to achieve the scientific objectives (to see that drugs get into the system so they work, and checking that they dont affect the heart and circulation) using the minimum numbers of animals. Because our experiments are run to satisfy regulators that drugs are safe, and we have a lot of experience in performing them over many years, so we know what numbers give the right sort of data the regulators require. Statisticians will be</p>

	consulted to assist with group size and dose design.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Non-human primates will only be used when they are scientifically the only appropriate species for a particular study and no other species (like the pig or dog) or non-animal alternative is available or acceptable to achieve the objectives of the study.</p> <p>For the management of animals undergoing recovery surgery, an aseptic approach will be used. These surgeries will be carried out by veterinary surgeons. Appropriate pain relief will be provided as required. Surgical procedures will be carried out in accordance with the principles set out in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017). This is the same sort of care that a patient in hospital would receive when undergoing an operation.</p> <p>Because primates have been employed in biomedical research over many years, there is a large pool of background knowledge which can be tapped into the advantage of both animals (in terms of welfare and environmental refinements) and to the researcher (in terms of the quality of the scientific data which results from refinement of technique). For each surgical procedure type, appropriate pain relief and balanced anaesthesia will be provided as required in accordance with a regime agreed in advance with veterinary clinicians. Again this is very similar to what would happen to a patient in hospital for an operation.</p> <p>In addition, care is taken to provide as much environmental enrichment as possible, and working parties that often test and introduce environmental enrichment e.g. introduction of specific between feed snacks for primates based on their preferences.</p> <p>All our staff carrying out these studies are fully trained and competent to do this work, otherwise they are not allowed to perform the experiments. They follow standard techniques which have been refined over many years of experience.</p> <p>Dosing and sampling procedures will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than short term discomfort and no lasting harm and will be the minimum consistent with the aims of the studies. This will often be similar to an injection you may receive at the doctors or in hospital, or maybe like having a blood sample taken in a doctors surgery. In addition, any suffering will be further minimised by implementing clearly defined humane endpoints. So if anything happens to adversely affect the animals they will be</p>

	humanely killed whether the experiment is finished or not.
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Project	Safety Pharmacology
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input checked="" type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>To fulfil regulatory requirements for new human drug submissions in the field of safety pharmacology</p> <p>To identify undesirable properties of test item that may have relevance for their human safety</p> <p>To investigate and further evaluate adverse effects observed and/or suspected</p>
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The principal benefit of the project is to assist in the assurance of human safety by conduct of relevant tests in animals, as described by current regulatory guidance in Europe and in other international markets. The successful conduct of tests will help bring to market test items which

	<p>are safe, and shown to be effective in the treatment or prevention of human diseases. Without these studies, progression of new medicines to early human studies and to patients could not occur in the current regulatory framework. Early cessation of programmes of work with unsuitable test items will reduce overall animal use in a drug development programme. Validation and refinement of test methods may be completed for specific techniques, and may be published to the wider scientific community.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Within the five year life of the project it is estimated that the following may be used: • 1500 rats or mice • 60 dogs • 15 pigs • 20 macaques</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The regulatory need is to demonstrate any relationship between the dose level of a test item and any adverse effect observed, include its onset and duration post-dosing. In order to do this, it is expected that one or more dose levels of test items will result in measurable adverse effects for animals such as increased breathing, heart rate, blood pressure or general activity. Potentially a toxic effect may be seen as shown by, for example, decreased activity or food consumption. Non-rodents may be surgically prepared with devices to enable collection of data on measures such as heart rate and blood pressure without any form of capture or restraint. Data collected in this way are more representative of the animals' true condition than if restrained, but animals may experience a degree of discomfort from surgery, which is minimised by appropriate veterinary care. Rodents will typically be humanely killed at the conclusion of a test. Non-rodents will typically be assessed by a veterinary surgeon for lack of significant or lasting effects, and may be re-used in multiple studies.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-</p>	<p>Current regulatory guidelines indicate the need for conduct of both non-animal methods and those which use animals, in this field of</p>

animal alternatives	regulatory work.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Requests for animal studies are reviewed by scientific staff to ensure that such work is required before it is agreed.</p> <p>Previous work has been conducted and published, to assess the statistical power of group sizes required for the various study types in the project.</p> <p>Control groups are commonly included to ensure that studies are robust and do not need to be repeated. In some cases, a single control group can be used for two or more test items, reducing overall animal use. Studies with non-rodents commonly use a design where each animal acts as its own control, by measuring effects in sequence, at different dose levels, and with no test item at all, in the same animal.</p> <p>Re-use of animals is employed in the project as a means to reduce overall animal use. This is particularly relevant where animals are surgically prepared; use of the same animal in multiple studies following initial surgical preparation reduces the total number of animals which are subject to surgery.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The regulatory guidance on safety pharmacology establishes the important body systems which must be assessed for human safety, due to their importance in life-supporting functions. Studies are designed to assess effects on these body systems; principally the heart, lung and brain functions.</p> <p>Rodents can be used for much of this work, but are not appropriate for some assessments of heart function, as they do not compare well with human risk in this area. Use of non-rodents including dogs, pigs and primates does allow a better assessment of human risk, enables comparison between different test items in the same animals by re-use of the animals, and reduces overall animal use as noted above.</p> <p>Non-human primates are used where the particular type of test items requires such species as the best option to assess human risk, due to</p>

	<p>the similarity of humans to non-human primates.</p> <p>Staff training and support documents allow identification of adverse effects if and when they develop.</p> <p>Prior knowledge may assist in development of appropriate animals monitoring schedules for specific test items. Additional assessments are included where such need is demonstrated by effects, as standard practice.</p> <p>Careful selection and escalation of dose levels will be used to ensure that adverse effects can be identified but should not cause life-threatening toxicity. Effects may be seen as described above, but will commonly be only for a short period; persistent moderate clinical signs will result in cessation of further testing at the relevant or higher dose levels.</p> <p>Where surgery is conducted it is done so subject to all relevant veterinary assistance in terms of use of sterile materials, provision of appropriate anaesthetics and suitable pain relief schedules</p>
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Project	Safety Testing of Medicinal Products Using Dogs and Minipigs	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	4 Years 8 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project licence authorises the conduct of studies in laboratory dogs and minipigs to evaluate the safety, quality and effectiveness of medicinal products for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening conditions in man, in terms of general toxicity and whole body system exposure.</p> <p>The expansion of scientific and medical knowledge has led to the development of drugs which can treat or alleviate the symptoms of many illnesses but there is still a need to</p>	

	<p>develop medicinal products to diagnose and treat many human conditions such as Heart Disease, Stroke, Obesity, COPD, Respiratory Infections, Cancer, Autoimmune diseases, Diabetes, Alzheimer's and Schizophrenia, amongst others. When these needs are combined with the growing threat from antibiotic resistant bacteria the advancement of science and the development of new products are as important as ever.</p> <p>The primary aims of this project are to support the development of these medicinal products through acquisition of data to; 1) Support selection of new candidate molecules for further evaluation and development. 2) Demonstrate the safety-hazard profile of a new medicinal product prior to the initiation of clinical trials involving humans. 3) Demonstrate the hazard profile of a medicinal product, in order to meet the regulatory requirements for marketing authorisation. Further aims include validation of new experimental conditions including the collection of blood/tissues to support drug development and the validation of non-animal alternative methodology.</p> <p>As a specially protected species, the dog is selected for safety assessment studies only after careful determination that it is the most biologically appropriate species with relevance to man, and that there is no other acceptable candidate species of lower neurophysiological sensitivity/status.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>People are exposed to medicinal products as patients, medical professionals, carers and during their manufacture. The products can be biological or non-biological materials and include diagnostic agents or substances associated with drug candidates e.g. metabolites, impurities and drug degradants. The principal benefit of this project is the provision of robust safety data to facilitate sound decisions by national and international Regulatory Agencies regarding human exposure to medicinal products. Without these studies, progression of new medicines to early human studies and to patients could not occur safely or in the current regulatory framework.</p>

	Validation and refinement of test methods may be completed for specific techniques and may be published to the wider scientific community.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year life of this Project Licence, it is estimated that 4,800 dogs and 2,300 minipigs will be used. These numbers include a small proportion of re-use of the same animals.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals used under this licence will be given the test substances in a similar way to that in which they are expected to be given to humans. As most medicines are taken orally the majority of animals will receive the test material directly by insertion of a flexible rubber catheter into the stomach via the mouth. Most animals are treated in this way once per day daily, although studies may occasionally require two or three doses within 24 hours. For some test materials the oral route of administration may not be appropriate; for example, it may be broken down, not tolerated or not absorbed during the digestive process. In these situations, alternative administration routes such as sub-cutaneous or intravenous injection may be more appropriate. Animals may be manually restrained for "bolus" administrations lasting a few seconds or minutes, or they may be habituated to sit in specially designed slings for longer intravenous administrations. For test materials which will be administered to humans intravenously over extended period of time due to their inherent toxicity or fast clearance from the body it may be more appropriate to surgically implant a permanent catheter (with appropriate anaesthesia and analgesia) to allow the animal free movement whilst being dosed in their home enclosure. Test materials intended to treat a localised condition may be administered to that body part or area for example to the skin. Blood and urine samples may be taken to measure the level of test material or its metabolites within an animal's circulatory system. These may also be analysed to detect any effects on body systems and organs for example liver or kidney function. Study animals are closely observed several times a day by highly trained technologists who monitor for any signs of discomfort. Other

	<p>measures such as food consumption and bodyweight are used to closely monitor for treatment related effects. Veterinary surgeons are employed on a full time basis and are available 24/7 to provide clinical treatment, guidance on animal welfare and the conduct of procedures including appropriate surgical technique, anaesthesia and analgesia. The majority of animals are expected to have mild adverse effects of treatment such as reduced weight gain or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more moderate adverse effects. The nature and type of effect varies dependant on the biological systems affected, however, these usually result in findings such as reduced food consumption, weight loss and changes in behaviour such as reduced activity. Humane endpoints will be applied or dose levels reduced if animals show excessive effects. Longer term studies are expected to have progressively fewer adverse effects. Many toxicological effects of the test material are not evident during the in-life phase of a study and do not impact the animals wellbeing. Only through microscopic examination of the tissues from each animal, can evidence of all toxicological changes be fully assessed and the scientific value of each animal maximised. In order to undertake these evaluations the animals must be put to sleep humanely at the end of a study, under terminal anaesthesia. Animals that do not need to be euthanased at the end of a study and have experienced no more than moderate effects and have met the requirements for keeping alive may be reused or rehomed under AWERB oversight, in line with ASPA/HO requirements. In the case of rehoming, animals will undergo a programme of training and socialisation to enable transition to a domestic environment. Potential adoptees will also be assessed for their suitability to provide for the animal's needs.</p>
Application of the 3Rs	
1. Replacement	There are currently no scientific and legally acceptable evaluations of systemic toxicity that

<p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>will satisfy regulatory requirements and provide sufficient safety data other than use of animals, though validated <i>in vitro</i> tests for specific organs are used wherever possible. As new <i>in vitro</i> methods become available and achieve regulatory acceptance during the course of this project they will be validated and used to replace <i>in vivo</i> procedures. Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.</p> <p>As a specially protected species, the dog is selected for safety assessment studies only after careful determination that it is the most biologically appropriate species with relevance to man, and that there is no other acceptable non-specially protected candidate species.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives, and regulatory guidelines. Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies.</p> <p>Where available, sensitive analytical techniques may be used to reduce animal numbers.</p> <p>In general, toxicity studies are initiated in rodents before progressing into larger animals. This approach, combined with background literature searches and looking across at other study types, can lead to earlier decisions on whether or not to continue development of a particular test material, refinement of study designs and reduced use of dogs and minipigs.</p> <p>In recent years, the general availability and use of minipigs has also increased, with an associated reduction in the proportional use of dogs.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use</p>	<p>Species choice and use of specific animal models is determined by the need to generate regulatory acceptable data. Where a choice of species is possible, care is taken to select the</p>

<p>are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>most biologically appropriate species, and the species which most closely relates to man.</p> <p>Animal studies are only considered where there is a direct legislative or regulatory requirement and after review of all other available information to ensure that no alternative is feasible. A step-wise approach is taken, with higher risk studies, when little may be known about the test material, being performed early in a programme and using only small numbers of animals. As confidence in the data and the level of information grows, longer term studies in larger numbers of animals may become necessary and the design of these studies, whilst adhering to regulatory requirements, can be refined and tailored to obtain the most relevant and valid scientific information from the fewest animals and with the lowest level of adverse effects possible.</p> <p>Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints.</p> <p>Socially compatible species are routinely group housed with environmental enrichment which encourages species specific behaviours without adversely impacting study outcomes.</p> <p>Individual studies are designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare. Any confinement or restraint is restricted to the minimum required to achieve the scientific objectives of the study and all study plans/protocols are reviewed for adherence to welfare guidelines and best practices by the site's Animal Welfare and Ethical Review Body (AWERB).</p>
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Project	Safety Testing of Medicinal Products Using Non-Human Primates	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	4 Years 8 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project licence authorises the conduct of studies in laboratory non-human primates to evaluate the safety, quality and effectiveness of medicinal products for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening conditions in man, in terms of general toxicity and whole body system exposure.</p> <p>The expansion of scientific and medical knowledge has led to the development of drugs which can treat or alleviate the symptoms of many illnesses but there is still a need to develop medicinal products to diagnose and</p>	

treat many human conditions such as Heart Disease, Stroke, Obesity, COPD, Respiratory Infections, Cancer, Autoimmune diseases, Diabetes, Alzheimer's and Schizophrenia, amongst others. When these needs are combined with the growing threat from antibiotic resistant bacteria the advancement of science and the development of new products are as important as ever.

The primary aims of this project are to support the development of these medicinal products through acquisition of data to 1) Support selection of new candidate molecules for further evaluation and development. 2) Demonstrate the safety-hazard profile of a new medicinal product prior to the initiation of clinical trials involving humans 3) Demonstrate the hazard profile of a medicinal product, in order to meet the regulatory requirements for marketing authorisation. Further aims include validation of new experimental conditions including the collection of blood/tissues to support drug development and the validation of non-animal alternative methodology.

As a specially protected species, the non-human primate is selected for safety assessment studies only after careful determination that it is the most biologically appropriate species, and that there is no other acceptable candidate species. This is usually based upon the test material's mechanism of action, target systems/receptor profile etc and an assessment of the appropriateness of the primate model in general. This is typically achieved via the availability of data (e.g. *in vitro* metabolism, early pharmacokinetics, or other supporting information) demonstrating that the test material or metabolite is effective in primates, that primates are the most relevant model to man, and that the purpose of the programme of work cannot be achieved by the use of animals that are not primates. A record of the scientific rationale for the use of primates is always retained.

With respect to the high specificity of large molecule biotherapeutics (such as monoclonal antibodies and antibody-drugs conjugates) to the human target, non-human primates are

	<p>often the only species exhibiting binding of the target and the desired pharmacological effect, and therefore, toxicology studies are most frequently performed in this single species.</p> <p>Thus, the use of primates in carefully selected studies is an essential requirement in the successful development of new medicinal products.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>People are exposed to medicinal products as patients, medical professionals, carers and during their manufacture. The products can be biological or non-biological materials and include diagnostic agents or substances associated with drug candidates e.g. metabolites, impurities and drug degradants. The principal benefit of this project is the provision of robust safety data to facilitate sound decisions by national and international Regulatory Agencies regarding human exposure to medicinal products. Without these studies, progression of new medicines to early human studies and to patients could not occur safely or in the current regulatory framework. With the increasing use of advanced drug technologies, targeting the immune system to combat life-threatening and debilitating illnesses like cancer and autoimmune diseases, the non-human primate is often the only suitable test species due to similarity of the immune system. Validation and refinement of test methods may be completed for specific techniques and may be published to the wider scientific community.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Over the 5 year life of this Project Licence, it is estimated that 4900 non-human primates will be used (980/year).</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The animals used under this licence will be given the test substances in a similar way to that in which they are expected to be given to humans. As most medicines are taken orally the majority of animals will receive the test material directly by insertion of a flexible rubber catheter into the stomach via the mouth. Most animals are treated in this way once per day daily, although studies may occasionally require two</p>

or three doses within 24 hours. For some test materials the oral route of administration may not be appropriate; for example, it may be broken down, not tolerated or not absorbed during the digestive process. In these situations, alternative administration routes such as sub-cutaneous or intravenous injection may be more appropriate. Animals may be manually restrained for “bolus” administrations lasting a few seconds or minutes, or they may be habituated to sit in specially designed chairs for longer intravenous administrations. For test materials which will be administered to humans intravenously over extended period of time due to their inherent toxicity or fast clearance from the body it may be more appropriate to surgically implant a permanent catheter (with appropriate anaesthesia and analgesia) to allow the animal free movement whilst being dosed in their home enclosure. Test materials intended to treat a localised condition may be administered to that body part or area for example to the skin. Blood and urine samples may be taken to measure the level of the test material or its metabolites to which the animal is exposed. These may also be analysed to detect any effects on body systems and organs for example liver or kidney function. Study animals are closely observed several times a day by highly trained technologists who monitor for any signs of discomfort. Other measures such as food consumption and bodyweight are used to closely monitor for treatment related effects. Veterinary surgeons are employed on a full time basis and are available 24/7 to provide clinical treatment, guidance on animal welfare and the conduct of procedures including appropriate surgical technique, anaesthesia and analgesia. The majority of animals are expected to have mild adverse effects of treatment such as reduced weight gain or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more moderate adverse effects. The nature and type of effect varies dependant on the biological systems affected, however, these usually result in findings such as reduced food consumption, weight loss and changes in behaviour such as reduced activity. Humane

	<p>endpoints will be applied or dose levels reduced if animals show excessive effects. Longer term studies are expected to have progressively fewer adverse effects. Many toxicological effects of the test material are not evident during the in-life phase of a study and do not impact the animals wellbeing. Only through microscopic examination of the tissues from each animal, can evidence of all toxicological changes be fully assessed and the scientific value of each animal maximised. In order to undertake these evaluations the animals must be put to sleep humanely at the end of a study, under terminal anaesthesia.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>There are currently no scientific and legally acceptable evaluations of systemic toxicity which will satisfy regulatory requirements and provide sufficient safety data other than use of animals, though validated <i>in vitro</i> tests for specific organs are used wherever possible. As new <i>in vitro</i> methods become available and achieve regulatory acceptance during the course of this project they will be validated and used to replace <i>in vivo</i> procedures. Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.</p> <p>As a specially protected species, the non-human primate is selected for safety assessment studies only after careful determination that it is the most biologically appropriate species with relevance to man, and that there is no other acceptable candidate species that is not a primate.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives, and regulatory guidelines. Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies.</p> <p>Where available, sensitive analytical</p>

	<p>techniques may be used to reduce animal numbers.</p> <p>In general, toxicity studies are initiated in rodents before progressing into larger animals. This approach, combined with background literature searches and looking across at other study types, can lead to earlier decisions on whether or not to continue development of a particular test material, refinement of study designs and reduced use of primates.</p> <p>The number of small molecule new chemical entity drugs (NCEs) developed using non-human primates has declined in recent years but is being offset by the proliferation of large molecule biotherapeutics (such as monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs)). The non-human primate is often the only relevant model for testing such materials due to the similarity with the human immune system but a reduction in the numbers of animals used per study is being achieved through knowledge gathering and refinement of study designs at an industry/regulatory level.</p> <p>As most studies involve the post-mortem examination of tissues following treatment, opportunities for re-use are limited. Nevertheless, this licence does include the potential to re-use animals, in compliance with Home Office guidance and, where possible, this is intended to help reduce the overall number of primates used at this facility.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Species choice and use of specific animal models is determined by the need to generate data that satisfies worldwide regulatory authorities. Where a choice of species is possible, care is taken to select the most biologically appropriate species, and the species which most closely relates to man.</p> <p>Animal studies are only considered where there is a direct legislative or regulatory requirement and after review of all other available information to ensure that no alternative is feasible. A step-wise approach is taken, with higher risk studies, when little may be known about the test material, being performed early in a programme and using only small numbers</p>

of animals. As confidence in the data and the level of information grows, longer term studies in larger numbers of animals may become necessary and the design of these studies, whilst adhering to regulatory requirements, can be refined and tailored to obtain the most relevant and valid scientific information from the fewest animals and with the lowest level of adverse effects possible.

Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints.

Non-human primates are routinely group housed with environmental enrichment which encourages species specific behaviours without adversely impacting study outcomes.

Individual studies are designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare. Any confinement or restraint is restricted to the minimum required to achieve the scientific objectives of the study and all study plans/protocols are reviewed for adherence to welfare guidelines and best practices by the site's Animal Welfare and Ethical Review Body (AWERB).

Project	Safety Testing of Medicinal Products Using Small Animal Species	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The expansion of scientific and medical and knowledge has led to the development of drugs which can treat or alleviate the symptoms of many illnesses. Whilst progress has been made there is still a need to develop medicinal products to diagnose and treat many human conditions such as Cancer, Ischaemic Heart Disease, Sepsis, Stroke and Alzhiemer's disease. When these needs are combined with the growing threat from antibiotic resistant bacteria the advancement of science and the development of new products are as important as ever. This project licence authorises the conduct of studies in laboratory small animal	

	<p>species to evaluate the hazard profile of pharmaceuticals in terms of general toxicity and potential life time exposure.</p> <p>The primary aims of this project are to support the development of these new medicinal products through acquisition of data to 1) Support selection of new candidate molecules for further evaluation and development. 2) Demonstrate the safety-hazard profile of a new medicinal product prior to the initiation of clinical trials involving humans 3) Demonstrate the hazard profile of a medicinal product, in order to meet the regulatory requirements for marketing authorisation. Further aims include validation of new experimental conditions including the collection of tissues from surplus stock animals to support drug development and the validation of non-animal alternative methodology.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>People are exposed to medicinal products as patients, medical professionals, carers and during their manufacture. The products can be biological or non-biological materials and include diagnostic agents or substances associated with drug candidates eg metabolites, impurities and drug degradants. The principal benefit of this project is the generation of safety data to allow regulatory decisions regarding human exposure. Without these studies, progression of new medicines to early human studies and to patients could not occur safely. Validation and refinement of test methods may be completed for specific techniques and may be published to the wider scientific community.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Over the 5 year life of this Project Licence, it is estimated that 55,900 mice, 86,200 rats, 8,000 hamsters and 2,960 rabbits will be used.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Animals will be given the “test material” under investigation in a way which mimics the intended human exposure. As most therapies at taken orally the majority of animals will receive the test material either mixed in their food or directly by insertion of a flexible rubber catheter in to the oesophagus. Most animals</p>

are treated daily in this way, occasionally studies may require two or three doses within 24 hours. For some test materials the oral route of administration may not be appropriate for example it may be broken down, not tolerated or not absorbed during the digestive process. In these situations, alternative administration routes such as sub-cutaneous or intravenous injection may be more appropriate. For test materials which will be administered to humans intravenously over extended period of time due to their inherent toxicity or fast clearance from the body it may be more appropriate to surgically implant a permanent catheter (with appropriate anaesthesia and analgesia) to allow the animal free movement whilst being dosed in their home enclosure. Test materials intended to treat a localised condition may be administered to that body part or area for example to the skin. Blood and urine samples may be taken to measure the level of test material or its metabolites within an animal's circulatory system. These may also be analysed to detect any effects on body systems and organs for example liver or kidney function. Study animals are closely observed several times a day by highly trained technologists who monitor for any signs of discomfort. Other measures such as food consumption and bodyweight are used to closely monitor for treatment related effects. Veterinary surgeons are employed on a full time basis and are available 24/7 to provide clinical treatment, guidance on animal welfare and the conduct of procedures including appropriate surgical technique, anaesthesia and analgesia. The majority of animals are expected to have mild adverse effects of treatment such as reduced weight gain or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more moderate adverse effects. The nature and type of effect varies dependant on the biological systems affected, however, these usually result in findings such as reduced food consumption, weight loss and changes in behaviour such as reduced activity. Humane endpoints will be adopted or dose

	<p>levels reduced if animals show excessive effects. Longer term studies are expected to have progressively less adverse effects. Many toxicological effects of the test material are not evident during the in-life phase of a study and do not impact the animals wellbeing. Only through microscopic examination of the tissues from each animal, can evidence of all toxicological changes be fully assessed and the scientific value of each animal maximised. In order to undertake these evaluations the animals must be put to sleep humanely at the end of a study, under terminal anaesthesia.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>At present there are no scientific and legally acceptable evaluations of systemic toxicity which will satisfy regulatory requirements and provide sufficient safety data other than use of animals. Validated <i>in vitro</i> tests for specific organs are available and used to replace or refine procedures wherever possible. As new <i>in vitro</i> methods become available and achieve regulatory acceptance during the course of this project they will be validated and used to replace <i>in vivo</i> procedures. Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives, and regulatory guidelines. Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies.</p> <p>Where available, sensitive analytical techniques may be used to reduce animal numbers.</p> <p>Wherever practicable, and by looking across studies, the combination of endpoints eg general toxicity, reproduction and developmental toxicity, safety pharmacology, mutagenicity etc in studies is considered, to</p>

	<p>reduce overall animal usage.</p> <p>As most studies involve the examination of tissues following treatment opportunities for re-use are very limited. Tissues are collected to support drug and in vivo developments from any surplus stock animals.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Species choice and use of specific animal models is determined by the need to generate regulatory acceptable data. Where a choice of species is possible, care is taken to select the most biologically appropriate species, and the species which most closely relates to man. Typically studies are performed on small animal species before testing progresses to larger animals such as dogs, minipigs and primates.</p> <p>Generally the rat is the rodent species of choice in safety assessment. There is wide knowledge of the response of rats to various substances and a wealth of background literature. Rats are large enough to provide repeated blood samples, thus requiring significantly fewer rats than mice to achieve the same objective. Mice (or hamsters) may be used when considered a more appropriate species, for example, if they more readily absorb the test material, are more relevant biologically or improved tolerance depending upon objective of the study.</p> <p>Rabbits may be used when considered a more appropriate species, for example non-pregnant range finding studies prior to conducting reproductive toxicology studies in pregnant rabbits; local tolerance or vaccine development studies as the actual intramuscular or subcutaneous human dose volume can be administered</p> <p>Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints.</p> <p>Socially compatible species are routinely group housed with environmental enrichment which encourages species specific behaviours</p>

	<p>without not adversely impacting study outcomes. Occasionally it may be necessary to single house animals for example to collect urine samples of for the administration of test substances. All such occurrences are conducted in accordance with project licence limitations and under the oversight of the local Animal Welfare and Ethical Review Body.</p> <p>Individual studies are designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare. Any confinement or restraint is restricted to the minimum required, under guidance issued by the site's Animal Welfare and Ethical Review Body (AWERB).</p>
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Project	Salmonella Virulence
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>In this project we will study mechanisms by which <i>Salmonella</i> causes disease and how our immune system works to resist this. Infection of humans with <i>Salmonella</i> can lead to gastroenteritis and typhoid fever, depending on the strain type. It is estimated that over 90 million cases of <i>Salmonella</i> gastroenteritis and 13 million cases of typhoid fever (with approximately 130,000 deaths) occur globally each year. There is no vaccine against <i>Salmonella</i> that cause gastroenteritis and current typhoid vaccines do not work well for everybody.</p> <p>Infection of humans with <i>Salmonella</i> Typhimurium usually results in self-limiting gastroenteritis but this strain causes a systemic</p>

	<p>disease in mice that is similar to human typhoid fever. This very useful model system has been exploited intensively over the years and has provided a great deal of information of the infection process, the basis of host defence and immunity, and bacterial virulence factors involved. Much of this information is known to be relevant to human disease and has been exploited in the design of a novel vaccine against typhoid fever, which has been shown to be safe in clinical trials in humans.</p> <p>A large part of <i>Salmonella</i> ability to cause disease is associated with its ability to grow inside host cells, and we make extensive use of cells to study the biochemistry and cell biology of infection (approx. 75% of our work). However, these systems can never fully represent the complex environment and host response to infection that occurs in an entire organism such as patients or mice. For example, an essential aspect of the host immune response involves communication between different cell types and their activation. These interactions and responses are highly coordinated in time and space, involve cellular migration and occur both within and between different tissues. Therefore, some work on living mice is unavoidable to properly assess potential virulence defects, bacterial population dynamics and spread throughout organs as well as immune and other cellular responses. We will study the effects of deleting genes of <i>Salmonella</i> with respect to its ability to multiply and spread in mouse tissues. We will study the immune responses of mice to these strains, taking advantage of mouse strains that are already available and which have known immune defects. Animals get sick and lose weight after inoculation with <i>Salmonella</i>, but their suffering will be minimised by close monitoring so that action will be taken to relieve their suffering. The use of mixed infections eliminates mouse-to mouse variability and hence the number of animals required to achieve statistical significance.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or</p>	<p>Through this research we are likely to discover new processes of pathogen and host cell biology, which could have implications for other</p>

<p>humans or animals could benefit from the project)?</p>	<p>important pathogens that propagate within our cells. Our work is also likely to provide valuable information for designing vaccines, which are still needed to provide effective long-term protection against <i>Salmonella</i> and other bacterial pathogens.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Approximately 6000 mice will be used over the 5 year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Depending on the dose, the inoculation route and the strain of mouse, between 2 and 180 days after inoculation the majority of animals inoculated with <i>Salmonella</i> will develop mild to moderate symptoms of infection. When the animals get sick, they will be closely monitored for specific symptoms and if they don't recover from these symptoms over one day or lose up to 20% of their body weight, measures will be taken to relieve their suffering. At the end of the experiment, mice will be humanely killed.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Our aim is to understand how <i>Salmonella</i> (a bacterial pathogen which causes a wide variety of diseases in humans and other animals) cause disease and to learn more about host processes that influence the outcome of infection. An essential aspect of this work involves testing the harm potential of different bacterial strains in the well-established mouse model of infection. This helps us to establish the importance of genes that play critical roles during infection and provides information about their interactions and possible use in vaccine design. Much of our work involves experiments in which <i>Salmonella</i> grows in in-vitro models of cells grown in the lab. However, these systems can never fully represent the complex environment and host response to infection that occurs in an entire organism such as patients or mice. For example, an essential aspect of the host immune response involves communication between different cell types and their activation. These interactions and responses are highly coordinated in time and</p>

	<p>space, involve cellular migration and occur both within and between different tissues. Therefore, some work on living mice is unavoidable to properly assess potential virulence defects, and immune and other cellular responses.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>All experiments will be designed so that the minimum number of animals necessary will be used. Breeding strategies are performed by qualified personnel and aimed to avoid unnecessary animal generation (animal surplus). An experiment has been designed to evaluate the virulence of mutants in which a 1 to 1 mixture of wild type to mutant bacteria is inoculated into each animal. Since the strains are always compared in the same animal, this approach eliminates mouse-to mouse variability and hence significant differences in the virulence of strains can be obtained using fewer animals.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the most appropriate species in which to model systemic infection by <i>Salmonella</i>. The mouse model is especially appropriate as it enables (1) measurement of bacterial virulence by counting the number of surviving bacteria at different time points and from different organs following inoculation by the oral, abdominal cavity or intravenous routes, (2) how the infecting population migrates and behaves overtime in the animal to be analysed by new methodologies involving mathematical modelling. Animal suffering will be minimised by regular checking of animals for relevant symptoms that constitute the end point of the experiment, and the use of mixed infections to eliminate mouse-to mouse variability and hence the number of animals required to achieve statistical significance.</p>

Project	Salmonella Virulence
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>In this project we will study mechanisms by which <i>Salmonella</i> causes disease and how our immune system works to resist this. Infection of humans with <i>Salmonella</i> can lead to gastroenteritis and typhoid fever, depending on the strain type. It is estimated that over 90 million cases of <i>Salmonella</i> gastroenteritis and 13 million cases of typhoid fever (with approximately 130,000 deaths) occur globally each year. There is no vaccine against <i>Salmonella</i> that cause gastroenteritis and current typhoid vaccines do not work well for everybody.</p> <p>Infection of humans with <i>Salmonella</i> Typhimurium usually results in self-limiting gastroenteritis but this strain causes a systemic</p>

	<p>disease in mice that is similar to human typhoid fever. This very useful model system has been exploited intensively over the years and has provided a great deal of information of the infection process, the basis of host defence and immunity, and bacterial virulence factors involved. Much of this information is known to be relevant to human disease and has been exploited in the design of a novel vaccine against typhoid fever, which has been shown to be safe in clinical trials in humans.</p> <p>A large part of <i>Salmonella</i> ability to cause disease is associated with its ability to grow inside host cells, and we make extensive use of cells to study the biochemistry and cell biology of infection (approx. 75% of our work). However, these systems can never fully represent the complex environment and host response to infection that occurs in an entire organism such as patients or mice. For example, an essential aspect of the host immune response involves communication between different cell types and their activation. These interactions and responses are highly coordinated in time and space, involve cellular migration and occur both within and between different tissues. Therefore, some work on living mice is unavoidable to properly assess potential virulence defects, bacterial population dynamics and spread throughout organs as well as immune and other cellular responses. We will study the effects of deleting genes of <i>Salmonella</i> with respect to its ability to multiply and spread in mouse tissues. We will study the immune responses of mice to these strains, taking advantage of mouse strains that are already available and which have known immune defects. Animals get sick and lose weight after inoculation with <i>Salmonella</i>, but their suffering will be minimised by close monitoring so that action will be taken to relieve their suffering. The use of mixed infections eliminates mouse-to mouse variability and hence the number of animals required to achieve statistical significance.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or</p>	<p>Through this research we are likely to discover new processes of pathogen and host cell biology, which could have implications for other</p>

humans or animals could benefit from the project)?	important pathogens that propagate within our cells. Our work is also likely to provide valuable information for designing vaccines, which are still needed to provide effective long-term protection against Salmonella and other bacterial pathogens.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 6000 mice will be used over the 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Depending on the dose, the inoculation route and the strain of mouse, between 2 and 180 days after inoculation the majority of animals inoculated with Salmonella will develop mild to moderate symptoms of infection. When the animals get sick, they will be closely monitored for specific symptoms and if they don't recover from these symptoms over one day or lose up to 20% of their body weight, measures will be taken to relieve their suffering. At the end of the experiment, mice will be humanely killed.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Our aim is to understand how <i>Salmonella</i> (a bacterial pathogen which causes a wide variety of diseases in humans and other animals) cause disease and to learn more about host processes that influence the outcome of infection. An essential aspect of this work involves testing the harm potential of different bacterial strains in the well-established mouse model of infection. This helps us to establish the importance of genes that play critical roles during infection and provides information about their interactions and possible use in vaccine design. Much of our work involves experiments in which <i>Salmonella</i> grows in in-vitro models of cells grown in the lab. However, these systems can never fully represent the complex environment and host response to infection that occurs in an entire organism such as patients or mice. For example, an essential aspect of the host immune response involves communication between different cell types and their activation. These interactions and responses are highly coordinated in time and</p>

	<p>space, involve cellular migration and occur both within and between different tissues. Therefore, some work on living mice is unavoidable to properly assess potential virulence defects, and immune and other cellular responses.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>All experiments will be designed so that the minimum number of animals necessary will be used. Breeding strategies are performed by qualified personnel and aimed to avoid unnecessary animal generation (animal surplus). An experiment has been designed to evaluate the virulence of mutants in which a 1 to 1 mixture of wild type to mutant bacteria is inoculated into each animal. Since the strains are always compared in the same animal, this approach eliminates mouse-to mouse variability and hence significant differences in the virulence of strains can be obtained using fewer animals.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the most appropriate species in which to model systemic infection by <i>Salmonella</i>. The mouse model is especially appropriate as it enables (1) measurement of bacterial virulence by counting the number of surviving bacteria at different time points and from different organs following inoculation by the oral, abdominal cavity or intravenous routes, (2) how the infecting population migrates and behaves overtime in the animal to be analysed by new methodologies involving mathematical modelling. Animal suffering will be minimised by regular checking of animals for relevant symptoms that constitute the end point of the experiment, and the use of mixed infections to eliminate mouse-to mouse variability and hence the number of animals required to achieve statistical significance.</p>

Project	Sarcomere proteostasis in titinopathies	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Muscle is a complex tissue that remodels itself according to the workload. This can be increased size that accompanies exercise in muscles such as the biceps, or heart muscle due to overload from high blood pressure. DNA mutations (faulty DNA sequences) have been found to cause disruptive remodelling of muscle tissue in inherited skeletal myopathies as well as cardiomyopathies. How these DNA mutations lead to disruption of the muscle tissue is still poorly understood. As a result of these mutations, misfolded toxic proteins and/or the failure of their clearance could lead to reduced efficiency and functioning of the muscle tissue. This leads to heart and skeletal muscle disease	

	<p>but the mechanisms behind this process are still unclear.</p> <p>The objectives of this project are therefore to understand the fundamental process involved in inherited heart and skeletal muscle disorders and learn more about the muscle's ability to clear toxic proteins and whether we can improve this mechanism in the disease state.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>We hope to understand how DNA mutations cause heart and skeletal muscle disorders, specifically those in the giant TTN gene, as these are more common than expected in the general population. Understanding how these variants lead to disease is critical in the effort to identify disease-causing mutations so as to provide a more comprehensive clinical genetics screening service. We are also looking at a mouse model of a human disease, REDACTED a complex multi-system disorder which affects both skeletal and heart muscle. REDACTED is caused by the lack of or mutation of a protein involved in the cell's quality control machinery and how this leads to the disorder is currently unknown. Studying the mouse model of this disease will advance knowledge on how disruption of the cell's quality control machinery causes disease in general. This could lead to discovery of potential therapies and in addition, the mouse model can be applied to screen for drugs or interventions to benefit patients pharmacologically.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use mice for our studies as we can alter or remove the same genes as in humans and assess what effect this has on their muscle size, structure and function. We aim to use the minimum possible number that provides statistically significant results, and expect to use no more than 1000 mice per year over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The majority of mice we use have no obvious defects and experience mild levels of severity. We often only see differences when the muscles of the mice are tested by increased exercise or muscle wasting. The mice anticipated to present similar symptoms to human diseases are expected to experience</p>

	<p>moderate level of severity and will be closely monitored and culled at the onset of disease symptoms to keep distress to a minimum. Animals undergoing drug administration or food withdrawal are expected to experience moderate levels of severity and will be closely monitored for adverse signs of distress or ill health and culled at the moderate limits of severity. All surgical procedures will be carried out using anaesthetic and pain relief appropriate to the age and species of the animals and closely monitored during post surgical recovery. Animals are expected to make a rapid and unremarkable recovery, any that fail to do so or exhibit signs of pain, distress or significant ill health will be humanely killed. At the end of the tests the animals will be humanely killed.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Muscles are complex structures, made of different complex cell types and fibre types to give different physical properties- this cannot be easily represented in non-animal models.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>All experiments have been designed to produce statistically significant results from the minimum numbers of animals possible. Using mice to generate models of inherited human conditions will allow us to assess the effect of the loss, or mutation of a single protein in a whole animal model. This allows us to analyse, for example, both cardiac and different types of skeletal muscle in a single animal, and thus reducing the total number of animals used.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are used as they can be readily genetically modified to produce animals lacking certain genes, or with disease-associated mutations as they have similar muscle structure and genetics as humans. We will monitor the animals that are expected to display the symptoms of human diseases closely and humanely kill any animals that begin to show any distressful symptoms to reduce potential suffering. Any animals undergoing procedures that may cause harm will be closely monitored and humanely killed if</p>

	they show any signs of suffering. All procedures have been refined to use the most humane techniques possible to reduce distress to the mice.
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Project	Schwann cell development, function and tumourigenesis in the nervous system	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our nervous system is divided into two parts, the central nervous system (CNS), the brain and spinal cord, and the peripheral nervous system (PNS). The nerves of the PNS carry information that allows us to move our muscles, breathe and sense our environment. Our size means that information has to be carried quickly over long distances and this is achieved by insulating nerve fibres in the PNS. This job of insulating the nerve fibres, also known as myelination, is carried out by specialised cells called Schwann cells in our bodies. Malfunction of Schwann cells occurs in many common clinical conditions, such as	

	<p>Charcot-Marie-Tooth peripheral de-myelinating neuropathies, Guillain-Barre syndrome and even leprosy. These conditions may lead to loss of myelin in the PNS and consequent death of nerve cells, causing a lack of sensory and/or muscle function or even complete paralysis. Additionally, approximately 50% of patients with diabetes will develop diabetic neuropathy leading to loss of both sensory and motor functions of the PNS, including a loss of bladder control, digestive problems as well as sexual dysfunction. The abnormal proliferation of Schwann cells following genetic changes may also cause schwannoma tumours seen in patients with neurofibromatosis types 1 and 2. Unfortunately, there are currently no effective treatments for any of these conditions.</p> <p>Peripheral nerve damage is seen in approximately 3-4% of all trauma cases and another part of Schwann cell biology we are keen to research is the regenerative role for Schwann cells in PNS repair. Although the PNS can repair itself, in practice this rarely leads to a full functional recovery for individuals; there is almost always some sensory or motor deficit. By understanding the processes in PNS repair, we wish also to improve the functional outcome in human nerve injuries.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Our aim is to move this basic understanding of Schwann cell function quickly into the use of potential disease modifying treatments and therapies for these debilitating conditions, accelerating peripheral nerve repair and the treatment of nervous system tumours such as schwannomas and meningiomas. The preclinical testing we will do in our mouse models will, we hope, pave the way for potential clinical trials in the future.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This project will last for 5 years. During this time, we would plan to use approximately 1600 mice per year and approximately 300 rats per year. For about 50% of the rats used, these would be for the preparation of cell cultures from neonatal animals. For the genetically modified mice, about 50% of these will have no abnormal phenotype and will be used as controls and the remaining</p>

	50% will have a mild or moderate phenotype.
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>During the breeding and during experiments with animals, the animals are monitored daily by staff with extensive training and expertise in animal care. In the unlikely event of an infection or significant discomfort, the named veterinary surgeon will be notified and the animal(s) will be given antibiotics and pain relief under veterinary supervision. If symptoms persist >24 hours, then the animal will be humanely killed. A significant proportion of the animals used for cell culture or tissue preparation experiments (30% approx.) will be killed humanely without any experimentation. Some animals will suffer some discomfort due to disruption or injury to a nerve on one leg, which is achieved by an operation using general anaesthesia, pain relief is used following surgery. A proportion of the mice will have altered function of the PNS due to genetic changes we will induce, the effects of which are generally mild and not detectable without detailed examination of nerves themselves, although some changes may be visible in those mice that have undergone nerve injury. In some experiments, simple measures may be used to assess the behaviour of the animal in tests that measure balance, footprint analysis or sensitivity to touch to measure any possible impairment. At the end of the experiments, animals are humanely killed.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>A significant amount of the work we do relies upon using cell culture systems, but the development and repair of the nervous system involves a complex interaction between many different cells types and therefore cannot, unfortunately, be accurately modelled in vitro. We can get useful information from the cell culture experiments but the validation of these results needs to be performed using animal models.</p> <p>The use of lower animal species is not possible for this work as their nervous systems do not show the same developmental mechanisms, response to injury, allow accurate measurement of functional recovery and thus do not produce translatable data for human nervous system</p>

	function and tumour development. This is why we require the use of mice and rats for this work.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of replicate experiments in our work is always kept to a minimum. In the case of cell culture experiments the maximum amount of PNS tissue is taken from each animal, expanded in culture as far as is possible and cells frozen for later use. Pilot in vivo experiments (2 or 3 animals of each genetic make-up) are performed and, if results positive, numbers expanded to n=4 or 5 to ensure statistically significant results that can then be published and shared with the scientific community and wider public. Precise animal numbers used for each experiment will depend upon the scatter of the data and the appropriate statistical test(s) used to analyse the data set; advice will be taken from medical statisticians where appropriate. Animals used will be randomly assigned to control or treated groups and the individuals performing tests to measure recovery or function in animals will be unaware of their treatment or genetic mak-up. By such good experimental design, we can minimise animal numbers used and still provide robust high quality data from our work.</p> <p>Strict training and supervision of licensed research staff will also ensure that procedures carried out are reproducible and consistent for all work carried out. Where possible, the strategy for breeding of genetically modified mice will be set to minimise the numbers bred and ensure the maximum number of mice bred are of the correct genotype.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use mice and rats for these studies as there are a large number of tools (antibodies, growth factors and molecular biology methods) available for these two species; far more than for any other species. Furthermore, mice and rats have a nervous system that is much more similar to that of a human compared to other model organisms such as fruitflies, fish or worms. In our work, we ensure the welfare of our animals to the highest possible level and review our surgery, aseptic technique, post-operative care and analgesia in collaboration with other groups in the UK and elsewhere to ensure best possible practice in our</p>

	<p>work. We have authored several book chapters REDACTED as a mark of our expertise. For protocols which have a need for repeated administration of substances by injection, we will adopt the use of osmotic mini-pumps to eliminate the need for such repeated daily injection of animals .</p>
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Project	Sea lice and amoebic gill disease in Atlantic salmon	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	<input checked="" type="checkbox"/> Basic research <input checked="" type="checkbox"/> Translational and applied research <input checked="" type="checkbox"/> Regulatory use and routine production <input checked="" type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This licence will allow us to develop treatments for two of the most significant health problems for salmon farming. Sea lice are a damaging parasite that are showing increasing resistance to current drugs, meaning costs are increasing and lice on farmed fish can threaten wild salmon. Amoebic gill disease (AGD) is an increasing problem as sea temperatures rise, and current treatments are either difficult or can be harmful to the salmon themselves.</p> <p>To develop more effective treatments, such as new drugs or physical methods, we need to be able to test them on infected fish in the laboratory, as we cannot grow sea lice without</p>	

	using fish we maintain colonies using infected fish
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Improving the health of farmed salmon reduces suffering and increase the supply of a healthy food. Salmon is a good source of marine omega-3 oils that help maintain human cardiac and brain health.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use Atlantic salmon because data from salmon will be required to gain approval of new drugs for use in salmon farms. We will only use young farmed salmon that have adapted to their seawater life stage. We expect to use 60,000 fish over the duration of the licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most fish used for sea lice research will be infected with such low numbers that there is only mild or no damage to the skin. As infection is due to many factors including the frequency of encounters with sea lice, and the susceptibility of the fish to infection, some fish will be more heavily infected and suffer minor skin damage. We observe all fish carefully, and any fish showing deeper skin damage will be killed immediately by a humane method. Fish affected by AGD show thickening of the gills causing them to breathe more quickly. We observe all fish carefully, and any fish showing moderate respiratory distress will be killed immediately by a humane method. For any new drug we may not know the safe dose in salmon and will have to expose fish to a range of doses. The potential toxic effects vary with the kind of drug tested but we will try to anticipate them and include any potential side effects in observations. Alternative treatments may have varied side effects during their development. For example, fast jets of water or a laser system for example that may dislodge scales causing minor irritation. All fish will be humanely killed, for example with an overdose of anaesthetic and sent for incineration.
Application of the 3Rs	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The sea lice we will use can only be grown on fish. Some of the pathogens involved in gill disease can grow but not cause disease on invertebrates such as crabs, but we need to use fish because as with sea lice the complex interactions between parasite and host determine how well any intervention will work.</p> <p>We are sometimes able to identify candidate medicines on sea lice without using fish, but all veterinary medicines must be proven effective and safe in the intended species before approval.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Sea lice produce large numbers of larvae that can be tested used without using fish to screen out molecules which are unlikely to be useful as medicines.</p> <p>It takes fewer fish to grow enough lice for a test on the lice themselves than it does by counting the lice on groups of treated and untreated fish. Checking sea lice from farms for drug resistance will be done as far as possible without exposing the fish to drugs.</p> <p>We follow standard protocols for testing on fish. The number of fish used to grow the required sea lice is adjusted based on the size of fish available, and we aim to use as few fish as possible without causing excessive skin damage.</p> <p>We will only grow the minimum lice needed for each strain to maintain it and supply planned experiments.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We will only keep sea lice species known to infect salmon, and only strains that will be useful for developing new medicines or investigating resistance to current treatments.</p> <p>We have control over the numbers of lice infecting fish because we add them to a tank directly. This means we can take into account the size of the fish, keeping infections lighter on smaller fish.</p> <p>We check the level of lice on fish after infection and will observe the fish at least</p>

	twice daily throughout. Any fish found to have an unexpectedly high level of infection or deeper skin damage will be killed by a humane method such as an overdose of anaesthetic and sent for incineration.
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Project	Sensation to action: Function of brain circuits controlling behaviour in zebrafish.	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The goal of this project is to understand how the brain processes sensory information from the outside world in a context-dependent way, to guide behaviour. Except for quite simple reflex behaviours, there are currently few examples where we understand the complete brain circuit that controls a specific behaviour. Our aim is to produce a comprehensive model of the complete brain circuitry that controls the selection and execution of behaviour, using larval zebrafish.	
What are the potential benefits likely to derive from this project	The primary benefit of the research will be to advance our scientific knowledge about how	

<p>(how science could be advanced or humans or animals could benefit from the project)?</p>	<p>brain circuits are organised and how they function. Secondly, diseases of the nervous system, and in particular neurodegenerative diseases such as Alzheimer's, are a growing human health concern. The development of effective treatments for neurological conditions is a massive challenge, due in large part to the great complexity of the nervous system: although basic neuroscience research has made considerable progress in understanding many aspects of neurobiology, including the cell and molecular biology of individual neurons, the most poorly understood aspect of brain physiology is how the ~100 billion individual nerve cells that comprise the human brain function together, as a network, to perform the computations that control our actions, emotions and thoughts. This lack of basic scientific knowledge represents a major obstacle to understanding how genetic abnormalities, trauma, degenerative loss of specific cell types, and pharmacological agents affect the computational functions of neural networks. In this project we propose to uncover fundamental principles about how entire neural networks are functionally organized, and how they carry out the computations that control behaviour. This understanding of how neural circuits function in the healthy brain is necessary for understanding circuit dysfunction during diseases, and for developing improved diagnostic tools and treatments.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This project uses zebrafish, a small tropical freshwater fish species. Adult fish are maintained for breeding purposes to generate embryos and larvae which are then used for experiments between 5 and 10 days of age. Over five years we expect to use 20,000 larvae for experiments and 60,000 animals for maintaining our fish colony.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The majority of experimental procedures will have mild or undetectable adverse effects on the larval fish and the maximum severity limit is moderate. Larval animals are tethered using gel and presented with natural stimuli (for example resembling their prey) whilst we monitor brain activity using non-invasive light microscopy. In some experiments we will use laser-surgery to</p>

	<p>carefully remove small numbers of brain cells to help to work out what role they play in processing information. Throughout all experiments we continuously monitor animal health and behaviour to detect any adverse effects. A small number of adult animals will have a `fin-clip` to remove a small piece of fin tissue for genetic analysis, which is done under analgesia. At the end of the experiments, all animals are euthanised using an overdose of anaesthetic. This is a humane procedure approved by the Home Office.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>How neural circuits are organised and the computations they perform are still very poorly understood. There are currently no in vitro systems or computer models that can accurately replicate the function of an entire nervous system. Therefore, to learn more about the patterns of brain activity that control specific behaviours we must perform experiments using animals where we are able to monitor brain activity in the context of a behavioural task. We use larval fish, rather than more sentient rodents, for these experiments.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We use the minimum number of animals required to obtain high quality scientific data. This is achieved by (1) using best practices in animal husbandry such that our fish colonies can be maintained with the minimum numbers of breeding adults (2) using cutting-edge microscopy techniques to image brain activity. This allows us to obtain large and comprehensive datasets from a single procedure in a single animal, which reduces the total number of animals required.</p> <p>(3) Using statistical tests, where possible, to ascertain the minimum number of animals required to produce statistically robust datasets.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having</p>	<p>We use larval zebrafish primarily because they are a simple vertebrate and have a tiny transparent brain. This allows us to use non-invasive optical imaging to observe patterns of</p>

<p>regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>brain activity whilst the animal views and responds to visual scenes. This approach to recording brain activity is harmless and is much less invasive than traditional techniques using surgery and implanted electrodes. Furthermore, it allows us to monitor many more cells at the same time, reducing the total number of experiments. To prepare animals for experiments we have to tether them using gel. The larval fish are briefly anaesthetised during this procedure to minimise stress.</p>
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Project	Sensory and emotional processing in the nervous system	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Chronic pain affects at least 20-30% of the human population, reducing patient quality of life and ability to work, therefore presenting a huge social and economic burden. Chronic pain can be a consequence of accidents, surgery, chronic diseases such as arthritis or drug treatments such as chemotherapy. Less than 50% of chronic pain patients achieve pain relief taking prescription drugs and this relief is often only partial and can be accompanied by unpleasant side effects including addiction. Moreover, depression and anxiety are often observed in many chronic pain	

	<p>patients.</p> <p>Chronic pain is also being increasingly recognized as a significant and underappreciated problem in companion animals. It is estimated that at least 20% of dogs over the age of one suffer from osteoarthritis, a very common debilitating condition associated with significant pain, and these dogs are offered a limited number of treatment options to relieve their pain. Osteoarthritis is also recognized as a common condition in cats, with some quoting an incidence as high as 90% in cats over the age of twelve. In America, only one non-steroidal anti-inflammatory drug is approved for long-term use in cats, a treatment unlikely to provide sufficient relief in long-lasting pain states.</p> <p>The quest for better pain relief for human and veterinary chronic pain patients therefore remains a significant challenge. One of the main hurdles to the development of novel analgesics is our limited understanding of the mechanisms that underlie chronic pain and our project will help to achieve a better understanding of these mechanisms.</p> <p>We work on the assumption that if we understand the molecular changes in the central nervous system in chronic pain states, and therefore the mechanisms that maintain chronic pain states, new treatments that will improve the pain will be generated. Importantly, we expect these treatments to improve not only the pain, but also affective conditions, such as depression and anxiety, often seen in chronic pain states. These new treatments will therefore lead to a significant overall improvement of pain patients' quality of life.</p> <p>In this project we will look specifically at the way the brain, the spinal cord and the 'pain' fibres themselves contribute to the pain experience and how the previous history (e.g. early life injury or trauma) and mental state can change the way pain is experienced.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could</p>	<p>Our project will uncover new targets for the development of drugs and new approaches to pain therapy that can alleviate pain in humans and animals. Our research will also investigate the influence of past experiences on pain</p>

benefit from the project)?	experience in adulthood. Injury, whether through surgery or accident in young infants and animals, is known to alter pain sensitivity in the adult but how this occurs is not known. Our work aims to uncover the mechanisms responsible for this altered pain sensitivity in adulthood and therefore is likely to identify potential biomarkers of the susceptibility to chronic pain in vulnerable individuals. This would be a significant advance in pain research as it is currently impossible to identify individuals likely to develop chronic pain following accidents or surgery. Identification of these individuals would ensure that maximum pain relief and adequate care is given at the time of injury, an approach that has been shown to reduce the likelihood of developing chronic pain.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use rats and mice because these species have told us quite accurately about many aspects of pain processing in humans. Genetically modified mice will also allow us to confirm results from molecular analysis on wild-type mice and rats. We will use approximately 1200 rats and 2000 mice during the course of the project (5 years).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will generate animal models with increased mechanical and thermal sensitivity but not in continuous pain. While some guarding of the sensitive paw is often seen, the models we generally use are the least severe available and levels of severity are never more than moderate. Experiments will not be continued for longer than is absolutely necessary. All experiments will be terminated using humane killing and we will take tissue for molecular analysis. We will always maximise animal use and dissect all tissue that we may need for future studies.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>In most of our studies it is essential to use animals rather than cultured cells in dishes. Cultured cells can tell us a lot and, if required, we use these approaches. However, the complex diseases we study require that we look at the behaviour of the whole animal. This is because all symptoms that we observe through our animal</p>

	<p>studies (pain and mental health) are fundamentally a reflection of how the brain deals with injury to the body during recovery. Fortunately, previous research in rodents has shown that these animals can shed considerable light on human diseases and indeed, as we have shown, even lead to new therapeutic approaches.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We are experts in experimental design and always use both our experience and statistical approaches (e.g. power analysis, Mead's resource equation) to estimate the number of animals we need in our experiments not to waste or overuse animals. Often we use both sexes of animal in our experiments, so our results can be applicable to the whole population. This is also taken into account when estimating our sample size. We also seek specialist statistical advice where required for new study designs. Control animals are shared between experiments when possible. Novel test compounds provided by our collaborators are never used in animals without being first tested in vitro.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We will use rats and mice because these species have told us quite accurately about many aspects of pain processing in humans.</p> <p>We have chosen models that have been widely used in the pain field for many years. This will allow us to build on previous data and to compare our work with that of others and will ensure that we do not need to characterise novel models that might not be suited for the study of both pain mechanisms and translational approaches for the treatment of chronic pain in humans. Importantly, we have started engaging with the patient community through patient and public involvement activities to ensure that our chosen models have translational value (i.e. that the animals display patient relevant symptoms), as we want our work to be of maximal benefit for the patients. Our behavioural approaches will also provide us with valuable information with high translational value.</p> <p>We will use great care in ensuring that rats and mice are well maintained and suffer minimal</p>

	<p>distress as well as using best practice surgical procedures. Environmental enrichment, such as carton tubes and wooden artefacts, will be provided though this may be tailored so as not to affect experimental measurements. Such environmental enrichment stimulates the animals and contributes to their physical and psychological well-being.</p>
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Project	Sex chromosomal control of development and disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Men and women are genetically the same, with the exception of their sex chromosomes. Men are XY and women XX. The sex chromosomes have a special role in making sperm and eggs. Abnormalities in these chromosomes are thought to be responsible for many cases of infertility, a condition that affects 15% of couples. Evidence suggests that sex chromosomes are also responsible for other differences between men and women, for example in their likelihood of developing cancer, their life expectancy and how they respond to medications. The overall aim of this project is to investigate how the sex chromosomes control these processes. More broadly, we also wish to understand how	

	<p>problems in chromosome behaviour in developing eggs and sperm give rise to conditions in offspring such as Down syndrome. Finally, many areas of medical research and agriculture require only animals that are male or female, and those of the opposite sex are therefore created needlessly. A good example is the dairy industry and egg industry, in which only female cows and chickens are required. We want to design a system for creating litters that contain only males or only females.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Ultimately, our studies of sex chromosomes could give insight into the causes of human infertility and of sex differences in disease. They could also help us understand why chromosome abnormalities are so common in humans, affecting 7-10% of all clinically recognised pregnancies. In turn, the discoveries could lead to new ways of diagnosing or treating these conditions. Finally, a method to create single sex litters would have a huge economic impact on the medical and agricultural industries, and would be a major step forward for animal welfare.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>For most of our experiments we will use mice. These are an excellent model system because their genetic make-up is similar to that of humans. Also, egg and sperm formation in mice occurs in a manner similar to that in humans. We also use REDACTED. The rationale for using both model organisms is that mechanisms common to both are likely to be of highest importance for understanding the diseases in which we are interested.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Our objectives require the creation of genetically altered mice and REDACTED. In the majority of cases, the effect on the animal will be observed only in the testis or ovary, and so the severity level will be mild. Also, most of our experiments will be performed on material obtained from animals post-mortem.</p>
<p>Application of the 3Rs</p>	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Our studies require the use of animals, because currently it is impossible to make germ cells (i.e. eggs and sperm) in a dish. This is probably because in order to form properly, germ cells require two-way interaction with other cell types in the gonad, as well as changing levels of hormones provided via the bloodstream. Nevertheless, one of our aims will be to try and make eggs and sperm types in the laboratory. If this succeeds, it could help replace the use of animals in the longer term.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We implement a number of approaches to reduce animal numbers. This begins with experimental design. Genes that we think are important for the processes we're studying are chosen based on published literature and data generated both by us and by other scientists. This vastly reduces the number of "false-leads". Genetically altered animals are only created if they not available from existing sources. If this is the case, the genetically altered animals are created in-house by highly trained personnel, and are usually maintained as small colonies. We plan our experiments so that each animal provides the maximum amount of material for analysis, and that tissue harvested post-mortem from a single animal can be stored and repeatedly reused in different experiments. This approach, together with statistical approaches, means we use few animals to address a specific scientific question. A major focus of our work is to design a system for creating single-sex litters. Many areas of medical research and agriculture require animals of a defined sex, and those of the opposite sex are therefore created needlessly. If successful, this approach would dramatically reduce the numbers of animals needed for our experiments, and for those of the international research community as a whole.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms)</p>	<p>Our work focuses on sex chromosomes. Mice and REDACTED are ideally suited for this work, because unlike other model organisms, their sex chromosomes, and the mechanisms by which their eggs and sperm are formed, are similar to those of humans. The mouse is also useful because it is the most tractable mammal with</p>

<p>to the animals.</p>	<p>respect to genetic manipulation. In the majority of our experiments, the genetic alterations we create impact only fertility, and thus do not appear to cause pain or distress. Furthermore, in most cases material will be acquired post-mortem. We use highly trained personnel to carry out protocols with moderate severity limits, e.g. induced ovulation, in order to keep animal suffering to a minimum. We cannot always predict the effect of a new genetic alteration. However, animals exhibiting any unexpected or detrimental effect will be killed by a Schedule 1 method, or in the case of new lines or individual animals of particular scientific interest, advice will be sought from the local Home Office Inspector.</p>
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Project	Sex-dependent obesity and metabolic complications	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Our project aims to begin to tackle the following current unknowns:</p> <ol style="list-style-type: none"> 1. How specific genes are involved in sex-dependent differences in obesity and the health problems linked to it. 2. What other genes and processes also involved in sex-dependent differences in obesity and the health problems linked to it. 3. Whether these genes are good targets for specific treatments to prevent or treat obesity and/or the health problems linked 	

	<p>to it.</p> <p>Overall Aims:</p> <p>To identify, the function of, specific genes involved in bringing about the sex-dependent differences in obesity and its problems. Also to identify better ways to prevent or treat the problems caused by obesity.</p> <p>Specific Objectives:</p> <ol style="list-style-type: none"> 1. To identify the genes involved in obesity and the health problems linked to it. 2. To discover the effects that changes in specific genes have on obesity and health problems linked to it. 3. To discover how specific gene changes affect sex-dependent differences in obesity and health problems linked to it. 4. To discover how gene changes in specific tissues affect sex-dependent differences in obesity and health problems linked to it. 5. To identify new drug targets and test if they can be used to prevent or treat obesity and/or the health problems linked to it.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Overall this project has the potential to increase our understanding of how the sex-dependent differences occur in obesity, metabolism, inflammation and the health problems linked to obesity. Specifically:</p> <ol style="list-style-type: none"> 6. Increase our understanding of how specific genes are involved in obesity and health problems linked to it. 7. Increase our understanding of how energy metabolism is controlled and how obesity is linked to its health problems . 8. Increase our understanding of the sex-

	<p>dependent differences in obesity and health problems linked to it.</p> <p>9. Increase our understanding of new ways to prevent or treat obesity and/or the health problems linked to it.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use up to 9,000 mice over the course of this project. These will include both normal and mice with specific gene changes.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>THE METHODS USED AND HARMFUL EFFECTS</p> <p>The main impact comes from eating too many calories (e.g. from high fat diet) over several weeks and becoming obese. This can speed up the development of long-term health problems linked to abnormal metabolism. These problems increase the risk of developing diseases such as obesity-related high blood sugar (Type 2 diabetes), fatty liver disease, heart (cardiovascular) disease and certain cancers. Our studies will focus on the early events that occur and link diet-induced obesity to its metabolic problems. These do not cause harm or suffering and are not life threatening. When fed a fattening diet some animals will begin to show signs of increased blood sugar (i.e. pre-diabetes and/or Type 2 diabetes). Similarly, the signs of fatty liver disease, heart disease and certain cancers are not externally visible - affected individuals can appear overtly normal. Therefore, specific blood tests are needed. Very small samples of blood will be taken during these tests to examine how well sugars and fats are handled. Some tests need animals to be fasted before taking blood samples. This will be temporary and its effects on loss of body weight will be limited. In obese mice, weight loss after fasting is mostly due to loss of fat weight and can be useful. We want to understand how food is handled in animals with specific changes in their genes (genetically altered). All animals under studies will be regularly monitored and no mice will develop life-threatening signs.</p> <p>By changing specific genes we can learn how</p>

they are involved in disease and importantly whether they could be good drug targets. None of the chosen gene changes in themselves are expected to cause adverse effects beyond minor. However, when combining the gene changes with feeding a high fat diet may result in increasing or reducing the speed at which obesity and/or its metabolic problems form. Also, both sex and age can affect this. We plan to identify and change genes that are likely to play important roles in sex-dependent differences in metabolism. Obesity and/or abnormal metabolism may only occur in one of the two sexes. To identify this, we will need to compare animals with specific changes in their genes and normal mice from both sexes. We will study how they handle their food intake, energy stores and their metabolism.

Since older females also experience changes in their metabolism linked to menopause, we will study the effects of surgical menopause on a selected group of animals. All surgeries, as for human surgery, are done with appropriately sterile conditions. Both anaesthesia and pain killers will be provided. All animals will be closely monitoring and care provided until fully recovered. This will minimise stress and suffering of the animals.

Finally, we will also study different ways to either, a) prevent diseases from occurring or b) treat them once early signs of disease have been observed. The methods we will use will include treating with dietary supplements (e.g. pre-biotics, pro-biotics or Omega 3/6 fatty acids), changing housing temperature (to promote energy use), or treating with specific drugs (anti-diabetics, inhibitors of lipid metabolism, antibodies, anti-inflammatory agents, sex hormones or selective estrogen receptor modifiers). Each treatment will aim to improve energy metabolism and/or sex hormone action.

WHAT WILL HAPPEN TO THE ANIMALS AT THE END?

At the end of each study the animals will be humanely killed and their tissues collected for

	<p>further detailed study. For example, we will measure the amount of fat build-up, factors linked to sex hormones, fat and sugar metabolism and specific signs of disease. Occasionally, animals with specific gene changes will be bred and transferred to other authorised establishments for further study.</p> <p>The results and new knowledge gained from our work will be made publicly available.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We need to use animals because;</p> <ol style="list-style-type: none"> 1. Obesity and its metabolic problems occurs at the level of the “whole animal” and not just in fat tissue or fat cells. It involves the communication of many different tissues to control food intake, energy use, fat storage, fat and sugar metabolism. Therefore, to understand how animals handle energy from food and obesity leads to signs of disease, we need to study the whole intact living animal. 2. We will try to do as much as possible, with isolated tissues and cells. However, such studies may also need to use animals with specific changes in their genes to provide specific cells and tissues to study in more detail outside of the whole animal. 3. We cannot use a lower life-forms, e.g. fish or insects, as they regulate energy balance, get obese and associated problems and have sex-dependent differences and immune function, very differently from mammals. We want to make new discoveries from these animal models of disease to allow the discovery of better treatments.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<ol style="list-style-type: none"> 1. Minimal numbers needed in each study group. By using standard methods and appropriate statistical calculations we will make sure the results are of a high quality and reproducible. This will allow us to provide clear conclusions, from a minimal number of animals. 2. Time-course study design. We will follow the same animals through the course of

	<p>each experiment from weaning, as it develops obesity, and/or metabolic problems and early stages of disease. This will reduce the number of animals needed to generate high quality data. The regular handling of the same animals also reduces stress felt by animals not used to being handled.</p> <p>3. New less disturbing technologies. We plan to take advantage of new less disturbing technology. For example, scanners to measure total fat mass without anaesthetics. This will allow time-dependent data to be produced in the same animals and reduces the number of animals needed. It also removes the need for repeat dosing with anaesthetics and produces high quality, reproducible data. These technologies are also designed to cause the least possible stress and pain to the animals.</p> <p>4. Using a cocktail of differentially labeled chemicals/nutrients, we can generate more information from a single animal and treatment. For example in one injection we can introduce two or more compounds/nutrients labeled with different colours. Each compound/nutrient can then be tracked in real time, as it enters tissues and is metabolised. This reduces the number of animals needed to generate robust data sets.</p> <p>5. Multiple tests in one sample. By using new multi-test technology we can now measure multiple factors in the same sample. For samples such as blood, this reduces the volume, number of samples and number of animals needed.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>1. Choice of animals: Mice have been chosen for the first studies because much is already known about them especially as models for obesity-related diseases. Mice have the added benefit that methods already exist to allow specific gene changes in them. This allows us to increase, reduce or remove specific genes in the whole animal from birth or in a controlled manner. For example in a specific tissue or at a specific time.</p> <p>2. Less disturbing technology For example, scanners to measure total fat mass without anaesthetics. This will allow us to collect many measurements to be taken of the same</p>

	<p>animal over a long period of time and as they gain weight. We will also look to buy new highly sensitive technology (e.g. thermal imaging camera) that may reduce the discomfort related to existing methods.</p> <p>3. Surgery (as for surgery in humans) will be carried out under sterile conditions in specially designed rooms and by experienced staff. The animals will receive proper anaesthetics, pain killers, close monitoring and appropriate care before, during and after any surgery as advised by the vet.</p> <p>4. The time needed for restraining animals will be kept to a minimum. For example, animals will be warmed up without holding them down in a warming box and then placed in a holding tube only for the time needed for blood pressure measurement. Small blood samples will also be collected without holding down animals. This reduces the stress felt by animals when they are held.</p>
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Project	SIGNALING IN SENSORY PROCESSING AND DRUG EFFECTS	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Pain is a complex medical and social issue with a poorly defined relationship between injury and the subsequent feeling of a painful sensation. It is the most common reason that drives us to seek medical help. However, currently available pain therapies, particularly in pain that won't 'go away', are highly unsatisfactory and this is unacceptable and inhumane. A survey conducted in 2006 revealed that nearly half of people seriously affected by chronic pain were not able to reduce their pain with available medications e.g., nonsteroidal anti-inflammatory drugs (NSAIDs), paracetamol, weak opioids	

	<p>(REDACTED). Therefore, pain has a major social and economic impact in terms of lost employment and medical costs. Our studies seek to understand the sensory processing mechanisms that cause an acute pain that remedies to become a chronic condition which can last a lifetime. We also aim to identify and characterise of novel drug targets and novel therapeutic strategies that will directly improve pain control in the clinic, also through improvement of currently available but not satisfactorily pain therapies (e.g., involving opioids). Also, better understanding of correlation between mood and pain suffering may lead to the improvement of patients experiencing life-long pain. Thus, this project aims to address fundamental areas of medical concern that are related to pain suffering.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The results from the proposed studies will provide us with important information, relating to how the body processes information about pain and how it responds to therapies aiming to reduce pain suffering. Thus, the proposed programme of work will aid the future development of better treatments for many current intractable pain conditions. Identifying new therapies will benefit people, especially since different forms of chronic pain due to e.g., ageing affect the increasing percentage of human population, and also animals. Thus, all my experimental work with animals will be carried out with a view to translating obtained results into new treatments for humans and animals.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>It is expected that no more than 3,600 mice and 3,000 rats will be used during the 5 year-course of this project. These numbers are derived from previous experience and anticipated research funds over this period. In designing our experiments, we are able to use approved methods (Power Analysis or Resource Equation) to carefully choose how many animals are strictly necessary for each data set.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the</p>	<p>In order to study changes that occur during the development of long-lasting pain, in some cases, it will be necessary to induce on-going pain by</p>

<p>likely/expected level of severity? What will happen to the animals at the end?</p>	<p>inflammation or by inducing nerve injury to mimic states of nerve pain (neuropathic pain). It should be noted that these procedures will be performed under anaesthesia, to minimize pain and suffering, and animals will recover for further study at a later time. EMLA cream that works by numbing the surface of the skin for a short time due to medicines called local anaesthetics that are active ingredients of this cream may be used as part of the postoperative care. These animals will not be in constant pain, they perform normal daily behaviours e.g. feeding, drinking, grooming and socializing. In behavioural tests where stimuli are applied to the affected area, they show localized increased sensitivity to mild mechanical and thermal stimuli. In some cases, procedures involving e.g., injection of opioids or implantation of lead to produced neurostimulation, will reduce pain. Any animal displaying noticeable distress/loss of normal behaviours as a result of the procedures will be immediately humanely killed using an approved procedure. All animals will be humanely killed at the end of the study using approved protocols.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The sensation of pain is complex and poorly understood. We are beginning to understand more about pain sensation but we are not yet in a position to fully replicate pain processes in models such as cultured neurones or to apply computational simulations. Thus, it is essential to use animals rather than cultured cells in dishes, as complex diseases require looking at the behaviour of the whole animal. In addition, previous research in rodents has shown that these animals can shed considerable light on human diseases and indeed as we have shown, even lead to new therapeutic approaches. However, in my lab we are trying to develop a new cultured neuron method that could aid our work and help to replace some animal use.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of</p>	<p>We need to use a minimum number in order to obtain meaningful outcomes that stand up to statistical testing and scrutiny by other scientists, but also not so many that the lives of animals are humanely killed for no purpose. In practice, the</p>

animals	<p>results will be regarded as satisfactory when a clear conclusion emerges: either a statistically significant difference or a sample size (guided by Power Analysis or Resource Equation) that should have revealed a difference should one exist. We will also make appropriate arrangement plans and conduct studies to enable them to be published according to the ARRIVE guidelines.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Species. I will use rats and mice for these studies since they can shed considerable light on changes that occur in patients with chronic pain as well as in rodents. There is substantial background information about the structure and function of the nervous system in these species that is not defined in lower organisms and importantly, rats and mice were shown to have similarity with different types of mammals including humans.</p> <p>Models. Presented here are models and methods of pain assessment that are widely used in the pain research field and I have an extensive experience working with them. Thus, the obtained experimental results will be relevant to my previous research work and to other laboratories using these animals in studies of pain worldwide. To maintain welfare and minimize adverse effects, these models of pain will produce mild to moderate pain but not in continuous pain and no other significant alteration in animal behaviour.</p> <p>Refinement. All surgical procedures will use anaesthetics and refined techniques to minimize the risk of post-surgical complications (e.g., infection). As part of the postoperative care, local anaesthetic cream (e.g., EMLA cream) may be used to reduce local pain by numbing the surface of the skin and improving comfort of the animal. Behavioural protocols will be performed in quiet environment after allowing animals to initially adapt to the test and handling. In our experiments, we developed a protocol to minimize exposure to thermal stimulus that is associated with reduction of test days that still allows us to obtain meaningful outcomes that stand up to statistical testing and scrutiny by other scientists. Doses of drugs and routes of</p>

	administration will be chosen so as not to have adverse effects.
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Project	Signalling pathways in cancer, inflammation, and metabolism	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>NF-κB denotes a family of proteins which govern the body defence responses to injury, infection and stress. NF-κB however has also been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development. The aim of our work is to gain a better understanding of how NF-κB promotes cell survival, tumour development, and inflammatory and metabolic diseases, and develop safer and better treatments selectively targeting NF-κB in patients suffering from these illnesses.</p> <p><i>Aim 1:</i> Investigate the role of NF-κB in tumour development, and gain a better understanding of the molecular mechanisms by which NF-κB promotes this process. We will investigate the involvement of NF-κB in both solid and blood cancers, and delineate how NF-κB</p>	

	<p>operates in these contexts.</p> <p><i>Aim 2:</i> Develop new therapeutic strategies to selectively block NF-kB in cancer, and inflammatory and metabolic diseases, and promote the development of new and more specific therapies, which are safer and more effective than the current non-specific treatments aimed at blocking NF-kB.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Currently there is an urgent medical need for developing improved therapies to treat various types of blood and solid cancers, as well as inflammatory and metabolic diseases. The work proposed under this License application will deliver these objectives in various areas of unmet medical need, both within and outside of oncology, including solid and blood cancers, and inflammatory and metabolic diseases.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Only mice (both wild type and genetically modified) will be used for this project. This is a 5 year project License, and we estimate that for all the procedures outlined in this License up to approximately 7,330 mice per year will be used.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>None of the experiments planned under this project License involve procedures that are expected to cause the mice severe distress or discomfort. All procedures have been designed to detect any animals that appear to be suffering and killed them, if required, by using humane methods. Genetically modified animals, including animals that display a genetic predisposition to tumour development, will be used and bred under this License. For this reason, mice will be carefully monitored for possible side effects and specific endpoints have been adopted to minimise the suffering of the animals and limit this to what is strictly necessary for the research. A small proportion of the animals used in this Licence will undergo bone marrow reconstitution, following irradiation. To avoid infections and suffering to them, these animals will be inspected daily and several refinement measures have been adopted in order to ensure the animal wellbeing. Drugs will be administrated to a small proportion of the mice used in this License in order to study their therapeutic effects on tumour growth. Additional humane endpoints have been designed in order to avoid any suffering to these animals. A very small proportion of animals will undergo a moderate surgical procedure (e.g. the implantation of small drug delivery devices under their skin). To</p>

	<p>minimize any associated suffering to these mice, we have taken the veterinary advice, will utilize anesthesia and analgesia to control any pain, and will terminate the experiments early, if required, should the animals show any sign of discomfort or distress. Some of the animals used in this License will be exposed to tumour challenge, whereby the tumour may arise either spontaneously or after the injection of tumour cells. This is necessary to help us to understand how tumours develop and grow, and to find better treatments for patients with cancer. Occasionally, we will need to monitor tumour growth in these mice by taking blood samples or using imaging techniques similar to those used in the clinical practice. For mice undergoing each of these procedures, additional humane endpoints have been adopted in order to avoid any unnecessary suffering to them. In the case of development of unusual or unexpected clinical signs or adverse effects, we will seek advice from the Named Animal Care and Welfare Officer (NACWO) and/or the Named Veterinary Surgeon (NVS) for taking the appropriate measures.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>While valuable studies of human cancer are performed using human tumour material, the mechanistic understanding of cancer pathogenesis requires the use of living animals. In particular the development and function of the immune system involves many different cell types interacting in a dynamic three-dimensional environment. Similarly, cancer development and spread involves a plethora of interactions between cancer cells and their surrounding cells, governed by multiple signals originating from both their immediate neighbours and from distant tissues.</p> <p>Use of refined animal models is thus the only valid way of both advancing basic understanding of cancer development and evaluating novel approaches to treat these diseases.</p> <p>A search of the Altweb web site(http://altweb.jhsph.edu/resources/searchalt/searchaltdata.html) and a subsequent search of the NIH web site (http://emice.nci.nih.gov/aam/mouse/how-and-why-mouse-cancer-models-are-used) have confirmed that, on the basis of the aforementioned considerations, there are no suitable alternatives to mouse models in research aimed at delineating the processes governing inflammation, immunity and oncogenesis, as required by</p>

	this project.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p><i>The following measures will be routinely adopted for reducing the number of mice:</i></p> <ol style="list-style-type: none"> 1. Mouse colonies will be closely monitored to avoid excess animals. As appropriate, we will plan effective mouse breeding strategies which can provide us with the required experimental mice and controls. 2. In the majority of experiments, post mortem tissue will be harvested at the end of experiments to gain invaluable <i>in vitro</i> immunological and molecular analyses. 3. In the majority of experiments, post mortem tissue will be harvested at the end of experiments to gain invaluable <i>in vitro</i> immunological and molecular analyses. 4. In the majority of experiments, post mortem tissue will be harvested at the end of experiments to gain invaluable <i>in vitro</i> immunological and molecular analyses. 5. <i>In vivo</i> experiments are carefully designed to use the minimum number of animals, whilst providing meaningful and statistically valid outcomes. 6. <i>In vivo</i> experiments are carefully designed to use the minimum number of animals, whilst providing meaningful and statistically valid outcomes. 7. Only certain strains of mice, which reduce the biological variability due to genetic factors, will be used. 8. Comparison will be made only between strain-, sex- and age-matched groups, and equal number of mice will be used in each group to avoid statistical artefacts. <p><i>Experimental design:</i></p> <p>For those experiments where outcomes in experimental and control groups of animals are compared, it is important that group size is sufficient to demonstrate whether there is a statistically significant difference, whilst keeping the numbers of mice as low as possible (https://www.nc3rs.org.uk/experimental-designstatistics). In all cases, we will aim to minimise animal numbers</p>

	consistent with achieving scientifically and statistically robust results.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Appropriate guidelines for good practice will be followed. Animals will be inspected regularly to ensure general wellbeing and any animal showing signs of adverse effects will be humanely killed by a Schedule 1 method. The NVS and/or NACWO will be consulted for advice where appropriate.</p> <p>We will follow the following guidelines:</p> <ul style="list-style-type: none"> ● Lasa Good Practice Guidelines on ‘Collection of Blood’ and ‘Administration of Substances’ will be followed. ● The NCRI guidelines for the Welfare and Use of Animals in Cancer Research for endpoints in experiments involving tumour development will be followed. <p>To refine the experimental conditions and to ensure minimal suffering, the following steps have been taken:</p> <ol style="list-style-type: none"> 9. Where possible, a short-acting general anesthetic will be used 10. Pre- and post-operative analgesia will be routinely administered 11. Where possible, vasectomized males will be replaced by genetically sterile males 12. Non-invasive imaging may be used for direct assessment of tumour growth in systemic tumour models. This is a refinement over indirect (e.g. loss of condition) indications of tumour growth 13. Non-invasive imaging may be used for direct assessment of tumour growth in systemic tumour models. This is a refinement over indirect (e.g. loss of condition) indications of tumour growth 14. Non-invasive imaging may be used for direct assessment of tumour growth in systemic tumour models. This is a refinement over indirect (e.g. loss of condition) indications of tumour growth <p>Bioluminescence and ultrasound imaging, two non-invasive methods which increases the quality and quantity of the experimental data obtained from a single experiment, will also be used on animals under general anaesthesia. This will dramatically increase the</p>

	information that can be accrued from each mouse.
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Project	Skeletal homeostasis, remodelling and repair	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Arthritis is a leading cause of disability. Joint injury due to trauma, and obesity, increasingly common in the western world, are important risk factors. Joint destruction is the result of an imbalance between tissue breakdown, often caused by inflammation or trauma, and repair. Throughout life, stem cells are specialised cells that maintain and repair tissues and organs of our body. Our knowledge of the location and functional regulation of the stem cells in the adult joint is limited. We propose to study the stem cells naturally present in the normal and diseased joint and to investigate their role in the maintenance, remodelling and repair of joint tissues in the adult life. For such studies, no	

	<p>system using a dish in the laboratory would be able to reproduce the complex environment of a living organism with continuous interactions among tissue and organ systems across the whole body.</p> <p>In clinical conditions characterized by extended tissue damage/loss (e.g., cartilage defects or advanced osteoarthritis), the stem cells present in the joint may not be sufficient to ensure repair. In these circumstances, the transplantation of stem cells that have been taken out of their tissues and grown in the laboratory would be necessary for replacement of missing tissue components. Studies in humans support the utility of stem cells for bone and cartilage repair. A major problem, however, is the large variability in clinical outcome, partly due to inconsistency of the stem cell preparations. There is, therefore, an unmet pressing clinical need for development of quality controls for efficacy of stem cell preparations, a prerequisite for routine use in clinical practice.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The ultimate goal of this research is to develop cell-based treatments for patients with skeletal disorders such as arthritis, including osteoarthritis and rheumatoid arthritis. These conditions are characterised by extensive damage of cartilage and bone. Current treatments are often unsatisfactory. Medications can halt inflammation but are unable to achieve regeneration/repair of the damaged tissues. Our research could lead to novel cell-based therapies for replacement of damaged tissue via transplantation of stem cells or via the administration of drugs that target the stem cells that are naturally present in our body. Over the 5 years of this project, we expect to deepen our understanding of the stem cell populations present in the joint tissues and their roles in the maintenance of joint health and arthritis development, and to use this scientific knowledge to identify and evaluate drug targets and gain insights into the mechanism of drug interventions.</p>
<p>What species and approximate numbers of animals do you expect</p>	<p>We will use mice, which are most used for preclinical studies and allow assessment of</p>

<p>to use over what period of time?</p>	<p>function via genetic modification. We have estimated to use up to 3,000 mice for experiments over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Joint injury, osteoarthritis and inflammatory arthritis in adult mice will be induced to study the role of stem cells present in the joint in these processes. The models of injury consist of damaging joint tissues such as the cartilage (a tissue that covers the bone end in the joint and is devoid of blood vessels and nerves) through minor knee surgery under anaesthesia. Mice recover rapidly (within hours) from the surgery and return to their normal activities. Depending on the mouse type or kind of damage, there is either repair (with return to a normal joint) or development of osteoarthritis long-term. Obesity is an important risk factor for osteoarthritis and to study the role of obesity, some mice will receive a diet rich in fat that will cause weight gain. Inflammatory arthritis is achieved via injection of substances that induce an immune reaction that affects the joints in a way that is similar to the human rheumatoid arthritis. In these models, mice may receive injections or undergo blood sampling to measure specific factors or detect cells under study. Mice may also undergo scanning such as x-ray under anaesthesia to monitor the disease. These conditions are typically well tolerated, and mice are monitored regularly. Like human patients with joint disorders, mice may develop joint pain and swelling. Side effects are rare and include skin lesions at the site of injections, opening of sutures (which can be successfully re-closed), and those related to the anaesthesia. Immune-deficient mice (unable of immune rejection) will be used for transplantation of human stem cells, obtained from adult individuals and grown in laboratory dishes, to assess their ability to form joint tissues such as cartilage and bone in vivo. These protocols are well tolerated and the risk of adverse effects in our experience is very low. Mice will be kept in protective housing and all interventions will be carried out aseptically. Appropriate anaesthetic and pain relief regimes will be given as needed according to a regime recommended by the vet. Surgery will be carried out aseptically. Animals will be humanely killed at the end of the proposed experiments and</p>

	tissues will be analysed.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Studies in living animals are necessary to study the stem cells that are naturally present in the joint and define their role in joint tissue maintenance, remodelling and repair. None of these experiments could be performed in a dish in the lab. Cell or tissue culture cannot mimic this, as the interactions between the different tissue and cell types within the joint and in the entire body are lost.</p> <p>To study stem cells in arthritis there is a requirement to look at the cells in the full picture consisting of continuous interactions across multiple cell types, tissues and organs in a living body with circulation through bloodstream of a myriad of molecules. There are no suitable experimental models of arthritis in non-protected animal species.</p> <p>In order to test the ability of human stem cells to make new joint tissues including cartilage and bone, we routinely employ several assays in laboratory dishes to determine the capacity of stem cells to form such tissues. However, evidence indicates that often they are an overestimation of the true in vivo capacity of stem cell populations. Hence, normally after extensive screening using dishes in the laboratory we proceed to confirm the findings obtained in the laboratory with appropriate experimentation in living animals, as this is a required step for any clinical translation.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The procedures involved in this study are well-established and can therefore be performed in a way that provides maximum information with the minimum number of animals determined using statistical analysis.</p> <p>Experiments are planned very carefully. The availability of different optimised and specialised mouse models allows choosing for each circumstance the model, strain, sex and age that best addresses the scientific question and gives the most robust and reliable outcome, thereby</p>

	<p>allowing to obtain the information sought with the minimum number of mice.</p> <p>The use of internal controls whenever possible (i.e. in a mouse arthritis is induced in one knee while the other knee of the same mouse is not treated and therefore used as internal control) eliminates the variability related to each individual mouse, and reduces the number of mice needed for the study.</p> <p>In vivo imaging allows studies in mice that are longitudinal with multiple assessment in the same mouse at different time-points, thus reducing further the total numbers of animals used when time-point analysis is required.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse is the most appropriate mammalian species and also now considerably more valued in preparation for clinical studies. The procedures in this study are internationally well-established and routinely used by academic and industrial institutions for preclinical studies and assessment of novel treatments. Several similar experiments have been carried out previously and have provided sufficient information to enable us to perform them in a way that provides maximum information but minimal distress to animals. Surgery is carried out by experienced individuals using sterile techniques to prevent infections. When needed, anaesthesia and pain-killers are provided. Mice are monitored regularly and, if needed, extra bedding is provided. When necessary, mice are provided with a diet with soft food for ease of eating. Veterinary staff is always accessible for advice and assistance in matters pertaining to the welfare of the animals.</p>

Project	Skeletal Mechano-Pathobiology	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Disorders of the skeletal system may result from hereditary or acquired pathologic processes. Impairments may result from degenerative processes as well as traumatic events. Two of the most prevalent skeletal conditions are osteoporosis and osteoarthritis, both of which will increase even further as a consequence of increasing longevity and lifestyles. Those conditions result in fracture, chronic pain, impaired quality of life, higher levels of morbidity and mortality and provide a challenge in terms of management and health economic costs. There is therefore a continuing need to advance our basic knowledge on the remodelling, repair and regeneration of the skeletal system in order to	

translate understanding of mechanisms to new clinical strategies in prevention and management of skeletal disease.

Our project uses animal models of these disorders, genetically modified rodents and a range of specific protocols to further understand the mechanisms and essential factors regulating skeletal tissues remodelling and repair from development to ageing with the ultimate goal of providing directions for drug developments to alleviate osteoarthritis and osteoporosis and the impacts of their consequences such as pain and fragility fractures. Our work under a previous Licence has indeed led to successful transition into proof-of-concept studies for the treatment of osteoporosis, with successful avoidance of ovariectomy- and neurectomy-induced bone loss with the compound REDACTED. Also in the alleviation of gait abnormalities that develop in line with advancing osteoarthritis in the STR/Ort strain of mouse in response REDACTED administration in vivo.

There is a still continuing need to translate understanding of mechanisms to new clinical strategies to improve prevention, diagnosis, control and treatment of skeletal disease. For example, despite considerable advances, the mechanisms controlling the response of cells to specific biological and defined mechanical stimuli have not been fully elucidated. This is true not only for bone tissue, which has been the initial focus of mechano-biological research, but also for other non-calcified musculoskeletal tissues where the lack of knowledge of these specific mechanisms is even greater.

Our aim is to build upon current knowledge and extend understanding to variables such as genetics, mechanical environment, hormonal changes and ageing. The fact that musculoskeletal disorders lead to significant human suffering, this work is focussed on furthering our understanding of the mechanisms controlling normal physiological function of joints and bone in the pathogenesis of osteoarthritis and osteoporosis. They also align with a One Medicine approach and seek to similarly inform drug development in the treatment/prevention of

	<p>canine osteoarthritis.</p> <p>In each of the protocols we aim to emulate some aspect of the human pathobiology in order to understand and interfere with the pathophysiological processes. In both osteoarthritis and osteoporosis, there is a multi-cell involvement with complex systems that cannot currently be addressed in tissue culture or non-animal model systems alone. In osteoarthritis, the main surgical model that we propose to use is akin to sports injury in people which go on to develop osteoarthritis, therefore these models are important paradigm for interventional studies to ameliorate the outcome.</p> <p>Our program of work will principally investigate:</p> <ol style="list-style-type: none"> 1. The identification of the molecular mechanisms and novel regulators involved in skeletal tissues repair and functions. 2. The response of specific skeletal tissues to defined mechanical and biological stimulation and how this response is compromised with age and disease. 3. The interactions between biological factors and mechanical loading for the maintenance of skeletal tissues during ageing. 4. The influence of age, disease, mechanical and biological factors on the repair processes of skeletal tissues and structures.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The aim of our project is to gain better understanding of the factors and their mechanisms of action, regulating bone and joint physiology for diseases in which these tissues are affected. Overall, our research aims to improve the quality of life and mobility of people with bone and joint pathologies. Ultimately, we hope to help develop directions for treatment options for those pathologies and for the associated pain.</p>
<p>What species and approximate numbers of animals do you expect</p>	<p>We will use a maximum of 9,000 rats and mice, mainly mice over a period of five years. The</p>

<p>to use over what period of time?</p>	<p>rodent species to be used are appropriate because their fundamental skeletal biology is very similar to humans in many regards and there is the advantage that genetic models, probes and antibodies are available. The most appropriate models of osteoporosis and joint disruption are in rodents. Sample sizes to be used are based on previous work and a calculation to estimate the minimum number of rodents required for establishing significant differences between groups.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>All procedures to be undertaken are performed in rodents and do not exceed "moderate" in severity. We have developed over the years appropriate animal models of skeletal diseases and protocols that aimed at investigating the remodelling responses of skeletal tissues to their mechanical and biological environment. All these experiments are performed by appropriately trained experimenters and are essential for the success of this project. Animals will be sacrificed by Schedule 1 at the end of experiment.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The processes involved in achieving adaptive changes in bone architecture and mass and those involved in the degeneration of joint that are likely promoted by mechanically-derived loads are incompletely understood and can only realistically be replicated using live animal models. <i>In vitro</i> organ culture systems appear capable of at least partly replicating the events whereby these mechanical stimuli are applied and may therefore be useful in examining the immediate and short term responses to such application, but they are completely incapable of replicating the longer term osteogenic response in bone to create functionally appropriate changes in architecture and mass. These <i>in vitro</i> approaches also fail to produce the range of structural abnormalities in joint architecture that can be seen, sometime after, in response to abnormal loading in the intact joint. Monolayer cell culture can sometimes be used to replicate selected aspects of both of these types of responses but they fall short of providing</p>

	<p>integrated, organ-level, physiologically intact environment in which such responses are normally coordinated.</p> <p>These <i>in vitro</i> and cell culture based alternatives have been, and will be, used by us as replacements wherever possible to examine some selected aspects of the responses we aim to more fully decipher. We have fully acknowledged their strengths, reviewed their use for others, but appreciate their limitations (see also 3Rs, above).</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We always aim to reduce the numbers of animals we use. Power analyses are applied in order to identify the minimum number of animals that we need to use in order to answer the specific question being posed. For instance, we have established that our tibial bone loading studies require group sizes of no more than eight to secure statistical significance. Wherever it is possible we will also exploit contra-lateral limbs as controls in order to reduce the numbers of animals required still further; the possibility of exploiting such controls is another area in which future reduction in numbers may be achieved. This may not always be possible, however, but efforts will be made in all initial investigations to secure the validity of internal control samples.</p> <p>The principles of our experimental design have been already established in our on-going programme of study and so there is little need in performing studies to modify our bone loading programme. This is not necessarily the case for joint loading but advances are being made all the time and it is our hope that during this particular programme of study that we will have identified an optimised osteogenic loading protocol; an important step, as it will mean that we more fully understand the mechanical drivers of osteoarthritis – a vital advance in our understanding.</p> <p>If loaded animals are also simultaneously treated with compounds that may modify bone remodelling then animals will be previously randomised and blinded (see also The 3Rs, above).</p>

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We have chosen to focus particularly on rodents. This decision has been made as it will provide us with the potential to explore the role of specific genes in the response we identify, through the use of mutant and transgenic mouse models. Indeed, the choice to develop the tibial bone (REDACTED) and joint loading model (REDACTED) in the mouse was made with this purpose firmly in mind. These models are being replicated by other groups and represent the fore-front of this <i>in vivo</i> approach to address questions in bone and joint mechanobiology.</p> <p>Animal suffering will be limited in our studies by our strict monitoring of severity limits and our use of protocols that do not produce excessive trauma or suffering. The alternative strategies which others have used to attain similar end-points frequently involve surgery and our use of surgical approaches will be kept to a minimum. Appropriate pain relief during our protocols will be achieved through appropriate levels of analgesia (see also The 3Rs, above).</p>
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Project	Sleep apnoea and cognition	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Sleep-disordered breathing is linked to many other illnesses and disorders, such as heart failure and cancer. It also has the potential to effect brain function, including cognition, though both sleep deprivation and neuroinflammation.</p> <p>We wish to understand the influence of sleep on cognitive function and neurodegenerative disorders. To do this we will expose wild-type and genetically-modified rodents to behavioural analysis in learning and memory tasks. We will do this in models of sleep apnoea to indicate whether this disease has a detrimental effect on cognitive function. We will also study cognition in models of Alzheimers disease with and without sleep apnoea, to indicate whether sleep</p>	

	apnoea hastens the progression of neurodegenerative disorders.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The human brain, like other organs, is affected by ageing. In severe case it can be effected by neurodegenerative disease which puts the person at risk of harm and jeopardises independent living, placing heavy burdens on families and society. Neuodegenerative disorders may be worsened by sleep apnoea, which increases in prevalence with age. There is therefore a great need to both understand the effects of sleep anoea on normal cognition and it's effect on the progression of neurodegenerative disease, and to develop strategies to limit the impact of sleep apnoea on brain function. An understanding of the factors and mechanisms that influence the degeneration of the mammalian brain will have impact on the care of vulnerable adults. Mechanistic insight into the influence of sleep apnoea on neurodegeneration provided through this project will reinforce – and explain - the importance of monitoring patients for what is considered a seemingly innocuous disease, but which is in fact is quite insipidous.
What species and approximate numbers of animals do you expect to use over what period of time?	10,000 Rodents over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The learning of a new behavioural task may initially be moderately stressful as the animal will be exposed to a novel, unfamiliar environment. After the behavioural testing animals will be killed humanely and brain tissue will be used for various in vitro analyses. To induce sleep apnoea we are going to kill cells in the brain. As most people with sleep apnoea are not aware that they have this disorder, this should not cause any stress to the rats and mice who only have apnoea during sleep. There are obviously other possible complications of general surgery, infection, pain, haemorrhage etc. We reduce these complications by making sure everyone on the project is extensively trained in how to perform the procedure and animal care. We also have pain medication and antibiotics readily available

	<p>to treat rats and mice when it is needed. We also work very closely with a veterinarian and other highly trained technicians to make sure that the animals are well looked after and do not suffer in any way. At the end of the experiment all animals will be humanely euthanised.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>This study assesses how entire physiological systems interact over a period of time to lead to, or exacerbate, disease states. Unfortunately for this initial study we must use animals, as we cannot replicate complex organ systems outside of the body. We are also required to study cognitive behaviour, which again cannot be studied in a dish. As it would also be extremely unethical to perform experiments on human subjects, there are no suitable alternatives to experiments in animals. However, when we begin to home in on a mechanism, we may be able to replace some of the whole animal work with other experimental material, such as cell lines.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use pilot studies to reduce the number of experimental groups, by only moving forward with experiments that lead to significant results in preliminary work. Using carefully design experiments to change specific parameters, the optimisation process will use the minimum number of experimental animals possible.</p> <p>The experiments are designed so that following the initial surgeries the different diseases can be assessed using non-invasive methods. Therefore, a single animal can be used for all experimental time points, thus significantly reducing the number of animals used. In addition, at the end of the study, animals will be used in either non-recovery experiment or their tissue will be collected for studying <i>in vitro</i>, to identify the mechanisms that link these diseases. By using a single animal for both the behaving and non-recovery/<i>in vitro</i> experiments we can halve the number of animal used. Also by assessing the animals before the terminal/<i>in vitro</i> experiment, we can assure that diseases</p>

	<p>we wish to study have occurred before the final experiment takes place, thus all terminal experiments will provide meaningful data, again reducing the numbers of animals used in this project.</p> <p>We will also make all tissue, not used by the primary investigator in the respective studies, available to other investigators, so as to reduce the need to repeat procedures for different studies. We base our sample size calculations on both our prior experience and that of others. This will allow us to generate robust, statistically significant data upon which to draw firm conclusions and in doing so both advance the field and iteratively adjust the sample size of future studies.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The process of refinement will be ongoing and assessed continually as new data arrive. We also take great care to keep up to date with animal welfare literature and work closely with animal welfare professionals to provide the best care possible.</p> <p>We will use pilot data from different models to find the best model in terms of both experimental and animal welfare. If less invasive models provide good quality data, then they will be used to carry the project forward.</p> <p>The use of terminal experiments will also help us to determine possible regions of interest in the brain, and may also help us to identify the mechanisms linking sleep apnoea to other disorders. We will use this data to refine our techniques, and to better design future experiments</p> <p>Wherever possible, we will endeavour to design the experiments that use the least number of animals possible, and that cause the least amount of pain, suffering, distress, and lasting harm to those that we do use.</p>

Project	Small animal models of cardiovascular disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This programme of work has 2 main objectives:</p> <ol style="list-style-type: none"> 1. to determine whether a novel compound which blocks the function of a specific heart cell membrane protein (ion channel) has a beneficial effect during a heart attack and 2. to determine whether a non-invasive protocol of temporarily disrupting blood flow to the limbs can modify re-modelling of the heart caused by high blood pressure 	
What are the potential benefits likely to derive from this project (how science could be advanced)	This project aims to improve our understanding of how the heart is damaged during a heart attack, and also to assess the efficacy of a novel group	

<p>or humans or animals could benefit from the project)?</p>	<p>of compounds to treat the immediate muscle damage and reduce the chronic adverse effects of a heart attack on the structure and function of the heart, which leads to heart failure. The project will also investigate the links between a newly identified protein on the cell surface in the heart (Kir6.1) and the hearts own in-built protection mechanisms. One way that this is thought to be activated is by bursts of exercise. This project will investigate how short periods of exercise can protect the heart from damage. Finally, it will also assess the potential for a non-invasive procedure (remote ischaemic conditioning – achieved by repeated temporary interruption of blood flow to a large muscle bed, e.g. a limb, using a tourniquet) to modify the enlargement of the heart due to high blood pressure or diabetes, which are among the most significant risks for heart disease.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>1,100 rats and 1,250 mice over 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Models of myocardial infarction (heart attack) and persistent hypertension (high blood pressure) are prepared surgically with full recovery of consciousness; therefore there are both anaesthetic and postop pain risks to the animals. Anaesthesia will be carefully monitored until full recovery occurs, and postop pain will be treated appropriately for as long as necessary. There is a small risk of intra-operative or immediate post-operative death in the animals undergoing the heart attack procedure, however these animals will likely die under full general anaesthesia therefore no suffering is expected to occur. Severity is graded in the 'severe' range for the heart attack model and 'moderate' for the high blood pressure models. Longer-term consequences, including the development of heart failure, will be routinely assessed by clinical signs and imaging where appropriate. Remote ischaemic conditioning (temporary interruption of blood flow to a muscle bed, e.g. a hind limb) should have no risk other than repeated sedation, and would normally be considered to be of mild/moderate severity. However animals transferred from the surgical protocols may be at</p>

	<p>increased risk of death during this procedure, therefore under these circumstances, this protocol has been classified as 'severe'. Induction of diabetes is expected to be of 'moderate' severity and will be used for additional studies. It may be combined with the models already described (heart attack, high blood pressure, remote ischaemic conditioning) conferring some additional risk, but this will be mitigated by increased monitoring whenever necessary. Exercise conditioning (protocol 5) is considered to be of moderate severity. All animals in this protocol will be purchased specifically for this protocol and will not be from other protocols in this licence. Animals from this protocol will be used in protocol 1 or for tissue harvesting following a schedule 1 procedure. No knockout animals will be used in this protocol. The severity for protocol 6, GA breeding, will be mild, however the severity for the second breeding protocol (7) is graded as 'severe' due to the fact that Kir6.1 knockout animals are prone to sudden cardiac death. As such, knockout animals that are bred in protocol 7, and used in protocol 1, will be maintained under terminal anaesthesia during the reperfusion phase and killed at the end of the procedure. All animals will be humanely euthanised at the end of the experiments by trained staff.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Diseases of the heart and circulatory system are multi-faceted and rely on interaction between several body systems. These include complex biochemistry, hormone changes and blood pressure effects and cannot be replicated in vitro.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Appropriate statistical tests will be applied to all experimental procedures. Once the proposed models are validated (pilot data) power calculations will be used to determine group sizes.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will</p>	<p>The techniques described are widely used in rats and I have substantial experience in this species. They are established in the scientific community as excellent clinical models and data generated</p>

<p>use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>are considered applicable to human disease. Welfare costs to the animals are significant but will be mitigated by extensive use of pain relief and by the use of sedation/anaesthesia whenever it is required. To investigate the effects of elevated glucose on cardiovascular function, we have refined our surgical protocol to look at acute hyperglycaemia, rather than streptozotocin-induced diabetes. This will have significant benefits to the animal by reducing the need for the induction of diabetes to achieve the experimental aims</p>
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Project	Specialist Aging, Support and Supply Service for Previously Approved Neuroscience Projects	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To assist in the provision of aged rodents for the discovery of new neurological medicines, and, where medications are already available, to provide more effective treatments with less side effects.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit)	This project will provide naturally aged animals as models for neuroscience research including research into disorders associated with aging. This project will assist projects in the sourcing Genetically Altered Animals with either	

from the project)?	spontaneously occurring phenotypes, or experimentally induced phenotypes which are relevant to aging conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	If every protocol on this licence were used to its maximum, then this project will use up to 60,500 rats and mice. A more realistic figure would be 36,000 animals, but this depends on the demands of the customer as they receive results of ongoing animal studies and clinical trials which may influence their demands accordingly in the future.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals will be allowed to age naturally and the effects will be those of a naturally aged animal. At the end of the study, the animals will either be humanely killed, and their tissues harvested, or they will be supplied to other projects where they take part in studies to look at the effect of ageing on neurological systems.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Naturally aged tissues come from animals that have been through the ageing process and provide samples that cannot be produced in a laboratory.</p> <p>Genetically Altered Animals will also help us obtain this tissue, and understand the effect of the presence and activity that a gene may have in the ageing process.</p> <p>None of this would be measurable in non-animal alternatives.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Studies are designed using the advice of an experienced statistician, to ensure that the number of animals used is kept to a minimum, while providing the data outcome required.</p> <p>By ageing animals in a barriered environment, and managing their access to feed, the number of animals are kept to a minimum as more will survive into old age allowing the study of ageing.</p> <p>Where possible homozygote animals are bred to maximise the number of animals produced for</p>

	<p>experiment while reducing wastage.</p> <p>By using superovulation, less animals are required to produce the numbers of embryos required.</p> <p>By cryopreserving the colonies, it will not be necessary to breed animals simply to maintain the reference gene stock.</p> <p>Best practices as published in current literature will be followed to ensure best practices are followed, such as those published by the Home Office, LASA and the IAT.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rats and mice used in the Project will go on to support other Neuroscience Projects. In these projects rats and mice may be easily trained to perform behavioural tasks which can be used to look at the effects of ageing. The animal's performance can easily be recorded, making them good models for this work.</p> <p>The animal's access to feed will be controlled ensuring they are less prone to being overweight, and reducing the incidence of tumours, circulatory dis-orders and cases of arthritic diseases.</p> <p>Whenever possible animals will be kept in social groups, unless they prove incompatible, or social housing would null the value of the experiment. Animals will be provided with environmental enrichment and nesting material to provide more stimulation in their environment.</p> <p>Genetically altered animals can be used to study the effects of a single chemical in the body, and its relevance in ageing diseases, or interactions with the medications being developed.</p> <p>Each animal will have its own lifetime history, which will be supplied to the scientist for future reference in their experiments.</p> <p>Animals will be aged in a facility specialising in the care of aging rodents, by Technicians who are specialised or can specialise in their specific care as they age.</p> <p>Transgenic animal tissues can be compared to</p>

	non-transgenic wildtype.
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Project	Splicing modulators as anticancer agents	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Despite tremendous efforts in biomedical research in the last 50 years there is still a high incidence of cancer and mortality due to cancer in the world, including UK. More than 90% of deaths related to cancers are attributed to metastasis – the process through which the cancer spreads from the initial site to various organs. Though many treatment options are available these days for cancer patients we are still far from curing the disease and most often we are barely able to slow its progression but not prevent people from dying of cancer.	

	<p>Therefore there is a lot of interest in <i>i)</i> understanding more of the basic biology of cancer and metastasis; and <i>ii)</i> use this basic information to find novel therapeutic targets and avenues.</p> <p>The drugs we are trying to develop relate to an important aspect of gene regulation, called “alternative splicing” - a process in which parts of a gene are assembled together in different ways.</p> <p>It is known that the DNA is the molecule that contains the genetic code to make proteins. However, it cannot do that directly – it is first transcribed in the nucleus of cells into another molecule called RNA; this is the messenger that gets the code from the nucleus to the protein building machinery in the cells’ cytoplasm. The proteins give functions to our cells.</p> <p>Before the proteins are built, the RNA may be edited – parts of it spliced and re-joined together – this is what is called “alternative splicing”. It is therefore possible to get multiple proteins – called isoforms – from a single gene.</p> <p>These isoforms are different in disease compared to normal ones. If we could switch back the splicing to the normal pattern, we can obtain a therapeutic effect – this is what we are trying to achieve with the substances used in this project.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This project addresses one of the most under-explored areas in cancer cell biology – the possibility to manipulate defective cell properties at a different level inside the cancerous cell than the ones used today in anti-cancer therapies. What we mean by different level is that anti-cancer drugs in clinic today are affecting various cell properties and/or proteins – like signalling inside the cytoplasm, capacity to repair DNA, proteins involved in cell division and multiplication – none of the known drugs are using “alternative splicing” as a target - which is under study in this project. This is the level of so-called “post-transcriptional” regulation in which a class of molecules named splicing factors play a major role. While a lot is known about the properties of splice factors in</p>

	<p>cell culture there is very little understanding to whether manipulation of splice factors is able to inhibit tumour growth in vivo as well as the spread to different organs. Therefore the research described in this project is essential for obtaining proof-of-principle that splice factors and connected molecules may be used as therapeutic targets to fight cancer</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>3000 mice over 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The most used model is the subcutaneous model – in this we will implant tumour cells into mice; this involves only a single injection under the skin. After a few days tumours start appearing under the skin at the site of injection and we measure them with a calliper. In this model tumours usually do not metastasize, and we do not expect to see adverse effects because of the tumours. Animals may also receive substances which are intended to alter tumour growth. These may be given by a number of routes, but often can be given in water or food. Careful monitoring of the animals is crucial for our studies, and we have strict criteria when animals are to be killed, depending upon the level of development of tumours. For example, if the skin over a tumour was seen to breakdown, the animals will be killed straightaway. Animals will be inspected daily and the presence of signs of distress assessed. Tumours will only be allowed to grow to a predetermined size and if the animals normal behaviour is altered, they will be killed. The design of our experiments will be such that we will minimise animal use but maximise data collection; often animals will act as their own controls, and repeated measurements minimises the numbers used. To minimize possible stress to animals, tumour measurements (e.g. by calliper) will be made maximum three times per week. To assure an effective and optimal monitoring of adverse effects in mice treated with various substances we have put in place a “welfare score sheet” that is checked and updated daily with a systematic monitoring of various signs and</p>

symptoms: weight, clinical signs post-anaesthesia (loss of coordination, abnormal breathing), swollen abdomen, gastrointestinal problems (diarrhoea), hunched posture, pilo-erection, restlessness, less mobile and alert, isolated, vocalisation, self-mutilation. All these signs and symptoms are scored and based on this appropriate action will be taken – for a low score close monitoring and/or analgesics; for higher score, termination. In some animals we might insert cancer cells by microsurgery into the prostate gland, breast or under the kidney capsule. Surgery is done under strict aseptic conditions and there is a protocol in place for analgesics administration to be sure animals do not experience pain. We will then monitor the animals carefully and make use of special imaging machines that allow us to repeatedly view the tumour cells in the living animals both in the primary tumour as well as during their spread in the organism. This is performed under anaesthetic (to keep the animals still) and is non-painful. To minimize the possible stress to animals provoked by repetitive anaesthesia, we will perform the imaging maximum twice weekly. This technique allows us to reduce the number of animals that we need to use. Animals may be administered substances, as described above. Most of the times the experiment will finish before any possibility of discomfort from the tumour growth and/or spread – this is because of the sensitivity of the in vivo detection device. Regardless, animals will be carefully monitored using the score sheet described above. In some mice we will inject tumour cells directly into circulation to study metastasis – the spread of cancer cells into the whole organism. The experimental data on how metastasis develops is also collected by in vivo imaging. Again, because of the sensitivity of the device, the experiment is most of the time terminated before there are any chances for the animal to display discomfort – nevertheless, we will closely monitor animals using the score sheet described above. The most common sites for metastasis are the lungs – in which case difficulties breathing may develop or liver – in which case collection of liquid and abdominal distension may happen – if we notice any of these clinical signs, the

	animals will be killed.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Tumours are as complex in structure and organization as organs are. Though the cancer cells form the bulk of the tumours volume they contain other types of cells e.g inflammatory cells as well as a sophisticated network of blood vessels. An important component of the ability of tumours to grow is based on the interactions with the host organism and the structures surrounding them (so called tumour microenvironment). Therefore it is essential to study tumour biology in vivo in animal models as only limited information may be obtained from culturing cancer cells in incubators (the only other alternative). Additionally, metastasis, the process of cancer spreading to other organs, is a process that happens in vivo in the whole organism and there are no other experimental alternatives</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Experiments will be done using a special design with repetitive measurements of the tumour volumes in the same animal. This has greater statistical power, and animals need only be killed at the end of experiments rather than at each time- point drastically reducing the numbers of animals being used.</p> <p>Transgenic (genetically altered) mice numbers will be kept to a minimum by using crossing designs that result in minimal animal numbers, demand will be assessed before breeding and crossing, colonies will only be maintained while there is an experimental plan and funding allocated</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Two animal models will be used:</p> <p>1. Nude mice – mice that have been genetically altered to inhibit their immune system; these mice are widely used and considered the best model across the world for human cancer cells implantation studies; the main reason is that human cancer cells would not be able to grow in a different species if the immune system would be intact; in particular for my project, this</p>

is the best model possible – we are investigating the response of various types of human cancers to different chemicals; we can grow human tumours only in this model

2. Genetically altered mice that harbour a so-called “splicing reporter” – a tool through which we can understand gene regulation in various tissues and cells just by imaging– compared to other species mice are considered the easiest to be manipulated genetically and therefore we used mice to genetically insert these reporters.

We are using mouse tumour protocols that have previously been used to study growth inhibition to reduce the number of experiments. Thus mice will be killed before the tumour load becomes large enough to impair health in these animals, thereby reducing the likelihood of pain, suffering, distress or harm. The following models are used:

1. subcutaneous implantation – tumour cells are implanted under the skin; this is the simplest model we can use to grow human tumours – there is virtually no suffering for the mice and they are killed when tumours are fairly small in size

2. metastasis model – we inject tumour cells in circulation through the tail-vein to mimic metastasis – usually to the lungs; this is the simplest model we can use for asking our scientific questions; there is virtually no suffering from the mice because we are monitoring metastasis very closely through imaging and mice are killed usually way before any clinical signs

3. orthotopic models for prostate, breast and kidney – sometimes tumours do not grow unless re-implanted back in the organ they originated from; this is done with surgery under anaesthesia and aseptic conditions; the project leader has more than 15 years experience with these models and the procedures and monitoring are designed so there is no suffering for the mice

The substances that we want to work with in this project are either known drugs or are chemicals that have been developed with the

intention to become drugs – they are therefore known to not be in general toxic. They have been tested extensively in cell culture and have been shown that are able to inhibit various functions of cancer cells. They are therefore strong candidates to be developed in the future as anti-cancer drugs. We hope to show that they inhibit tumour growth or spread of cancer cells in the organism.

Because these substances are known and studies before, we do not expect any important unwanted effects when administered in mice. However, for added safety, for any substance that we did not use before in mice, we will follow a staged approach, in which we will perform pilot studies for tolerability.

The introduction of repetitive imaging procedures reduces the number of animals that need to be used for tumour experiments and reduces the burden on those animals. Tumours can be detected when smaller than palpable, and metastases can be imaged before signs of distress occur. Since the objectives of these experiments are to determine the mechanisms underlying splice factor importance for tumour growth and metastasis in animal models of disease we will be investigating the early time points of tumour growth, when the least adverse effects are seen. Therefore these experiments are designed to cause the least pain, suffering, distress or lasting harm possible to achieve the objective. Furthermore, if the animals appear to be suffering, in pain or the tumours show evidence of harming the animal, the experiment will be terminated by killing the animal.

Project	Standards in Virology
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input type="checkbox"/> Basic research
	<input type="checkbox"/> Translational and applied research
	<input checked="" type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective is to make the materials necessary to be able to test the quality and effectiveness of biological medicines, such as vaccines. These tests are essential to ensure the vaccines are safe and effective before being administered to humans.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The systems and assays used to control vaccines are well established and of demonstrated effectiveness. The reference materials and working reagents that are essential to the provision of effective vaccines can currently only be generated by the use of animals. The materials made under this licence contribute to the control of the potency and appropriateness of the vaccines produced by manufacturers and form a central part of

	<p>the process by which satisfactory and protective products are marketed globally. The consequences of using a vaccine of low potency or inappropriate strain are that it will fail to protect recipients and disease burden in the human population could increase.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Embryonated eggs domestic fowl 2000 Ferrets 100 Turkeys 60 Sheep 150 Rabbits 20 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Typically the majority of animals used are not expected to experience significant ill-effects. The overall severity for all but a small number of ferrets (up to 25 animals over the course of 5 years) will be mild. For the ferrets that will be infected with virulent influenza, the severity may be moderate the outcome of these infections can be unpredictable and so animals will be monitored very closely, the animals will be treated with antiviral medication to limit the symptoms they experience due to the infection. Any animal that has any significant adverse effect will be humanely killed using an overdose of anaesthetic. For animals being immunised there may be some local irritation at the site of inoculations particularly where adjuvants are used (adjuvants are chemical compounds that increase the immune response to an inoculation). This will be monitored and advice from a vet will be sought if any adverse effect is seen. All animals used under this licence will be humanely killed at the end of the study, or earlier if it is deemed necessary for the welfare of the animal.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Specific antibodies are required for use in tests to characterise viruses and evaluate viral vaccines. These can only be generated in protected animals. We are investigating more modern methods of producing antibodies in the laboratory, using bacteria, but research in these areas is still at an early stage and is not yet ready for use.</p> <p>Materials prepared for evaluation of vaccines</p>

	<p>must be prepared on the same substrate as vaccines. Embryonated eggs are currently the most common way by which vaccines against influenza are manufactured.</p> <p>Turkey blood is a critical component of assays used to assess viruses and viral vaccines and the immune responses they elicit.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Use of ferrets: - there will be sharing of production with other organisations requiring sera to avoid replication.</p> <p>Few animals are required to generate the sera that are needed, but experience has shown over the years that several animals must be immunised or infected to be sure that there are sufficient suitable sera. Between two and four ferrets will be used in infection experiments and the same number of sheep for immunisation with non-replicating antigens. The numbers of animals proposed is regarded as the minimum necessary to produce the desired result, based on the experience of many years.</p> <p>We are aiming to survey vaccine manufacturers to ask if they can use less volume of sera to test their products, and if so we will be able to reduce the amount we provide and thus reduce the number of sheep used to make sera.</p> <p>The number of eggs required is more predictable and is regarded as the minimum to assess the material needed to test vaccines. The volumes of blood from turkeys are those required based on years of experience.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Ferrets are among the few animals other than primates whose response to infection with influenza reflects that of humans; both the immune response and the clinical signs closely resemble that seen in humans. Methods for observation of clinical signs have been developed for earlier recognition of onset of disease allowing earlier intervention with the use of medication to relieve symptoms or</p>

	<p>termination as appropriate.</p> <p>We will use pain relief medication (non-steroidal anti-inflammatory, similar to ibuprofen used by humans) to relieve symptoms in all infected animals, and antiviral medication to reduce the clinical symptoms in animals infected with virulent influenza.</p> <p>It is recognised that group housing is preferable for optimum well-being of ferrets and wherever possible they will be group housed. There are situations where single housing is required due to husbandry needs or for safety reasons (for example where two male animals would otherwise fight and potentially injure each other). There are also occasions where an animal has been infected with a virus and must be housed singly to avoid infecting other animals. In these situations wherever possible animals will be housed in cages in rooms with other ferrets.</p> <p>We will also try to use animals that need to be housed singly for husbandry reasons for the experiments that require an animal to be isolated due to being infected with a virus. This will minimise the number of animals that are not group housed.</p> <p>Sheep are required to generate the large amount of serum required for a reagent for global use. The effectiveness of alternative adjuvants has been and will continue to be explored with the aim of minimising or removing the need to use Freund's adjuvant. Mice are too small and the response too variable to make them a practical alternative.</p> <p>Embryonated eggs are the least sentient animal available and are used at < two thirds incubation whenever possible.</p>
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Project	State-dependent neural processing	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to understand how processes in the rest of the body influence information processing in the brain. Brain networks control instinctive behaviours such as parenting, feeding and aggression, but the animal's current (internal) state profoundly affects their function. Hungry animals for instance will be strongly attracted to food, whereas animals that have recently eaten typically ignore or even avoid food. We will (i) perform behavioural experiments to uncover which behaviours are affected by state changes such as pregnancy, (ii) determine which brain areas and neurons are affected by these states, (iii) record and manipulate the activity of these identified neuronal populations and (iv) aim	

	to identify the cellular mechanisms by which state changes can permanently remodel the brain.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The main benefit that will arise from this project is knowledge about how internal states affect the brain, and more generally about how the brain processes information. Internal states such as pregnancy, hunger, or aggression are highly similar between mice and humans, and so are the brain networks controlling many basic behaviours. Therefore, the results of this project will give fundamental insight into the function of the human brain. Gaining knowledge about how internal states and hormones affect the function of the healthy brain is essential for understanding what goes wrong in the diseased brain, such as in types of depression or –anxiety that frequently occur after giving birth. This project will also increase our knowledge of mouse animal behaviour, and in particular will identify states that can profoundly alter brain function and behaviour – this can be used to refine future animal experiments. Moreover, we will develop new tools (e.g. maps that visualise where hormones bind in the brain) that will be of interest to scientists across a broad range of neurobiology.
What species and approximate numbers of animals do you expect to use over what period of time?	This work will use less than 7000 laboratory mice over 5 years. Mice are an ideal model for this programme, since their parental, aggressive and feeding behaviours are strongly affected by the animal’s current state, and because a large range of cutting-edge techniques are available for recording and manipulation neurons in the mouse brain.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In order to record from neuron networks in the brain, mice undergo surgery under deep anaesthesia to implant recording devices, fixed externally to their skull. They are cared for after surgery and receive pain relief until they recover completely and adapt to the devices; there are no pain receptors in the brain so complications may only arise if the device detaches from the skull, in which case it is repaired or the animal is killed. In a subset of experiments, the mice are then exposed to (social) situations in which a threatening stimulus can be present (e.g. an unfamiliar mouse intruding into their space) and

	<p>the simultaneous activity in their brain is recorded. In some studies, the function of specific neurons may be knocked out by using chemicals (such as pharmacological drugs or by removing cells) in order to confirm which regions are responsible for certain behaviours. In some cases, the hormonal state of the animal might be altered surgically (e.g. ovariectomy) or chemically (e.g. stimulation with pregnancy hormones), which might lead to mild transient distress/pain. Mice will always be fully recovered before entering further experimental/behavioural studies. In all these studies, it is critical that the mice exhibit natural behaviours so it is essential that the surgical procedures do not, in themselves, cause adverse effects which interfere. Repeated exposure to threatening stimuli may increase generalised anxiety but the recording sessions will be limited in duration and frequency to ensure no lasting harm. At the end of experiments, or if mice show signs of ill health, distress or suffering, they will be humanely killed. Brain tissue will be collected from animals post mortem in order to study the relationships between behaviour, neuron recordings and anatomy. Since anatomical, recording and behavioural experiments will be performed from animal at different stages of gestation (as well as after gestation), we will carefully determine any adverse on the pregnant animal, as well as possible adverse effects on unborn or newborn pups. In some behavioural experiments, pups (age typically P2-4) will be exposed to females of different reproductive stages (e.g. virgin female, lactating female). Although the vast majority (>95%) of females will either ignore the pups or be parental, a subset of animals might start showing aggressive behaviour, in which case the animals will be immediately separated. In the rare case that a pup should be wounded during such interactions, it will be immediately and humanely killed.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>This project aims to understand how processes in an animal's body influence information processing in the brain. This requires studying the intact brain in mice. It is therefore impossible to avoid the use of animals for addressing these questions,</p>

	<p>because other approaches such as neuronal cell cultures do not replicate the connectivity structure and of the brain, and preclude behavioural measurements. However, as the project – and thus our knowledge – advances, we will increasingly be able to use computer models as a replacement for subsets of experiments.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use several state-of-the-art methods simultaneously, together with powerful data analyses, to get the maximal amount of data and information collected from each animal. In addition, the statistical power of each experiment will be increased by collecting functional, anatomical and cellular data from the same animal. Also, in most procedures the experiment and control can be performed in the same animal, which further increases statistical power and reduces the number of animals used.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>All experiments will be done in mice. To minimise harmful effects, we will use recording and stimulation techniques that are well established and with which we have expertise. All experiments in the brain will target specific areas so that effects on other areas and functions are minimal. Surgeries will be done under aseptic conditions with appropriate anaesthesia and pain management. Experiments in awake animals will only be performed if the animals are stress-free and experience no visible discomfort.</p>

Project	Stem cell mechanisms for lung development and regeneration	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our lungs are built and maintained via the action of specific stem cell populations. The ability of the stem cells to produce new cells of the correct types in the correct places determines how our lungs are maintained throughout life. Stem cells are controlled via interactions with their neighbours and a number of cell-cell signalling pathways. If the control of stem cell behaviour goes wrong it can lead to a variety of lung diseases including cancer, when cells divide too much, and chronic obstructive pulmonary disease, when stem cells change their behaviour and do not make the normal	

	<p>types of new cells. Conversely, there are also opportunities to use stem cells to deliver treatments to diseased lungs, such as for individuals with Cystic Fibrosis. This project aims to understand how lung stem cells are normally controlled throughout life. In particular, we will investigate in detail how one specific signalling pathway (Receptor Tyrosine Kinase signalling) controls lung stem cells. Secondly, whether the phenomenon of cell competition can be harnessed to make stem cell transplants into the lungs a serious possibility. Thirdly, if mechanisms that control lung development in the embryo can be harnessed to improve the regeneration of diseased lungs.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This project will lead to an improved understanding of how lung stem cells are regulated. It will focus on very specific aspects of this regulation and will therefore contribute to the growing understanding of lung stem cell behaviour that is being gained by scientists world wide. In time this knowledge will contribute to new therapies for lung diseases, such as chronic obstructive pulmonary disease and Cystic Fibrosis.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This project will last 5 years and use genetically altered mice of all ages (embryonic, growing, adult, ageing). We expect to use 8900 mice over the 5 year project.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>This project will rely on the use of genetically altered mice which will be bred and maintained throughout. The genetically altered mice are used to supply animals with a specific genetic make-up for studying lung stem cells. The majority of animals bred for this project (88%) are expected to undergo no procedures, or minimally invasive procedures meaning that they will suffer only transitory pain/discomfort, such as an injection. They will be used for analysing the effects of specific genetic alterations in lung stem cells in otherwise healthy individuals, and also for supplying animals with specific genetic alterations for use in the other procedures on the license. 12% of animals will undergo more invasive procedures and are expected to show signs of pain and</p>

	<p>discomfort for a period of a few days, including losing weight, ruffled fur and lethargy. Examples of these procedures are minor lung injuries (where lung cells are damaged by the instillation of a chemical into the lungs; damage to the lung cells results in activation of the lung cells and allows us to study their injury-repair response) and surgical procedures (in which the animal is briefly placed under general anaesthetic so that stem cells which have previously been grown in a dish in the lab can be grafted into the lungs, or another site on the mouse, to study their behaviour in the body). At the end of each experiment, all animals will be humanely killed by an approved method and their lung tissue harvested for further analysis in the lab.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>This work is aimed at the better understanding of human lung stem cells and for this reason we use human lung cells grown in culture as much as possible for our experiments. All preliminary experiments will be performed on human lung stem cells grown in the lab using a culture system that we have developed. However, some experiments have to be performed in the context of the whole lung in the intact body because the lung stem cells are highly regulated by interactions with their neighbours including nerves, blood vessels, immune cells and extra-cellular material which can not easily be replicated in a dish.</p> <p>For experiments in intact lungs in the body of a living animal we use a mammalian species, as only mammals have a lung which is similar to our own. We cannot use humans for ethical reasons. We therefore use mice as the species of choice as they have a similar lung structure and cell organisation to humans. They can also, be readily genetically manipulated and kept in the lab.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>It is essential when animals are used in experiments to ensure that too many animals are not used unnecessarily, but equally that a sufficient number of animals is used to allow clearly experimental conclusions to be drawn</p>

	<p>and ensure that experimental animals are not wasted. For this reason, we perform pilot experiments (small-scale studies) to test hypotheses and only perform large-scale studies if the small-scale show an effect. In addition, the small-scale studies allow us to calculate the minimum number of animals needed for larger-scale experiments. All studies are designed carefully with the inclusion of age and sex-matched control groups to ensure that the maximum amount of information is extracted from the smallest numbers of animals.</p> <p>To ensure that only the minimum numbers of animals needed are bred for our research we keep careful breeding records and monitor closely to prevent over-breeding.</p> <p>We will also trial new methods for genetically manipulating stem cells directly within the lungs of adult animals which require a brief period of anaesthesia and the administration of a gene-altering agent directly to the lungs. If these are successful, they will reduce the number of animals bred for research as we will not have to breed so many complex genetically-altered mice, some of which cannot be used because they do not have the correct combination of genetic alterations</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use mice as the species which have a similar lung structure and cell organisation to humans, can be readily genetically manipulated and kept in the lab. In addition, mice are relatively short-lived compared with humans. This ability to genetically alter mice easily, together with their short life span, means that we can study the effect of changing a gene in the lung relatively quickly in both young and old mice. This is very important as many lung diseases that affect humans are only seen in older people.</p> <p>The majority of our experiments will result in a small number of genetically altered cells being produced within each mouse lung. These cells are labelled with a fluorescent protein and can easily be detected and their behaviour tracked by staining the lungs of the animals after death. This is a very informative experiment</p>

scientifically because we can study the behaviour of the genetically-altered cells in comparison with their unaltered 'control' neighbouring cells. Moreover, because only a small number of genetically altered cells are produced within each lung, the lung function of the animal remains normal and therefore there are no adverse impacts on the health and welfare of the animals concerned. This is a refinement over studies in which many lung cells are genetically altered in which the lung function of the animals can deteriorate thus making them sick.

For the more invasive injury experiments which involve stimulating the lung stem cells to repair tissue damage by using a chemical to injure the lungs, we have chosen methods that will provide the required experimental data, but which result in the lowest possible level of animal suffering. One such improved methodology was developed in the course of our previous licence in order to refine the previous method of chemical lung injury that was used in this lung stem cell research. This resulted in the animals experiencing less pain because their lungs were less injured, but at the same time the response of the stem cells to injury could be studied.

A small number of surgical procedures will be performed to deliver drugs, or cells, to the animals by the most appropriate route. These surgical methods will only be used when a less invasive alternative (such as simply injecting the drug or cells) is not suitable. Surgical methods will always be performed in aseptic conditions by suitable trained individuals.

In every experiment where animals are required to undergo an injury, or a surgical procedure, we will provide pain relief to the animals under the supervision of a vet.

Project	Stem cells and niche cells in lung regeneration, repair, and cancer	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our lungs are built and maintained by the actions of tissue-specific stem cells. Their behaviour must be controlled with exquisite precision so that they produce new cells of the correct type at the correct time in the correct place. Incorrect control of stem cell behaviour can be a contributory factor to several degenerative lung diseases and to lung cancer. The objective of the project is to advance our understanding of the regulation of lung stem cell behaviour. Specifically, we will study the regulation of lung stem cells by cell-to-cell signalling pathways and by their neighbouring	

	cells.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	<p>The British Lung Foundation estimates that approximately 12.7 million people in the UK have a history of longstanding respiratory illness including lung cancer and idiopathic pulmonary fibrosis (IPF). Lung cancer is the third most common cancer in the UK (2014), accounting for 13% of all new cases. In 2012, about 32,500 people had IPF in the UK and the incidence has continued to rise. One of contributing factors is that these diseases are multifactorial diseases that likely result from complex interactions between genetic and environmental factors. An improved understanding of cellular interactions and their impacts on stem cells during tissue regeneration and injury repair will advance not only the fundamental stem cell biology of the lung but also the better therapeutic development. The long-term goal in this work will advance our understanding of stem cell contribution to human lung diseases including IPF, Cystic fibrosis, and lung cancer for developing potential therapies. In addition, many lung diseases have a poorly understood initiating cause and progression and significantly impact upon quality of life. Signaling between cells is important to the control of various biological processes, including lung development, maintenance, and damage repair. These signaling interactions can become uncontrolled during lung disease progression. Works by other scientists are increasingly demonstrating that the mechanisms used during lung development are being re-used during maintenance and repair of tissue after damage. Therefore, it may be helpful to investigate these processes in the hope of finding ways to induce lung regeneration. Our studies will focus on the important cell interactions described and compare development and damage repair to discover therapeutic targets for lung disease.</p>
What species and approximate numbers of animals do you expect to use over what period of time?	<p>This project will use mice, including genetically modified strains. The maximum number of mice that could be used over a 5 year period would be 21,150.</p>
In the context of what you propose	<p>The project includes protocols to investigate cell</p>

<p>to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>behaviour in regeneration, repair, and early tumour formation. To do this we will use techniques such as drug administration to genetically modify animals in order to either label cells or induce specific cell type damage, which will allow us to understand how stem cells generate specific cell types. Sometimes we may replace naturally occurring cells with our modified and traceable cells using transplantation techniques, so that we can precisely study how these cells behave. All animals are carefully checked regularly and if there are any concerns animals are examined and weighed. The majority of the animals will be used in protocols under which they suffer either no, or very mild, adverse effects. For example, transient pain or discomfort following an injection. A smaller number of animals (<5% of the total) will be exposed to protocols of moderate severity to model different aspects of human lung disease. Some weight loss with or without other signs of ill health may be seen. In these cases, animals will be humanely killed.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Lung stem cells are regulated by their surrounding environment. This includes other lung cell types, nerves, blood vessels, and immune cells from the circulation. This complex environment cannot yet be fully recreated in a dish in the laboratory and so needs to be studied within the context of the whole organism. Nevertheless, some aspects of lung stem cell regulation can be studied in cultured cells and where possible we use this non-animal alternative. Importantly, we have recently established a lung organoid co-culture system that visualises stem cell behaviours and recapitulates cell-cell interactions between stem cells and neighbouring cells in a cultured dish. We will extensively use this powerful tool prior to use of animal models to prove our findings.</p> <p>Whenever possible, we will implement <i>ex vivo</i> (in tissues outside of body) techniques to obtain preliminary data to inform experiments with animals. Importantly, we have been educating more than 100 scientists from academia and</p>

	industry to grow <i>ex vivo</i> lung organoids REDACTED.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will perform preliminary experiments on lung stem cells which are grown in organoid culture system that we have established. We use age-matched animals of the same background strain to minimise variability within each experiment and therefore reduce the numbers of animals required. We will also use statistical techniques to calculate the minimum number of animals which are required to obtain a conclusive result in each experiment. By labelling cells using fluorescent markers in the confetti mouse model, fewer mice can be used compared to conventional approaches where an increased number of animals were required to understand changes in cell behaviour.</p> <p>The study of the lung tissue during development provides a good model to understand the changes in cell behavior occurring during extensive tissue growth or/and regeneration. The molecular mechanisms regulating tissue repair or/and expansion may be identified in developmental models and subsequently validated in a limited and informed number of experiments using injury repair and cancer models.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>To study lungs in the context of the whole animal, we are restricted to studying an air-breathing vertebrate animal. We choose mice as a species as they have lungs very similar to our own and are also easy to study and make genetic changes to in the lab.</p> <p>All methods proposed in this project use refined techniques that minimise animal stress. These include refined cell damage approaches with minimal side effects as opposed to the traditional usage of chemical-induced lung injury models. The lowest dose that is sufficient for observing stem cell activation with mild clinical signs will be determined and used in our experiments.</p> <p>Cell based-studies will be used to plan experiments with animals, reducing number of</p>

animals and refining the experimental design.

A new method of causing short-lasting damage is now included. This method will specifically damage the upper airway (trachea) and this localised damage will be repaired within one week. Therefore damage is both refined and only short-lasting. Being able to use this method is important as, once it causes damage, we will be able to study how unique cell types found in the trachea contribute to the repair process and how this process could go awry in lung disease initiation.

Project	Stem cells in tissue regeneration and cancer	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Liver disease is the 5th commonest cause of death in the UK, and costs Scotland more than £1 million per day. The only cure for end stage liver disease is organ transplant, where demand far outweighs supply. Thus, there is an unmet need for new liver disease therapies. We aim to understand processes controlling liver disease and repair to discover new ways to limit damage, enhance repair and detect damage early before it becomes late stage liver disease and/or cancer.</p> <p>Our animal models of liver disease will be used to meet the following objectives:</p> <ol style="list-style-type: none"> 1. To understand how liver stem cells (LSCs) 	

	<p>contribute to liver repair in conditions of liver injury.</p> <ol style="list-style-type: none"> 2. To understand how cells form a supportive environment enabling LSCs contribution to liver damage/repair. 3. To define the role of LSCs and their environment in the development of cancer. 4. To develop new ways to non-invasively analyse liver injury/repair.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Liver injury patients have limited treatment options. Therefore, it is imperative to translate findings in the laboratory to the clinic. We are well placed to achieve this as we frequently care for patients with advanced liver disease and have been involved in trials for novel therapies for these diseases. By identifying new processes that influence LSC behaviour, we aim to develop novel therapies for liver disease that target these processes. Our results will allow the development of pharmaceutical and cell-based strategies to influence liver repair and optimise the “healing response” following liver injury. These strategies may be translated into the clinic rapidly as in this group of patients current treatment options are limited. REDACTED. Our investigations under this license will allow us to identify:</p> <ul style="list-style-type: none"> - Pathways that allow mature liver cells and liver stem cells to repair the liver during short term injuries (such as paracetamol poisoning which we observe in patients) and long-term injury (which leads to cirrhosis, as is seen in patients with alcoholic liver disease). - Mechanisms by which LSCs interact with their environment and influence the development/resolution of liver inflammation and scarring. - New pathways that control the development of liver cancer. - Enable development of non-invasive analyses (such as new ways of imaging liver disease or discovery of new blood markers of injury) of organ injury/repair for ongoing assessment of effects of therapeutic interventions, allowing reduction in animal use. Results will be published in peer-reviewed journals, enabling the scientific community to learn and advance.

<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>30,250 mice and 400 rats 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Expected adverse effects: Models of liver disease can result in weight loss and loss of body condition at least in short term. These effects are dependent on the type of mouse used and is taken into account when designing experiments and duration is limited accordingly. Animals are monitored for bodyweight loss, which is used as a humane endpoint. Cancer models: To properly judge the effects of cancer development on rodents, we will monitor weights which are judged against normal growth rates for healthy rodents. We also assess clinical signs such as body condition or atypical behaviour which could be associated with the development of cancer. Surgical models: Animals normally recover within 24 hours. Animals which don't recover promptly from surgery, where pain appears uncontrolled, have significant surgical complications, or whose general health deteriorates will be humanely killed. Post-surgery monitoring will take place daily by assessing general behaviour, coat quality, skin tone, abdominal distension, jaundice and wound healing. Endpoints: Mice are humanely killed at the end of their breeding life or at the end of the experiment</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>It is increasingly recognised that the factors controlling organ injury and repair are complex and mediated through multiple cell types and signals. This response is dependent upon the liver environment. In order to understand complex liver disease and repair mechanisms, and also to evaluate potential therapeutic use of cells and other agents in liver disease and cancer, animal experiments modelling human diseases are required. Prior to performing experiments in animals, we undertake detailed studies in human and rodent cell lines and also utilise tissue from patients and previous rodent experiments to inform the parameters of subsequent animal experiments in order to refine and reduce the amount of work involving</p>

	animals.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Group sizes are calculated by examining previous data from our laboratory and published studies. Experiments are designed based on the effectiveness of the procedure and the historical size of the effects and the primary variable that is being measured (e.g. the degree of liver scarring or the number of liver stem cells).</p> <p>We seek to use numbers of animals that are likely to give meaningful results.</p> <p>We have extensively tested measures of outcome for all experimental protocols - This allows us to maximise the information gained from any particular experimental procedure.</p> <p>Tissues generated from previous experiments are archived and stored appropriately, ensuring that experiments are not repeated unnecessarily.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use mice and rats for most of our experiments as they accurately model human diseases within a timeframe that is suitable for research progression. Furthermore, there is an extensive range of genetically modified mice to specific effects of modifying single genes in complex environments</p> <p>We ensure correct handling of animals to minimise stress.</p> <p>Animals exhibiting unexpected harmful effects will be humanely killed, or advice sought from the local Home Office Inspector.</p> <p>Local guidelines for surgery/post-surgical care (including appropriate anaesthesia/ pain relief protocols) will be followed at all times. We use several support measures to refine our models, in collaboration with veterinary and technical staff, such heated cabinets/heat pads to ensure animals maintain body temperature, fluid supplementation to avoid dehydration and using more palatable food.</p> <p>To minimise animal suffering we utilise strict humane endpoints</p>

Project	Strategies for orthopaedic translational research II	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The objectives of this licence are to:</p> <p>Determine the response of skeletal tissues to mechanical and biological stimulation.</p> <p>Enhance the integration of prosthetic implants with tissues.</p> <p>Develop strategies to regenerate skeletal tissues.</p>	
What are the potential benefits likely to derive from this project (how science could be advanced or	Musculoskeletal conditions (MsCs) are a major burden to the individual, society and the health service. One in five of all general practitioner	

<p>humans or animals could benefit from the project)?</p>	<p>consultations involves a patient with an MsC. The main consequences of MsCs are chronic pain and physical disability. The burden of MsCs on society will continue to rise as life expectancy increases. Orthopaedic conditions and disease in animals is also common. In veterinary surgery, MsCs are a common reason for referrals with the caseload dominated by trauma in younger canine patients and degenerative diseases in older dogs. The goal of this licence is to help to drive forward understanding of the principles of musculoskeletal disease, to investigate the complexities and limitations of regenerative techniques in this area and to investigate new materials that are used to replace and regenerate musculoskeletal tissues. The skeletal system is uniquely responsive to changes in the mechanical environment. Such mechanical perturbations are relevant to both aetiology of pathological changes but also to the management and control of skeletal disorders as well as the normal development, adaptation and repair of this system throughout life. The work proposed in this project will continue to advance the basic knowledge on the remodelling, repair, regeneration and replacement of the musculoskeletal system in order to translate understanding of mechanisms to new clinical strategies in prevention and management of skeletal disease.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Over 5 years: Mice 200 Rats 300 Sheep 700</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In terms of severity, the models used are predominantly surgical and as such fall into the "moderate" category. Wherever possible there is a staged approach of the work, where the investigation of new materials, biologics such as stem cells and orthobiological materials (materials that are obtained from tissues or are a replica of tissues) is first carried out in relatively simple models before investigating in more functional models where for example, a segmental defect is created and repaired with either man-made materials, biologics such as stem cells and orthobiological materials or</p>

	<p>combinations of these materials. The use of relatively simple models before using more complex models, is consistent with minimising severity. In some cases, the staged approach is not possible for example, in cartilage repair, regeneration and replacement the only way to investigate this is to make a defect in the cartilage and repair the defect with materials, biologics, and orthobiological materials or combination of these. In certain procedures the response to will be measured longitudinally using imaging techniques or by measuring the animals' recovery using gait analysis. The animals are humanely killed at the end of the protocols and samples of musculoskeletal tissues are sent to laboratories for close examination to get the maximum information from the study.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The integrated physiological environment of the living animal is still required to elucidate biological mechanisms prior to advancing clinical management of musculoskeletal. Alongside this licence we have developed predictive computer models and tests in the laboratory enabling us to reduce the number of different treatments and the number of animals used in our experiments. Examples include the development of computer models to investigate the effect of changing the porosity of metal implants on the integration of tissues and the skeleton. Another example is the development of an in vivo skin model used to investigate soft tissue interaction with metal implants used for percutaneous devices. These models refine the materials thereby leading to the reduction of animals used.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of protocols has been reduced from 12 in a previous licence to 6 in this application. We no longer require these protocols because the research questions have been answered. This overall research progression means that fewer animals will be used. Statistical analysis and experience gained by using similar protocols in a previous licence means that a minimal number of</p>

	animals will be used in order to achieve statistical differences.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>A number of specific protocol steps have been refined to enable us to investigate the objectives. It is anticipated that new materials and concepts are tested initially in relatively simple surgical models and after this the proof of effectiveness is investigated in more functional models. We have refined the protocols; for example, we no longer require the use of sheep that have been treated to reduce their bone density and these protocol steps have been removed from this application. All models have been developed in previous licences. An example of one of the refinements that has been developed is the way that analgesics are used. In sheep we use topical analgesic patches pre and post operatively, whilst in rats we give analgesia in jelly that the rats eat. These interventions reduce the stress levels in animals because injections to deliver analgesics are now rarely used. Animals also now receive inter-operative analgesics injected into the wound site which is a technique we now consistently employ.</p>

Project	Strategies for orthopaedic translational research	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The work proposed in this project will continue to advance the basic knowledge on the remodelling, repair and regeneration of the musculoskeletal system in order to translate understanding of mechanisms to new clinical strategies and devices in prevention and management of skeletal disease.</p> <p>This project licence incorporates a number of procedures designed to elucidate the mechanisms that control remodelling, repair and regeneration of tissues and structures in the musculoskeletal system.</p>	

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>1. Determine the response of specific skeletal tissues and composites of skeletal tissues to defined mechanical and biological stimulation. 2. Elucidate the influence of mechanical, biological and material factors on the remodelling and repair processes of skeletal tissues and structures. 3. Define the influence of mechanical, biological and material characteristics on the integration of prosthetic implants with skeletal tissues 4. Develop strategies to regenerate skeletal tissues and structures through manipulation of the mechanical and biological environment to attract, cue and selectively differentiate multi-potent cell populations.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Sheep 1300 Goat 300 Rat 550 Mouse 250 Rabbit 300 All over the five year period</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The models used are predominantly surgical and as such fall in the overall "moderate" severity category. However, the staged approach of the work is consistent with minimising the severity within this category wherever possible. At the end, animals will be killed under Schedule 1 of ASPA.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Many aspects of our work are being achieved through the use of laboratory based in vitro cell culture, tissue culture, computer modelling and bioreactor studies using novel materials and chemicals. These techniques allow identification of the most likely treatments to be validated in vivo. This replacement reduces the numbers of animals used. The integrated physiological environment of the living animal is still essential to elucidate the patho-physiological mechanisms prior to application in advancing clinical management of musculoskeletal conditions in both veterinary and human patients.</p>
<p>2. Reduction</p>	<p>We have ascertained in our study designs that in most experiments minimum group sizes in</p>

<p>Explain how you will assure the use of minimum numbers of animals</p>	<p>the order of six animals are required to evaluate levels of effect that have both statistical and translational significance. Standard models have been developed and will be used where appropriate for comparative data to avoid replication of some control groups.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We shall adopt a hierarchical approach using simple models before using complex implants. The strategy used would be further minimised by use of appropriate analgesia protocols. Our previous experience and refinement of models used indicates that NO procedures would be required at the substantial severity level. The complex surgical models would be at the moderate severity level with appropriate protocols and end points to manage pain and infection.</p>

Project	Strategies for the restoration of sight in retinal dystrophies	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Photoreceptors (rods and cones) are the retinal cells in our eyes that transform light into electrical activity. There are cases where they do not function properly because of genetic mutations, and as a result, they begin to die, resulting in serious visual impairment and in extreme cases, complete blindness. These diseases are grouped under the common name “Outer retinal degeneration” (ORD). When photoreceptors have completely degenerated, one of the most promising therapeutic approaches is to replace them with new photoreceptors derived from stem cells, and the development of this approach is the main aim of this project. Our recent work has shown that it possible to make, in the lab,	

	<p>structures that resemble the human eye and that contain several eye parts (retina, cornea, lens) from human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC). We will work with mouse models of ORD and will investigate how the retinal cells generated from hESC and hiPSC can potentially lead to the restoration of vision in these animals. Very recently it has been shown that exchange of cellular material from transplanted donor cells helps the endogenous retinal cells to function more efficiently. We will research whether this phenomenon occurs during transplantation of hESC and hiPSC derived cells. If indeed it does, we will proceed with subretinal injections of material that is expelled by the hESC and hiPSC derived cells and we will investigate if this material is integrated by host cells and whether this helps restoration of vision.</p> <p>In parallel, we will investigate the effects of the natural spice saffron in slowing down the death of photoreceptors in these same mouse models. Saffron is known to protect photoreceptors from dying through antioxidant and anti-inflammatory actions. ORD is also characterised by the proliferation of glial cells (support cells in the central nervous system), and it has even been suggested that the activation of this process precedes and induces photoreceptor death. Hence, we will investigate whether inhibiting the activation of glial cells with pharmacological agents (such as neurostatin) has a potentially beneficial effect on the progression of ORD. We estimate that the duration of this project will be five years.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>ORD with photoreceptor loss is the final common pathway of irreversible visual loss in many diseases. Recent estimates indicate that the global number of people with sight loss is 285 million, of whom 39 million are blind. Although gene therapy strategies to delay visual loss are under development, this approach requires that photoreceptors remain alive, otherwise there is nothing to target with the replacement genes, and it is therefore ineffective following complete photoreceptor loss. Currently, there are no effective therapies to reverse the underlying pathological changes in ORD and, therefore,</p>

	<p>there is a requirement for new therapies to treat patients once visual loss is established. Cell transplantation therapy is an important option for patients suffering from currently incurable forms of blindness. The results from this work will allow a deeper understanding of the behaviour and biology of transplanted cells within the diseased retina so that the protocols and techniques can be optimized prior to clinical application in humans, improving visual outcome using this therapy. If the process of cellular exchange between donor and host cells is proven, new therapies of treating blindness can be realised through controlled injections of materials obtained from the cells (but not cells themselves). Moreover, this project will also provide important knowledge about the use of natural remedies such as saffron or gliosis inhibitors such as neurostatin in slowing down, or even stopping degenerative processes in ORD disorders.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We anticipate using a maximum of 632 animals over the duration of this license.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Genetically altered mice wild type and those that develop retinal degeneration and blindness will be bred and maintained. Some of the mice will be immunosuppressed with drugs, including use of surgically implanted devices (in the eye), and will have stem cells, cell extracts, or drugs injected in both eyes under anaesthesia. In addition, we propose to do dual injections in each eye to increase the likelihood of having large numbers of integrated cells. Functional visual tests will be performed to evaluate whether these procedures could successfully restore visual function. Animals will be monitored for up to 16 weeks and then humanely killed and their tissues examined in-vitro. Risk of infection post transplantation will be minimised by using sterile instruments under aseptic conditions. For cell transplant procedures, post-procedure pain will be controlled using appropriate analgesics. Animals showing signs of poor recovery from surgery under anaesthesia will be closely monitored. Animals that are recovering will be carefully monitored and if they exhibit weight loss greater than 20% (compared with similarly operated cohorts), or show marked</p>

	<p>signs of distress, e.g. marked piloerection, dehydration, hunched appearance, subdued behavior, solitary—for more than one day, will be killed. Some of the animals that will receive human stem cell transplants will be immunosuppressed which may increase the risk of infections. This will be minimised by housing them in conditions appropriate for immunosuppressed recipients. Furthermore, injection of cyclosporine to pregnant dams may affect the development of pups, although this may be rare and sporadic. Affected pups will be humanely killed. To date there are very few studies describing the effects of cyclosporine on pups following injection of dams; the effects are described as due to poor crossing of cyclosporin through the placenta. The only notable impact is a transient decrease in thymus weight and cellularity if injections are performed every day from coitus to birth. All these changes disappear 30 days post weaning. There is no expected direct adverse effect of neurostatin, the gliosis inhibitor. The level of severity is moderate. The animals will be sacrificed at different time points with the longest procedure lasting four months.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We will investigate the fate of photoreceptor progenitors (cells that will give rise to photoreceptors) derived from hESC/hiPSC or of cell extracts following subretinal injection into several different murine models of ORD. Recent reports have shown that hESCs and hiPSC can be coerced to become, or ‘differentiated’ into retinal photoreceptors <i>in vitro</i> with good efficiency. Notwithstanding these advances, <i>in vitro</i> results cannot shed any light on the survival, engraftment and functionality of cells following transplantation into the degenerate retina. Hence, <i>in vivo</i> investigations are required to answer these questions.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>All attempts have been made to reduce the number of animals and procedures to minimise suffering yet achieve clinical and statistical significance. In each experiment, both eyes will be injected with stem cell-derived retinal cells, vehicle control or cell extracts, thus minimising</p>

	<p>the number of animals used. Each of the experimental groups comprise 9 animals based on power analysis.</p> <p>There are a large number of tests to be performed in these animals including functional vision tests (visual cliff, light avoidance test and optometry), electrophysiological (multi electrode arrays), engraftment studies (immunohistochemistry) and to reduce the number of animals that need to be sacrificed, we will perform injections in both eyes. Since the GA mice that will be used in this study are blind and the adverse effects associated with the procedure are minimal, dual injections in both eyes will not cause added sight deterioration.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Most experiments will be performed on genetically modified mouse models of ORD. These experiments cannot be performed on patients, but their outcome will provide extremely valuable new knowledge, paving the way for future clinical trials. The models we plan to use are well recognised for faithfully representing common human ORD disorders and therefore, they will provide important new knowledge about how to rescue or repair retinas with advanced ORD.</p> <p>The protocols have been designed to keep suffering to a minimum. Where subretinal and intravitreal transplants will be carried out, appropriate anaesthetics and analgesics will be administered pre- and post-operatively. Any loss of condition will indicate removal from the procedure and killing by a Schedule 1 method. Transplants will be performed using cells that have been differentiated at least for 30 days ensuring that virtually all stem cells have differentiated into retinal cells. However, a small risk of tumour formation exists from any remaining undifferentiated stem cells in the grafts, thus animals will be closely monitored for this possible occurrence. We will also investigate the possibility to use extracts from stem cells rather than the cells themselves to achieve the same goal. If this approach is successful, it will remove any potential risk of tumour formation. Animals that do not recover well from surgery or develop an intraocular tumour will be humanely</p>

killed.

There is no expected direct adverse effect of neurostatin, the gliosis inhibitor.

We will also add a new type of wild type mouse (NOD) that can accept human cell injection and integration without the need for immunosuppression. This will avoid secondary effects related to immunosuppression and will aid to minimise procedures carried out in mice.

Project	Stress response and multivariate evolution in a fish model	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	In biology, 'stress' is often a poorly defined concept, but one that carries negative connotations for health in humans and animals. However, stress responses actually play an important positive role in maintaining viability and health. When challenged by a threat in the environment – perhaps a predator, disturbance, or adverse conditions – a set of neuroendocrine pathways trigger physiological and behavioural responses (e.g. fight or flight behaviours) that have evolved under natural selection to counter the threat. Nonetheless, while these acute stress responses are thought to be adaptive, it	

	<p>is also well documented that chronic stress exposure can reduce the health of individuals. Because chronic stress responses are typically bad for fitness (survival and/or reproduction), natural selection should act over evolutionary time to get rid of them. The fact that they are widespread, being found in vertebrates ranging from fish to humans, therefore poses an important question– what constrains the further adaptation of stress response towards a state where these harmful, or maladaptive, effects do not occur? The goal of the proposed work is to address this question by conducting studies to understand ‘genetic integration’ among components of the stress response, and aspect of organismal performance which they affect (e.g. growth). ‘Genetic integration’ in this context refers to the fact that different traits can depend on the same underlying genes. This means that traits cannot respond independently to selection, often resulting in trade-offs that constrain adaptation. In simple terms genetic variants that provide advantageous effects through effects on one trait, also have effects on another trait that are disadvantageous. We will use small fishes, and primarily the guppy (<i>Poecilia reticulata</i>) as a model system studying both patterns of genetic integration within populations. We will also compare findings across populations (and species) that have evolved under different stress environments. Practically, the work will involve experimentally manipulating stressors in the environment, for instance imposing periods of stress by chasing some fish with nets or exposing them to a model predator. We will then determine how different individuals, genetic types, and populations respond through behavioural, hormonal and physiological processes, and determine the consequences of this variation for performance traits linked to fitness (e.g. growth, longevity).</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>In the short term the principal outputs of the research will be academic, benefiting the broad set of biologists working from an evolutionary perspective across disciplines and levels of biological organisation. This is because the project will tackle fundamental scientific questions about the nature of evolutionary</p>

	<p>adaptation and constraint, a phenomenon that is far wider in scope than the specific context of understanding maladaptive stress responses. However, the work will also provide us with a better understanding of the genetics of stress response and health and performance traits that are impacted by exposure to stress in the environment. In turn this should yield tangible benefits for improving welfare in captive animals. For instance, the presence of genetic variance for damage caused by stress exposure opens up the possibility of using artificial selection as a tool to improve animal welfare in captive populations (e.g. livestock, aquaculture, scientific research). Understanding the genetic architecture of the stress response, and how this impacts the health and performance of individual animals, is a necessary first step towards this.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Over 5 years we expect to use approximately 5100 fish. Most will be guppies (n=3300, <i>Poecilia reticulata</i> and/or congeners <i>P. wingei</i>, <i>P. obscura</i>). We will also use their close relatives in the family Poeciliidae, including fish from the swordtail/platie species complex (<i>Xiphophorus</i> spp, n=900) and black-barred limia (<i>Limia nigrifasciata</i>, n=450). We will also use some small freshwater fish from the minnow (cyprinidae) family (specifically zebrafish n=450).</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>For the majority of fish used adverse effects experienced are expected to be mild. A typical individual will be subject to tagging for identification (and in some cases fin clipping to obtain samples for DNA analysis). These procedures, which will be conducted under anaesthetic, are routine in fish studies and complications (e.g., unsuccessful recovery from anaesthesia) are rare. Data on behaviour (including cognitive performance), size/growth, maturation time, hormone levels, metabolic rate and longevity will all be collected using completely non-invasive methods. Since longevity and reproductive performance are traits of interest in this study we expect most animals subject to licensed procedures (e.g. tagging under anaesthetic) to remain “on</p>

license” for the duration of their lives. Euthanasia criteria (agreed with the named veterinary surgeon) are in place to provide a humane endpoint for any diseased, injured or senescent fish. Where fish are not required for further breeding or data collection they will be euthanized.

A subset of up to 1000 fish will be subject to a chronic stress assay as part of the trait data collection. This is expected to cause moderate adverse effects in the form of weight loss (or reduced growth rate) and potentially also in reduced reproductive performance (e.g. number of offspring). We anticipate detectable effects on weight or growth in approximately half of the fish tested in this assay. In the most extreme case a fish could experience up to a 20% loss in weight. As a humane end point, any fish experiencing weight loss of this magnitude will be checked closely for abnormal behaviour and signs of disease or distress, and then euthanised if these are found. We won't use weight loss as the sole basis for making such decisions as most of the fish used will be livebearing species in which pregnancy and giving birth can lead to dramatic shifts in live weight (e.g. a female guppy may appear to lose up to 30% of her weight by giving birth).

In addition to collecting data on traits, a relatively small number of fish (up to 30) will be euthanised as healthy adults to provide tissue for a targeted study of chromosome structure and complement. This study is needed to help determine the best statistical analysis methods for the main data set. Euthanasia will be within hours of exposure to colchicine dissolved in the aquarium water. This substance stops cell replication mid cycle allowing chromosomes to be visualised using microscopy. Since it interferes with cell replication, colchicine exposure can cause serious health problems (e.g. cancer) over the long term (e.g. months). However, because fish are euthanised within hours of exposure, no adverse effects are expected over the relevant timeline.

Finally, up to 120 juvenile fish will be raised in aquaria containing synthetic testosterone dissolved in the tank water. Unlike mammals,

	<p>sex determination in many fishes - including guppies - can depend on both genetic and environmental factors. Here we manipulate the environment (by adding testosterone) which results in all juveniles maturing as males, regardless of whether they are carrying a typical male (XY) or typical female (XX) complement of sex chromosomes. On maturation these males will be mated to control (i.e. normal XX) females to help us understand sex-specific inheritance. As with the colchicine treatment this experiment is needed to help determine the best statistical analysis methods for the main data set. No adverse effects of this treatment are expected over the timeline of the experiment. However, as a precaution against any unanticipated late life consequences of juveniles being exposed to testosterone we will euthanise the treated fish once they have been mated to females.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>There are no non-sentient alternatives that could be used and in vitro approaches are not applicable to organism level questions. The study aims to test evolutionary theory about the pathways linking animal behaviour, physiology, health and the environment (the source of stress) in vertebrates. It is only possible to do this using a vertebrate model.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Studying the evolutionary genetics of traits in wild-type populations generally requires large sample sizes and our study is no exception. Experiments have been designed using computer simulations to determine the minimum sample sizes that will allow powerful testing of our hypotheses. In doing this we have also ensured that the statistical methods we will use to analyse the data are the most powerful ones available. To reduce animal numbers further we will not address all objectives in all of the study species. Rather guppies will be used to test all our key hypotheses, while other species will be used for smaller parallel studies where they add particular relevance. For example, by verifying our findings in zebrafish we can assess the potential for genetic</p>

	improvement to improve welfare in captive populations of this widely used scientific model.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The fish models to be used are exceptionally robust, easy to care for, and highly fecund under laboratory conditions. In all these regards they are more suitable than other possible vertebrate models (e.g., mice, rats).</p> <p>There are no alternative procedures of lower severity to those proposed. In fact the majority of procedures to be applied are mild or sub-threshold. These will include tagging for identification purposes, and non-invasive studies of behaviour and physiology (e.g., with stress hormones measured from water in which fish have been kept rather than from blood samples). However, some individuals (up to 20%) will also experience a chronic stress assay that involves deliberately exposure to stressful conditions. This is unavoidable as we do need to study performance under stress, but we will use the least severe endpoint possible, measuring performance under chronic stress from reductions in growth (and or weight loss).</p> <p>General welfare will be ensured by maintaining housing conditions and husbandry standards (e.g. daily inspections, frequent water changes, a robust program of water quality testing) that meet or exceed all HO requirements.</p>

Project	Stromal function in the tumour microenvironment	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Cancers have developed many ways in which to prevent our defences, that is our immune system, from destroying a tumour. New therapies that ‘kick-start’ our immune system back into action are proving promising, but these still only work on some patients in a few cancers. A tumour is much more complex than a collection of cancer cells. Many other cell types make up the tumour – these cells support the tumour in many ways and are known as the stroma. These stromal cells may help a tumour by acting on our immune system to prevent it from working properly, or by attracting bad immune cells. There is still very little known about how the stroma may do this, but from the evidence we do have, it is likely to have a key role. This project aims to</p>	

	<p>identify how stromal cells act upon the immune system as a tumour develops compared with normal tissues, and to use this knowledge to enable us to better and more specifically treat cancer.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>From this project our primary benefit will be the increased understanding of how supporting cells found in a tumour act, specifically how they work to switch off the immune system. These findings will provide immediate benefit to the research community sharing potential mechanisms and features that could be used for development of new or improved treatments that use our immune system to target a tumour. New mouse models and experimental systems developed will offer potential to benefit other scientists, and the knowledge we gain will begin to benefit clinicians, Biotech industries with drug discovery pathways, hopefully being initial steps towards the patient benefiting.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use a maximum of 6300 mice in this project covering breeding and experimental protocols over a period of 5 years. Mice represent the least complex system relevant to human disease and the complex nature of cancer, especially the complexities of tumour immunology. Genetically altered mice develop tumours with similar features as human disease, share patterns of spread and contain a functioning immune system that can be compared with human.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Most animals under the authority of this PPL will develop tumours that have been induced by a variety of methods such as cell implantation, inducing genes in genetically altered animals, using chemical carcinogens or using viral vectors. Tumour growth will be measured and blood samples may be taken. To investigate how stroma contributes to tumours, tumour-associated tissues may be imaged on a microscope where substances such as labelled cells, blocking antibodies or cell tracers may be administered by a range of routes such as intratumoural injection or application to skin. To help identify key populations, animals may also be irradiated to remove cells which are replaced with altered cells to allow us to identify those needed for tumour development. Healthy mice without tumours may also undergo similar procedures to help us understand how a tumour</p>

	<p>changes normal tissues and stroma to help disease development. In the majority of cases, tumours will not significantly impact animal welfare or normal behaviour and will be classified as mild. Rarely, mice may develop more clinical signs such as tumour ulceration or weight loss. If these are seen, mice will be killed immediately to limit any suffering experienced. However, in the vast majority of cases, our endpoints occur before any of these adverse effects have the chance to develop thus most animals be experience mild severity in their lifetime, and will not exceed a moderate severity limit.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Tumours contain tumour cells, but they are in fact much more complex systems including fibroblasts, blood endothelial cells, lymphatic endothelial cells, pericytes, adipocytes, smooth muscle cells, fibroblast and immune cell populations all of which interact, adapting as a tumour develops and evolves. We work routinely with in vitro models, making them as complicated and accurate as we can to generate as much information as possible with regards to the tumour microenvironment. The model systems we use aim to complement and help design mouse experiments appropriately. They help to identify key populations to investigate in mice, doses, potential responses to therapy before using mice. They also critical for us to home in on specific mechanisms following data generated in mice. However, the complexity of complete tissues and a changing tumour environment cannot be fully recreated in a plastic culture vessel. To do this we require a living system with aspects of the immune system comparable with humans. Such a system does not exist in non-protected alternatives.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>Before conducting any experiments, we perform statistical analyses to determine the fewest mice needed to produce useful data. We extensively use complex in vitro systems as a means to generate data without animals, but these are still not able to recreate a living animal. Thus we need to use animals. However, from each mouse we will collect multiple tumours and associated tissues i.e. lymph nodes that are analysed using multiple methods. Tissue from the mice also help to make cells which</p>

	<p>are further used the in vitro systems described above, which complement and help refine our research design. Moreover, the genetic models used can develop multiple tumours at differing stages per mouse as occurs in human disease. These approaches allow us to gain as much information from each animal as possible and reduce the numbers we need to use.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We need a model with a complex, functional immune system as is found in humans, and which is lacking in lesser species. Thus, mice represent the least complex system that possesses the parameters needed to yield meaningful data translatable to human disease. We have extensive experience in mouse tumour models and work closely with staff at the animal facility. We have refined models so that animals largely develop superficial tumours and experience very few clinical signs. This means our experiments end before adverse effects can develop. As animals are monitored very closely, by well trained staff, any welfare concerns raised can be dealt with rapidly. Mice are also housed in social groups and are given multiple bedding types for nesting, chew sticks, fun tubes and platforms to provide a rich living environment.</p>

Project	Studies of brain development in the mouse
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input type="checkbox"/> Basic research <input type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	
What species and approximate numbers of	

animals do you expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	

Project	Studies of Atherosclerosis and Aortic Aneurysm Formation and Progression	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Heart attacks cause more deaths in the UK than any other disease and survivors of a heart attack have a reduced life-span and quality of life. Heart attacks are most commonly caused by a disease called atherosclerosis (hardening of the blood vessels supplying the heart). Aneurysms are localised, blood-filled swellings (balloon-bulge) of a blood vessel and are linked to atherosclerosis. Furthermore, as aneurysms increase in size, there is an increased risk of them bursting, often resulting in sudden death without previous symptoms. Burst aneurysms account for 4-8% of all deaths per year in the UK. The formation of	

	<p>these diseases is in part due to death of cells and a process called inflammation, yet there are no effective anti-inflammatory treatments currently in use to treat these common diseases.</p> <p>Inflammation is directed by white blood cells found within our blood (called monocyte/macrophages) and we have recently identified a certain type of bad white blood cell which we believe causes heart disease and bulging of large blood vessels. Learning about how these bad blood cells are regulated will provide useful information for the development of new medicines for the treatment of these underlying causes of such cardiovascular diseases, and possibly find diagnostic tests that will identify people at increased risk.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This project will advance understanding about how white blood cells and their associated molecules are involved in normal processes within the body and contribute to inflammation and, ultimately, deadly cardiovascular diseases. As Work within this project may in the mid-to-long term lead to the development of safer and more effective preventative therapies for heart attacks and the bursting of aneurysms. Also, work within this project may tell us if we can count and characterise the number of bad cells in the blood of patients and therefore predict their risk of a heart attack or aneurysm.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This project proposes to use mice. Approximately 5900 mice will be used over five years (1180 mice per year).</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Approximately 20% of mice will be maintained for breeding purposes and to provide animals for atherosclerosis and aneurysm experiments, and this procedure is therefore classified as mild as no adverse effects are expected. Approximately 80% of animals will be given a single injection and fed a high-fat diet for up to 16 weeks for them to develop atherosclerosis. This procedure is mild and produces no outward signs of distress or discomfort to the animals. In addition, approximately 40% of these mice will undergo surgery (10-15 minutes) to implant a device or small pieces of sponge (1cm³) under the skin that</p>

	<p>release a substance slowly to cause the mice to form aneurysms or allow the collection of white blood cells respectively. The mice are expected to recover quickly and will be given painkillers and post-operative care just like people recovering in hospital. Due to recent refinements, these procedures are considered moderate as no significant adverse effects are expected, although a small number of mice (<2.5%) will die suddenly due to aneurysm rupture. Animals will be killed at the end of experiments by an approved Home Office humane method and blood, tissues and cells taken for further laboratory study and scientific analysis after death.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Laboratory studies using isolated cells and tissues from either mice or humans will form a major part of the proposed studies and will be used alongside our recently developed aneurysm model which uses human vessels in a laboratory setting (ex vivo) to determine potential interventions in advance of any work in live animals. However, the complex biochemical changes that occur in vascular disease cannot all be modelled in isolated cells or ex vivo tissues, because they are influenced by a wide range of physiological factors that are unique to living animals. The study of whole tissues or cells isolated from those tissues facilitates replacement within our studies, as certain interventions and agents can be used that would not be possible (for reasons of toxicity, rarity or cost) in living animals. It also enables us to reduce our use of animals because many cells can be isolated from a single tissue and used for multiple studies.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of animals will be minimised by conducting initial studies in cells and tissues in the laboratory, with strictly controlled conditions to minimise experimental variability. Our extensive experience of such studies means that we can use historical data to perform power calculations to ensure that the experimental designs are biologically and statistically rigorous. In general, the experimental design will involve comparison of a control group with one or more intervention groups using statistical tests appropriate to the</p>

	<p>data. We will regularly review our designs in the light of the data generated to ensure that the results are statistically rigorous but do not involve the use of unnecessarily large groups of animals.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice that develop vascular lesions (due to alterations in their genes) are virtually unique amongst experimental animals in that they develop spontaneous unstable atherosclerosis (the root cause of most cases of heart attacks in humans), and aortic aneurysms upon administration of drugs which increase blood pressure (angiotensin II), so we have chosen them for our studies of the biological basis of cardiovascular diseases. Moreover, we will use a new refined approach in our studies through the use of a single injection of a substance (PCSK9-AAV) which makes mice blood cholesterol levels raise when they are fed a high-fat diet, and therefore greatly reduces (50%) the breeding required to generate experimental mice for atherosclerosis and aneurysm studies. Mice are also an appropriate species due to the availability of genetically modified strains and active inhibitors. We have extensive experience in all of the models and methods to be used in this project and are confident that they are the most appropriate to address our research questions.</p> <p>We will always use the least invasive procedure alongside anaesthetic and analgesic in such procedures to minimise animal suffering. We will also continuously monitor the outcome of our procedures in order to effectively minimise this suffering.</p>

Project	Studies of calcium channel function and roles in disease
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To understand the role of calcium channels in normal physiology and various diseases including chronic pain and epilepsy
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We may gain a greater understanding of potential therapies relating to these diseases
What species and approximate numbers of animals do you expect to	mice ~7500 rats ~500 over 5 years

use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In most of our experiments, mice and rats will be used as a source of tissue for in vitro experiments for our studies on mechanisms of ataxia and neuropathic pain. For these experiments, the level of severity will be non-recovery, as the animals will be killed immediately by methods specified here. Most of the mutant mice to be used are expected to have either no behavioural or other symptoms, or symptoms classified as mild severity. However, it is possible that symptoms will be classified as moderate in some mice. In some experiments, nerve injury models of chronic pain will be used, in order to study how this pain develops and how it can be treated better (experiments are classified as moderate). Experiments will be of the minimum duration necessary for this type of study. All animals will be killed immediately at the end of the experiments, and their tissue will be used.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	Animal models of disease (specifically neuropathic pain and cerebellar ataxia in our case) often require the use of mammalian models in which the symptoms can be monitored and relate to human disease. Mice and rats are the most widely used species for this and cannot be replaced without losing relevance to human disease.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	Most of our work involves in vitro experiments, in which either wild-type or genetically modified (mutant) mice are only used as a source of tissue of in vitro experiments. The minimum number of mice will be used to provide sufficient material for statistically significant results to be obtained. Experiments will be grouped so that the maximum amount of tissue for different experiments will be taken from each mouse.
<p>3. Refinement</p>	The choice of species is mice, because they are the smallest mammal that is routinely

<p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>genetically modified, and rats, because they are a standard animal used in many laboratories. We will take all necessary general measures to minimise welfare costs (harms) to the animals, such as frequent observation, handling by trained staff, and use of anaesthesia to reduce pain.</p>
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Project	Studies of cancer inflammation and immunity in vivo	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our main objective is to elucidate which signals trigger anti-tumour immune responses, and to distinguish mediators favouring tumour elimination from those that support cancer progression.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The recently reported unprecedented efficacy of immunotherapy in a proportion of cancer patients has revolutionised the way we treat cancer. In turn, this has highlighted the need for more mechanistic studies to determine why some patients show partial response or do not respond. In this context, the study of the	

	<p>mediators that regulate the function of immune cells in the tumour microenvironment, distinguishing mediators promoting tumour immunity from those that support tumour growth, is critical to our ability to maximise the efficiency of therapy for cancer patients. The benefits of this project will be: 1) The generation of genetic evidence to show if and how the immune system blocks cancer development in mouse models of cancer that very closely recapitulate the human disease. 2) To inform and advance our ability to identify cancer patients likely to respond and benefit from cancer therapies aimed at harnessing the anti-tumour activities of the immune system. 3) The generation of novel therapies to disrupt immune suppression and enhance the efficacy of conventional and immune-based cancer treatment in patients.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Species: mouse Number of mice: 9200 Period of time: 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Mice may have tumours implanted under the skin or induced by chemical agents. The growth of tumours will be assessed by either imaging with ultrasound or in the case of subcutaneous tumours using callipers. On occasions the tumour cells may be injected into a vein. Some mice will be irradiated to destroy their own immune cells and replaced with specific populations of cells from donor mice. Chemical agents, such as existing cancer therapies, potential new therapies or agents that help measuring specific endpoints may be administered by a variety of routes including orally or by injection. The majority of animals (up to 95%) are not expected to show signs of adverse effects that impact on their general well being apart from the development of tumours. The vast majority of the procedures will result in no more than transient discomfort and no lasting harm. However, some mice in which we are investigating the effects of treatments upon the spread and progression of tumours (~3% based on recent experience) will be unexpectedly found dead over night when monitoring indicates that they are normal. This is due to a sudden impact of the spread of cancer cells from the</p>

	<p>original location to other body organs. The health of all mice will be observed daily. Notwithstanding this, all the mice will be humanely culled at the end point of the experiments.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The development and function of an inflammatory and immune response involves many different cell types interacting in a dynamic three-dimensional environment. For example, the progression of an immune response within a whole organism involves changes of antigen expression and presentation that evolve with both time and spatial distribution. Similarly, cancer development and spread involves a plethora of interactions between cancer cells and their surrounding cells, governed by multiple signals originating from both their immediate neighbours and from distant tissues. These factors combined with the involvement of multiple host cell-types and the clonal expansion and migration of effector cells mean such research cannot be carried out in tissue culture alone or reproduced <i>in silico</i> and can only be addressed with the use of animals.</p> <p>The mouse is one of the model organisms that most closely resemble humans. The human and mouse genomes are approximately the same size, and display an equivalent number of genes, which are functionally conserved. Further, mice have genes not represented in other animal model organisms (e.g. <i>Caenorhabditis elegans</i>, i.e. nematode worm, and <i>Drosophila melanogaster</i> i.e. fruit fly) such as those involved in adaptive immune responses. Mice can be genetically altered, there is extensive literature concerning the topics of our investigation, and our own studies can be enhanced by combination with many complementary models developed by others in the field. Definitively, mouse models are important for placing the findings of <i>in vitro</i> studies or correlative analysis of human samples into an appropriate and meaningful <i>in vivo</i> context. It is the combination of <i>in vitro</i> and <i>in vivo</i> studies that provides the insight needed to</p>

	<p>understand cancer biology and develop new therapeutic approaches, and there are no effective approaches to hand that can replace the <i>in vivo</i> studies, as these allow the <i>in vitro</i> findings to be tested in an appropriate environment.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The use of mice will be minimised in several ways:</p> <p>By doing as much preliminary work as possible in culture models <i>in vitro</i> and <i>in silico</i> analysis prior to engaging in <i>in vivo</i> studies.</p> <p>By minimising variability in results through utilising inbred strains and by housing them under identical conditions to limit variability.</p> <p>By performing pilot studies using few mice when no information is available in the literature so that the number of mice utilised in experiments is reduced to minimal levels.</p> <p>By considering on-going statistical estimation of power requirements in each of the studies, using prior results in order to use the minimum number of animals while retaining sufficient numbers for statistical significance.</p> <p>By incorporating as many test groups as possible within a single controlled experiment, reducing the number of controls required compared to a series of smaller experiments.</p> <p>By utilising tissues from different sites on one mouse for both treatment and control samples.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The use of inbred and fully backcrossed mice not only reduces intragroup experimental variability but also eliminates incompatibility when cell transfers are carried out between various knockout, transgenic and wild-type strains.</p> <p>The cancer mouse models that we will use very closely recapitulate the human disease and thus allow to understand the molecular and cellular events and steps involved in the activation of tumour immunity and tumour-related inflammation during tumour development and progression. Some mice in which we are</p>

investigating the effects of treatments upon the spread and progression of tumours (~3% based on recent experience) will be unexpectedly found dead over night when monitoring indicates that they are normal. This is due to a sudden impact of the spread of cancer cells from the original location to other body organs. The health of all mice will be observed daily. We believe we can justify this because we have seen unprecedented outcomes with our approaches in multiple malignancies, including some once thought to be treatment refractory.

Where possible, procedures will be undertaken under anaesthesia with the administration of analgaesia to minimise the experience of pain.

We constantly work to improve husbandry and procedures to minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. Mice will be maintained in individually ventilated cages under barrier environment, to avoid infections.

When considering which route of administration of substances to employ, we will strive to use the least invasive route whilst maintaining direct control of dose. The choice of route to administer a drug or cells will be such as to achieve "best practice", i.e. to minimise or avoid adverse effects, reduce the number of animals used, and maximise the quality and applicability of substances and cells to achieve the scientific objectives.

Project	Studies on the neurobiology of sensation	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Chronic pain is widespread, estimated to affect 1 in 5 of the population. Most of this pain is not well treated by existing medication because the drugs we have shown only limited effectiveness and they all have side effects. By understanding how the brain generates sensory experiences, we hope to identify ways in which pain can be better treated.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit)	New treatments for pain depend on understanding what causes it in the first place. By providing that understanding with this work, we hope to facilitate the development of novel	

from the project)?	analgesic drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use approximately 2500 mice and 100 rats per year. The majority of the mice are however not undergoing any unpleasant procedures, but are simply being born “transgenic”, e.g. with a modified mouse genome. Such transgenic animals are most often indistinguishable from regular mice. For actual procedures, we will undertake power calculations to estimate the minimum number of animals we need to study to obtain statistically robust results.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The work to be undertaken under this licence has three connected themes, which are: 1. to Identify novel pain mediators and develop new analgesic strategies for a variety of pain states. 2. To ask how much the immune system interacts with the nervous system in the production of pain.. 3. To define the ‘circuitry’ in the brain and spinal cord that is responsible for pain sensations. We will do this by studying a range of animal models of different human pain-producing diseases. The models will be created in rats or mice. The diseases we will model are: a) Neuropathic pain. Damage to the nervous system, from disease (eg diabetes), chemical toxicity (eg some cancer treatments) or traumatic injury can all produce neuropathic pain. Neuropathic pain is common affecting up to about 8% of the population. b) Cancer pain. Many cancers are associated with significant pain in humans. We will focus on one type of cancer pain, that associated with cancer that spreads into bone. To do this we take rodent cancer cells and transplant them into one of the leg bones of a rat or mouse. c) Pain associated with inflammation. Inflammatory pain is commonly experienced following infection. But it also occurs without infection for instance when the immune system begins to attack our own bodies, as in rheumatoid arthritis. We can mimic these processes in a number of ways. Sunburn produced by sunlight is one such model that we use. e) osteoarthritis is very common in humans, and its incidence increases with obesity. Wear and tear of the joints is the underlying cause. We can recreate this form of pathology in

animals for instance by making a small cut in a ligament that normally stabilizes a joint. These models are widely used in pain research. We will use them in adult animals of both sexes. We will often be testing the effects of a particular treatment or drug or we will be asking if a particular gene is important in the model. This latter question is often asked by using animals where a particular gene or biological processes has been inactivated or switched off and this allows us to judge whether a new analgesic strategy might be effective. A new form of treatment we are interested in is the use of electrical stimulation – so called neuromodulation – to change responses in the brain. We evaluate the effectiveness of our treatments in several ways. One important measure is how the animals behave. In most of our models, the animals become more sensitive to, for instance, heating the skin. For such experiments we heat the skin of, typically, one paw of the animal until the animal decides to withdraw from the stimulus and that point is taken to indicate how sensitive the animal is to painful stimuli. Analgesic drugs reduce this sensitivity. We are testing new measures of behaviour – how the animal behaves in its home cage, as determined by infrared video recording, might reflect ongoing discomfort in the animal. We also measure directly or indirectly the activity of the nervous system in these models and we do this in anaesthetised animals. After these tests are done the animals are humanely killed. Throughout the experiments, animals are monitored for adverse effects. As we are studying the mechanisms of pain, some discomfort is unavoidable in these experiments but in all cases this will be mild or moderate. We know that some drugs can make animals unwell and we will monitor their behaviour for this. Some surgical procedures may cause post-operative pain. We will minimise this by the use of analgesic drugs. For most of our behavioural tests, the animals are free to terminate the stimuli. There can be some adverse effects of anaesthesia and for this reason the frequency of anaesthetic inductions will be kept to a minimum. Rodents tend to be curious, exploratory and sociable in behaviour. Subdued behaviour and isolation are indicative of

	discomfort, pain and distress. Animals showing subdued behaviour, even when provoked, and little peer interaction, or persistent abnormal posture or coat changes or excessive weight loss will be humanely killed
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We are principally interested in how the nervous system generates sensations in health and disease. These sensations are multidimensional and emerge from the integrated action at multiple levels of the nervous system. Therefore, while some information is available from the study of single cells in vitro (which we will exploit), a full understanding can only be obtained by studying intact organisms. A limited number of studies can be undertaken in human subjects (and we actively undertake such work), but for ethical reasons not all studies can be done in people. We will therefore study rodents, where a great deal of previous work has been undertaken.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Animal numbers will be kept to a minimum by carefully planning all studies to ensure that the group sizes are kept to the smallest possible size at which a significant effect could still be obviously detected. Additionally, as much data will be obtained from each individual as is possible; this will include data from a number of simple behavioural tasks to assess post-injury function, data from neurophysiological recordings and detailed anatomical data collected from the same animal.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rats will be used as they closely mimic the pathology of human nervous system. Mice will be used when the use of a particular genetic modification can reveal valuable information (transgenic mice).</p> <p>The models we will use will either be discrete injuries of nerve fibre pathways in order to gain understanding of how specific sensory projections respond to injury and various experimental therapeutics; or, we will use clinically relevant models which closely mimic</p>

the pathology, disease progression and functional readouts observed in human patients; in these cases we can test promising therapies in these valuable pre-clinical models as a first step towards translating a therapy to the clinic.

In all the injury models selected for this project, the most substantial effects on animal welfare will be during the initial post-injury phase (up to 1-week post-injury) after which substantial recovery of general health will be observed in all animals along with significant functional improvements in the vast majority. All animals will receive intensive care, particularly in the acute post-injury phase, to ensure high standards of welfare are maintained. This will include cages remaining on heated mats, administration of analgesics and saline, provision of soft, easily digestible food.

Project	Study of immune responses to infections in humanised mice	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The main aim of our project is to produce mice which can potentially replace the need for non-human primates to be used in the testing and evaluation of new drugs and treatments for infectious diseases prior to clinical trials.</p> <p>Response in humans to disease can be very different from that in non-human animals. We aim to create a type of mouse which is 'humanised' i.e. genetically altered to respond to infection (such as hepatitis, HIV) in the same way as humans.</p>	
What are the potential benefits	Infectious diseases are among the main causes	

<p>likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>for human deaths worldwide, resulting in 10 million deaths in 2010 alone. To better understand why this is, we aim to produce improved mouse models which can be used to study them and better understand how chronic viral infections interact with the human cells. Such models would also enable more reliable assessment of new drugs, therapies and vaccines before use in clinical trials on humans. Mice which mimic human disease responses would additionally greatly benefit a variety of scientific disciplines, including the development of new diagnostic methods and medical imaging systems, drug and vaccine safety, advancements in tissue and organ regeneration, biology of growth and development, computer modelling and genetics.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice, 17,000 over 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>All regulated procedures performed under this license fall under a moderate severity limit. Humanised mice could develop 'graft versus host disease' leading to tissue or organ damage. While some aspects of this does not cause any discomfort or lasting harm, in particular the loss of fur or drying of the skin, all animals exhibiting neurological symptoms (e.g. circular movement patterns) will be culled by a humane method (schedule 1). Mice exposed to high dose radiation may become unwell and will have reduced disease resistance. We will minimise effects by applying high dose radiation in two separate cycles, monitoring animals after exposure. We will use sterile handling techniques at all times and antibiotics where necessary. Some mice will have surgery to implant tissue or cells. Surgery and anaesthesia may result in adverse effects. We will use sterile surgical techniques with close follow-up care and pain relief where necessary. Induction of inflammation or liver injury may result in weight loss or pain. Where possible we will preempt these potential effects using pain relief drugs and monitor mice closely for any adverse effects. Genetic modification of mice may result in adverse effects which can be unpredictable. If unexpected adverse effects should occur, advice</p>

	<p>will be sought from the Named Animal Care Welfare Officer, Named Veterinary Surgeon and Home Office. Tail vein injections of large liquid volumes required for hydrodynamic injection may lead to irregular heartbeat, lung abnormalities and death. We have refined this procedure and see adverse effects in less than 5% of animals. To limit pain and suffering, this procedure is carried out under anaesthesia and animals are closely monitored. Infection may result in adverse effects ranging from weight loss, abnormal organ function, disorientation, bleeding, pain and eventually, death. All animals will be closely monitored and killed by a humane method. At the end of each experiment, all animals will be humanely killed and tissues will be taken for analysis.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Human immune responses and disease is impossible to model <i>in vitro</i> (in the laboratory) as whole organ systems are needed. The analysis of human tissue specimens is an important tool we use but these do not allow us to study the effects of drugs or treatments in the laboratory. We are currently engaged in large-scale research programmes to develop microscale laboratory (“organ on a chip”) models which could be used to study many aspects of disease. However, there are other aspects of immune function which cannot yet be modelled in the laboratory.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We will carry out statistical calculations to ensure that we only use the minimum number of animals and will endeavour to minimise numbers of “control” animals (standards of comparison for checking results) by correlating experiments.</p> <p>Power calculations based on a desired statistical power of 0.05 and an estimated effect size of 0.5 have been performed and resulted in the necessary use of 5 animals per cohort. Since human cells will be used in experiments, these will be repeated with three human cell donors to account for biological variability in the used cell donors.</p>

	<p>We will revisit statistical calculations in light of experimental results and reduce numbers where feasible. Excess breeding will be avoided through close collaboration with animal technicians and mice will be enrolled in experiments at the earliest possible time. All experiments will be carried out in line with ARRIVE guidelines.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use mice in this study as they represent the lowest vertebrates with considerable similarity in our field of study to humans.</p> <p>Whenever possible we will choose minimally invasive techniques for substance administration, such as addition to diet. To reduce pain, suffering or lasting harm to all animals, we will use an agreed scoring system to identify pain or distress. Veterinary advice will be sought in the event of any cause for concern and any animal failing to respond to non-invasive treatment or whose conditions deteriorate beyond defined humane endpoints will be humanely killed.</p>

Project	Studying Gene expression in development and Disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>(1) Understand the processes that maintain normal healthy growth, development and function in the body</p> <p>(2) Identify changes that can contribute to the development of diseases that are relevant in humans</p> <p>(3) Test for potentially new and more effective approaches for preventing or treating common human diseases</p> <p>These studies are necessary to understand how normal function is maintained in healthy individuals and changes the can cause common</p>	

human diseases. The human body, like many higher organisms that develop from a single fertilised egg, must undergo very tightly controlled changes that give rise to the highly specialised and complex organs (e.g. brain, heart, liver, kidney) and maintain function under different conditions. However, because of the complexity of such organisms, identifying the changes that lead to abnormal functions or diseases can be very challenging. Our research is specifically aimed at understanding how normal specialised cells and tissues are produced and maintained from the common blueprint DNA code, which is found in every cell of an organism. Such unique functions are linked to specific regions of DNA, called genes, which carry the code that is used to produce different cellular proteins. However, the DNA must first be copied (transcribed) to produce an RNA template, which is in turn, used as the instructions to produce specific cellular proteins that carry out specialised function in different cells. One of the most important levels of control in the cell is the production of RNA from DNA (called gene transcription) and is largely driven by specialised proteins called transcription factors, which govern the assembly of the machinery that increase some genes while blocking others from being made. In this regard, transcription factors can be considered as master regulators that control cell fate and when altered can cause abnormal gene production that contribute to disease. Therefore, it is important to study how such master regulators act in different cells in the whole animal but also to identify disease-causing changes. Such knowledge can be harnessed for early detection and prevention or treatment of specific diseases.

Different organs in the body must work very closely together to maintain normal function in healthy organisms, so it is important that we identify and study disease-causing changes in a whole animal rather than in isolation, to understand what happens during the transition from health to disease. The master regulators that we are studying are evolutionarily conserved e.g. 90- 95% similar between mouse and humans. This means that by studying animal models we can gain essential information

	<p>on the changes that underlie common diseases in humans but also identify more effective ways of preventing / halting the progress of such diseases or looking for new ways of treating many diseases.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Increase of knowledge: One of the main benefits of these studies will be to increase our knowledge and understanding of how complex and highly specialised organs arise and function normally but also identify changes that lead to abnormal function associated with common human diseases such as cancer, diabetes and heart disease. Such increased knowledge can lead to more effective ways of preventing or treating such diseases. This is very important because many common human diseases develop over a long time, but in general, these are not detected until later stages when damage has already occurred in cells and tissues.</p> <p>Potential application for patients: The benefit for patient can arise by increasing our understanding what controls important cellular events such as cell survival, division and death, which when uncontrolled can lead to human diseases. Mouse models are particularly important because mutant strains with changes in specific master regulators have already shown relevance to human diseases including abnormal heart formation in babies (congenital heart disease), death of specialised cells such as heart muscle after stress and stiffening or abnormal function in blood vessels. Other relevant diseases such as cancer could also benefit from better understanding using some of the proposed studies. Results from using such models could help in early detection of disease-causing changes by identifying potential biomarkers but could also highlight new approaches to treatment at early stages and thereby prevent long-term damage. The zebrafish provides some unique advantages for studying heart development at very early stages that are not possible in mouse and humans. This can be particularly beneficial for understanding heart defects, which can account a large proportion of miscarriages or heart defects in newborn babies. Secondly, the zebrafish has a unique capacity to repair specialised tissues e.g. heart muscle after</p>

	<p>damage, which not seen in higher organisms such as human and mouse. Such damage, which commonly occur after a heart attack, can lead to heart failure and death in humans so studying the factors that help to prevent cell death and promote regeneration in the heart will provide significant benefits in treatment of patients with heart diseases.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This project will mainly use mouse models because existing models have already provided preliminary data that is relevant to human diseases. We will use existing mouse mutants but also produce targeted mutants using available and tested technologies and we anticipate that approximately 4000 mice will be used over the duration project. Rat and zebrafish models will also be used for specific studies where these are experimentally warranted with up to 2000 of each species used during this project.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In general, we do not expect adverse effects during pre-treatment phases of the experiment (e.g. drug administration) but will be carefully observed at all stages during any treatment. Pain relief will be given to animals deemed to be suffering (using defined scoring sheets). Similarly, pain relief will be given to animals undergoing any procedures that are judged to result in pain and discomfort and especially in animals recovering from operations. To ensure that no adverse effects arise from procedures undertaken under anaesthetic, all animals will be monitored closely during and after surgery, until the conditions are stable. Potential risks of infection or other complications during procedures can be minimised by using clean surgical equipment and aseptic techniques. All procedures will be undertaken by qualified and experienced staff who can observe experimental animals closely and identify and treat any pain and discomfort at early stages. Any animal that is deemed to be suffering unduly, will be promptly culled. By keeping monitoring records, we can identify any procedures with adverse effects and seek advice from the named veterinary surgeon. Additional steps will be taken to ensure comfort of animals after any</p>

	<p>procedure e.g. extra clean bedding and provision of mashed food and water. Majority of the experiments to be undertaken will be carried out under the mild or moderate severity and experiments involving higher severity level will be limited as much as possible.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>When trying to understand the changes associated with human diseases, it is necessary to consider the complex interaction between different organs of the body under normal conditions and identify changes that may contribute to abnormal responses and disease formation. Such complex crosstalk between organs and tissues are essential for normal health and function and can only be studied in intact animals. Similarly, to identify changes that cause common human diseases, it is important to mimic the whole-body conditions as closely as possible. For these reasons, experiments using animal will only be undertaken when it is necessary to recapitulate the complex internal environment or mimic the interaction and crosstalk between different tissues or other physiological changes seen in an intact organism that closely mimic the human systems.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Careful experimental design will be carried out to minimise the number of animals used in all experiments. For all studies, animals will be matched in terms of age, gender and weight to minimise variation and therefore reduce the number of animals used. In addition, whenever possible control studies will be performed on the same day to maintain environmental conditions (e.g. temperature) as closely as possible. Whenever possible, in-vitro models such as cell lines and three-dimensional cell cultures will be used to confirm findings and undertake in-depth cellular and molecular analyses that can then be progressed to primary cell culture, which uses fewer animals to derive many experimental points and test the number of interventions from specialised cells (e.g. heart or nerve cells).</p> <p>In addition, wherever possible, we will use carry out serial measurements and paired analysis in</p>

	<p>order to reduce animal numbers at a different time points/duration of treatment.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>For all experiments, relevant models will be selected based on the unique properties in relation to the human diseases being researched. For example, mouse models will be the primary systems used for the studies because these models are extremely well characterised but also because many processes that regulate development and/or function of different tissues are highly conserved between rodents and humans. On the other hand, the zebrafish will provide a better model for experiments aimed at understanding the factors that control heart regeneration.</p> <p>For the different studies, we have used information from existing literature and previous studies to refine the scoring system that will be used to monitor experimental animals. Such guidance, when combined with regular monitoring for clinically relevant measurements, will ensure that changes can be detected early and treatment given before adverse effects can occur. For all experimental animals, steps will be taken to maximise animal welfare with provision of additional bedding, pain relief, easy access to food and water, if necessary. Most of the experimental plans are designed to be carried out in a staged manner so to avoid any undue pain and suffering. In all cases, experimental studies will only be undertaken by experienced researchers with appropriate training trained for any specialised techniques.</p>

Project	Studying lameness causing foot lesions in dairy cattle	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Maintaining good foot health is one of the most critical challenges the dairy industry faces today. This is because poor foot health leads to reduced mobility of the animal and causes lameness. The latter is a debilitating and painful condition, and is described as one of the clearest indicators of compromised welfare in dairy cattle and one of the most important factors for the involuntary replacement of animals. No other common condition is associated with such visible signs of pain and, as such, cow lameness damages the public's perception of the industry. Recent studies have suggested that nearly half the dairy cows in the UK face reduced mobility and lameness issues at some point in their	

life, costing the dairy sector approximately £250 million annually. Painful foot lesions account for more than 90% of reduced cow mobility and lameness cases. These lesions broadly fall under two categories: infectious diseases such as digital dermatitis (DD) and interdigital phlegmon, and non-infectious claw horn disruption lesions (CHDL) such as sole haemorrhages, sole ulcers and white line disease.

Despite the importance of CHDL, the reasons leading to their development have not yet been fully described. The anatomic structure of the foot, animal hormonal and immune profiles, inflammation around calving, animal metabolism, and management practices have been thought to contribute. There are also genetic differences between individual animal susceptibility to CHDL or DD development. In this project, all these factors will be studied together using a large number of animals (3,000 Holstein cows) raised in four UK commercial farms. As a result, the project will (i) determine and quantify the impact of different factors affecting the development of CHDL, (ii) identify and quantify the genetic background of animal resistance to the development of CHDL and DD and (iii) develop practical breeding strategies and tools to reduce the incidence of CHDL while maintaining improvement in other important animal traits (fertility, resistance to other diseases and productivity). Additionally, we will identify novel metabolic biomarkers associated with the development of specific lameness causing foot lesions and describe the role of the foot skin and gut microbiota in the development of lameness causing foot lesions (**in adult and young animals**) and identify any host genotype/ microbiome interactions.

To achieve its objectives, the project will draw on complementary expertise in animal science, veterinary medicine, genetics, biotechnology, bioinformatics, molecular pathology, immunology, microscopy and epidemiology.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Outcomes of this project may improve considerably animal health and welfare by underpinning the development of efficient management practices, new breeding tools and novel pharmaceutical interventions.
What species and approximate numbers of animals do you expect to use over what period of time?	Cattle – 2,500 Calves – 1,000
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All procedures carried out as part of this study are classified as mild. The techniques involved are blood sampling, and biopsy collection which are routine procedures in veterinary practice. There are few adverse effects from the procedures involved. Good handling of the cattle and the experienced veterinary surgeons undertaking the procedure will minimise any discomfort to the animal. For blood collection, there will inevitably be the mild discomfort associated with venepuncture and the possibility of haematoma. Biopsies may be associated with mild, transient lameness. After all procedures, all animals will be discharged from the controls of the Act and returned to stock on the farm.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cattle are the animals which suffer these severe diseases (claw horn disruption lesions and digital dermatitis). Therefore, they are the most appropriate animals to study the disease; these animals may benefit from future preventative measures such as farm management practices and genetic selection for increased resistance. No disease model exists for these diseases and there is little need for it given the high prevalence of the diseases throughout ruminants across the UK and the minimally invasive protocol we are proposing to use for our studies.
2. Reduction Explain how you will assure the use of	We assure the use of minimal animal numbers by use of calculations based on statistical significance and use of power calculations as

minimum numbers of animals	valid.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Ruminants are the animals which are continually suffering with these severe diseases. Therefore, given the high prevalence of this disease ruminants are the most refined choice for this work. The general measures taken to minimise harm to the animals include Good handling of the cattle or sheep and the use of experienced veterinary surgeons undertaking the relevant procedures will minimise any discomfort to the animal. The procedures involved e.g. blood sampling, vaccination and biopsy are the kind of procedures normally conducted in veterinary practice.</p>

Project	Studying neuron-glia interactions during nervous system formation, function and repair in zebrafish																
Key Words (max. 5 words)																	
Expected duration of the project (yrs)	5 Years 0 Months																
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<table border="1"> <tr> <td data-bbox="603 678 630 768"></td> <td data-bbox="630 678 1396 768">Basic research</td> </tr> <tr> <td data-bbox="603 768 630 857">X</td> <td data-bbox="630 768 1396 857">Translational and applied research</td> </tr> <tr> <td data-bbox="603 857 630 947"></td> <td data-bbox="630 857 1396 947">Regulatory use and routine production</td> </tr> <tr> <td data-bbox="603 947 630 1077"></td> <td data-bbox="630 947 1396 1077">Protection of the natural environment in the interests of the health or welfare of humans or animals</td> </tr> <tr> <td data-bbox="603 1077 630 1167"></td> <td data-bbox="630 1077 1396 1167">Preservation of species</td> </tr> <tr> <td data-bbox="603 1167 630 1256"></td> <td data-bbox="630 1167 1396 1256">Higher education or training</td> </tr> <tr> <td data-bbox="603 1256 630 1346"></td> <td data-bbox="630 1256 1396 1346">Forensic enquiries</td> </tr> <tr> <td data-bbox="603 1346 630 1480"></td> <td data-bbox="630 1346 1396 1480">Maintenance of colonies of genetically altered animals</td> </tr> </table>		Basic research	X	Translational and applied research		Regulatory use and routine production		Protection of the natural environment in the interests of the health or welfare of humans or animals		Preservation of species		Higher education or training		Forensic enquiries		Maintenance of colonies of genetically altered animals
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	Regulatory use and routine production																
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	Preservation of species																
	Higher education or training																
	Forensic enquiries																
	Maintenance of colonies of genetically altered animals																
What's the aim of this project?	The goal of this project is to use zebrafish as a model organism to better understand the mechanisms that control how the nervous system is formed, how it functions, and how it is repaired following damage. This work will help understand how to help treat human diseases of the brain.																
Why is it important to undertake this work?	At present we have a limited understanding of the complex mechanisms that underpin the formation and healthy maintenance of a functional nervous system. This limits our ability to prevent or manage the effects of developmental and degenerative disorders of the nervous system, which represent major societal burdens. Part of the reason for our limited																

	<p>understanding of nervous system lies in the vast complexity of the brain and the many interactions between cells of the nervous system that are required to orchestrate its formation and function. The formation and function of neural circuits in the vertebrate central nervous system is incredibly complex. About half of our brain cells are neurons, the electrically active cells that drive communication across our nervous system and bodies. The other half of our brain cells are called glial cells, and there are different groups of glial cells with many different functions, from providing nutrition to neurons, allowing our neurons to talk to our immune system or our blood vessels, and in supporting the function of our neurons. We will use the relatively simple zebrafish model to help discover how the brain is formed, how it functions, how it is disrupted in disease, and how we might develop strategies for its repair. We are particularly interested in studying the interactions of neurons and the glial cells that make myelin in our nervous system. Myelin is a substance made by glial cells called oligodendrocytes in our brain and spinal cord that is wrapped around the electrical cables of our neurons. Myelin allows our electrical cables to transmit signals properly. Myelin is damaged or lost in many human diseases, including multiple sclerosis, and we need to find ways to help prevent myelin damage or loss, or to help regenerate myelin after it is lost. The zebrafish is a vertebrate with many similarities to humans. Zebrafish have many of the neurons and glial cells that we do, and the myelin-producing glial cells that we do. They also have an array of experimental advantages for studying neurons, glial cells and their various interactions the intact living nervous system.</p>
<p>What outputs do you think you will see at the end of this project?</p>	<p>The work of this project will teach us how nerve cells (neurons) and nerve supporting cells (glial cells) of the brain interact to ensure normal nervous system formation, health, and function. We will also learn how neurons and glial cells respond to disruption to the nervous system, when we create animals that model specific aspects of human diseases. Our ability to study zebrafish that model human disease also allows us to carry out discovery projects that aim to identify new strategies to treat disease, be they manipulations to genes or drugs. We will publish our findings that describe our new insights into brain formation, function, disruption and repair in scientific journals, all of which will be open access to the public. In addition, we have generated and will continue to generate genetically</p>

	<p>altered zebrafish to share with researchers around the world. We will prepare press releases and social media-based outputs to convey the findings of our work to the public in a digestible manner. We will work with collaborators in both academic and industrial institutions on drug development projects . Our long-term goal is to help find treatments for developmental and degenerative disorders of the human nervous system.</p>
<p>Who or what will benefit from these outputs, and how?</p>	<p>Numerous groups will benefit from the outputs of our studies. The scientific community directly involved in studying mechanisms of brain development and disease will benefit from new knowledge that can be integrated into our growing understanding of the nervous system. Pharmaceutical companies will benefit from our research and our work could lead to bring us closer towards finding treatments for human diseases. Therefore, we hope that patient groups, families, carers and the wider community will ultimately benefit from our work, through the development of strategies to cure disease. During the course of the project the general public will benefit from knowing that progress is being made in tackling major areas of unmet need in human health.</p>
<p>Will this work be offered as a service to others?</p>	<p>No</p>
<p>How will you look to maximise the outputs of this work?</p>	<p>We maximise the output of our work by collaborating widely with groups who have diverse expertise in technology or the use of different model systems. We collaborate with both academic research groups and major pharmaceutical partners to help increase the impact of our work in so far as possible. We make our findings publicly available through open access publications. In addition, we present our work widely at local, national, and international meetings, and through various media outlets. Furthermore, and importantly, we work closely with medical charities to convey our work to the public, and regularly contribute to information dissemination campaigns.</p>
<p>Explain why you are using these types of animals and your choice of life stages.</p>	<p>We use zebrafish as a model organism to study the formation and function of the nervous system, and also to assess the response to nervous system damage and to find strategies to bring about repair. Developmental and degenerative diseases of the nervous system</p>

	<p>represent a major burden to society, and there are currently very few treatments for disease. This is in part due to the complexity of the brain and in part due to the difficulty of observing key biological events in real time in most animal models. Furthermore, the feasibility and cost of carrying out large-scale discovery projects in most vertebrate models is prohibitive. We use zebrafish as a model to help overcome these challenges. Zebrafish are vertebrates that exhibit remarkable conservation in terms of their molecular and cellular makeup with humans meaning that they often have shared mechanisms related to diseases and respond to gene and drug based manipulations in similar ways. We primarily study young developing zebrafish in the laboratory, because they are small, relatively simple, develop quickly, building a functional nervous system in a week, are optically transparent, and can be generated in very large numbers. These features, together with our ability to create genetically altered animals, and treat young zebrafish with drug like compounds, means that we can directly see into the brain and observe brain development, brain function and even brain pathology as it occurs over time. This then allows us to investigate how experimental manipulations, for example drug treatments affects such processes in real time. Thus the use of zebrafish allows us to gain insights into biological events that are difficult to achieve using other systems and to identify strategies to treat disease at an unprecedented scale.</p>
<p>Typically, what will be done to an animal used in your project?</p>	<p>The majority of animals that will be used in our project will be genetically altered zebrafish that have fluorescent proteins in cells or tissues of interest that allow us to track biological events in real time using a range of cutting edge microscopes. In addition, many of the animals that we use in our project will have further genetic alterations that change the function of specific genes of interest, e.g. a gene associated with a human disease. The combination of genetic alterations that allow us to see cells of the nervous system in the context of manipulation of specific gene function allows us to gain great insight into how specific genes affect nervous system formation, function, disruption and repair. In many cases, we will also treat zebrafish with chemical compounds to assess how they affect the animal, and in particular the formation and health of its nervous system. We have a robotics-based microscopy platform that allows us to assess how treatment of large numbers of zebrafish with large numbers of compounds affects specific biological processes, e.g. the</p>

	regeneration of brain cells in disease-like states.
<p>What are the expected impacts and/or adverse effects for the animals during your project?</p>	<p>Due to the small size and rapid development of young zebrafish, and the fact that they can get nutrition from the maternal egg for the first week or so of life, we can carry out the majority of our experiments in largely non-invasive manners, and typically with little and often no signs of adverse effects on the animal. We are, however, interested in treating diseases of the human nervous system, and so we have created models of certain aspects of human diseases using zebrafish. These include systems to ablate cells of the nervous system, or the alteration of gene function that disrupts nervous system formation or function. In some cases, such animals can exhibit adverse effects including disrupted development or motor outputs. In the majority of cases, we can study animals with such adverse effects at stages prior to their being considered sentient enough to experience any suffering.</p>
<p>What are the expected severities and the proportion of animals in each category (per animal type)?</p>	<p>We do not study animals that exhibit severe adverse effects.</p> <p>We occasionally maintain animals that exhibit moderate adverse effects, due principally to genetic alterations, because very few of our experimental protocols elicit adverse effects of their own,. Such moderate effects may be due to neurodegeneration and manifest as impaired motor outputs, such as disrupted swimming, or could be due to complex interactions of cells of the brain and body and be manifest in increased stress. We would only study animals experiencing moderate adverse effects for short periods of time. However, even brief analyses of zebrafish can be very informative, due to their rapid development and the ability to directly watch biological events in the animal in real time. We expect that less than 1% of all the animals that we will use during our project would experience even this level of effect.</p> <p>We also study animals with mild effects. Such effects could be manifest as subtle deficits in behaviours, or mild stress due to being restrained during microscopy. We expect that up to 5% of the animals that we will use might experience this level.</p> <p>Our experience to date indicates that the vast majority (>90%) of animals will not exhibit evidence of experiencing an adverse effect that is observable. The vast majority of animals that we use are for breeding</p>

	and maintenance reasons, and we do not keep animals that exhibit any significant evidence of suffering for these purposes.
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	The formation and function of neural circuits in the vertebrate central nervous system is incredibly complex. Brain development, function, and healthy maintenance involves intricate interactions between neurons, between neurons and glial cells and between these cells and our immune system and cardiovascular system. Many of these cellular interactions are not yet possible to study without animal models, because they are so hard to recreate in a dish. The consequences of damage or disruption to the nervous system also triggers very complex cellular responses and interactions that cannot easily be reconstituted without animal models. Therefore, to be able to understand how neurons and glial cells interact to construct the nervous system we need animal models and to see how these cells are influenced by the immune system and vasculature in disease, it is also currently essential to employ animal systems. However, in using zebrafish, we make use of arguably the simplest vertebrate model in which the complexity of the nervous system can be directly interrogated.
Which non-animal alternatives did you consider for use in this project?	Certain aspects of neuron and glial cell development, and even certain aspects of myelination can be studied without using animals, by studying cells grown in the dish. We work closely with colleagues who have expertise in studying cells in the dish and we have gained many insights from such work. However, this current project aims to use the simple zebrafish model to begin to study the complexity of brain formation, function and disease as it occurs in the natural setting. At present there are no other suitable non-animal alternatives to do so. We considered using cell culture techniques that now allow mini-brain-like "organoids" in the dish, and are hopeful that in years to come technologies will become so refined that we they can be used to study neuron and glial cell interactions and neural circuit function. Such cell culture systems may even allow us to model certain aspects of disease in the dish, and we look forward to incorporating such models

	into our work.
Why were they not suitable?	Brain organoids are beginning to show great promise as an experimental tool for neuroscience, but have not yet been established to the point where they have all of the neurons and glial cell types that would be required to be able to probe the mechanisms of nervous system formation and function. Furthermore, in the context of modelling disease, brain organoids have not yet been developed with a vascular system and the full complement of immune cells that are known to influence pathology and repair.
Enter the estimated number of animals of each type used in this project.	zebra-fish: 150,000
How have you estimated the numbers of animals you will use?	<p>We have calculated the number of animals that we are likely to use based on our current use of zebrafish as a model system. One of the major advantages of using zebrafish as an animal model is that many distinct genetically altered lines of zebrafish can be maintained by single laboratories and that the system can be used for large-scale discovery projects that are not feasible using mammalian models. Therefore, we plan to use a large number of animals through our project. Of the animals we project to use, that majority are for breeding and maintenance purposes. We have calculated the projected number of fish, based on our current use of approximately 800 tanks of zebrafish per week, in which we keep an average of 20 fish. We aim to refresh stocks once per year, meaning that we will use 5 separate generations of each stock over the course of the project, giving a total of 80,000 animals. We maintain >150 distinct genetically distinct lines of fish, many used by several users, and constantly monitor our stocks to ensure that we are not maintaining lines that are not in use.</p> <p>The second protocol projects the use of up to 20,000 zebrafish for the generation of new genetically altered animals. This number largely reflects the new ability to target gene function at scale using new tools, including "CRISPR-cas9" gene editing. We can now assess the effect of changing gene function in animals very soon after injecting reagents that can edit the genome. In the past, if we were interested in a gene's function, we would have to edit the genome and grow animals up to sexual maturity, maintain them through subsequent</p>

generations, and test if they affected a biological process of interest. Now we can look for the effects of disrupting gene function within days of such "editing." Although the number of animals we are likely to use may increase, the length of time that animals need to be maintained will be greatly reduced, representing an experimental refinement. The ability to target gene function directly means also that we can quickly assess how individual genes affect many different biological functions. For example, we can see how editing different genes affects different cell types or disease states because we can directly edit genes in animals with fluorescent reporters in their neurons, glial cells, immune cells, cardiovascular system etc. Testing the effects of 200 genes over the course of this project in 3-5 assays using 10-15 animals per assay will require 10,000 animals. We will also generate stable mutant lines from genes that exhibit particularly important functions when assessed by acute gene editing, and expect to generate up to 20 such lines, with current estimations that we need to grow up 50 animals to successfully do so. In addition, we are currently generating animals in which gene function is disrupted in a cell-type specific manner. At present we need to screen through many animals in order to find suitable ones for in depth study, thus meaning we require about 200 animals per new line we establish. We anticipate establishing 20 such lines over the course of the project, and continuing to establish further new transgenic reporter and effector lines, and have thus estimated that we may use up to 10,000 animals for this purpose.

In addition to the 10,000 gene-edited animals that we will study directly after their gene editing, we predict using a further 40,000 animals in our experimental analyses. This is driven in large part to our success in establishing a fully automated screening system that allows high-resolution imaging-based analysis of larval zebrafish. Using this system, we can screen how drug-like compounds, as well as genes, affect up to 1000 fish per day. Although we anticipate that many of our studies will continue to be carried out at embryonic stages (5 days after egg fertilisation and under) before zebrafish are considered sentient enough to require legislative protection, we have established transgenic models in which we can ablate myelin, i.e. cause demyelination, which we study after 5 days of age. With this demyelination system, we can use our screening system to search for drug-like compounds that can, for

	<p>example, promote myelin regeneration. We anticipate using up to 30,000 animals in our screening system, and have predicted using a further 20,000 for other experiments, based on our current use. We carry out very careful calculations to define how many animals are needed to find statistically meaningful effects in our experiments, and will continue to do so for new studies.</p>
<p>What steps did you take during the experimental design phase to reduce the number of animals being used in this project?</p>	<p>The principle step that we take in our experimental design is to determine what questions we can address using zebrafish at unprotected stages. The use of zebrafish at unprotected stages greatly reduces the number of animals on experimental protocols, and we will continue to pursue this strategy. However, the modelling of disease and the analysis of some aspects of neural circuit maturation are not possible to carry out at unprotected stages, nor are breeding and maintenance protocols, nor the generation of new stocks of genetically altered lines. However, we can assess the efficiency of transgenesis and gene editing at unprotected stages, which reduces the number of animals taken on to protocols. Another important way in which we can reduce animal number is through live imaging of individual animals over time. Through time-course or time-lapse imaging, we can gain a wealth of information about the dynamic nature of biological events from single animals that would otherwise require multiple animals being assessed at many different time-points.</p>
<p>What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?</p>	<p>We will continue to work to optimise the efficiency of transgenesis and gene editing, particularly cell-type specific gene editing, which we hope will reduce the number of animals that we use in our work. We will also work closely with our aquarium staff who are implementing trials that aim to adapt husbandry procedures to ensure more reliable sex ratios in our breeding stocks. Skewed sex ratios are prevalent in zebrafish stocks and definitive protocols to balance male and female generation have not yet been established. This would help reduce the number of animals that we need to use to generate sufficient breeding stocks for our experiments. In many cases, we will also be able to carry out pilot experiments on zebrafish that can be shared by other users who have the authority to do so.</p>
<p>Which animal models and methods will you use during</p>	<p>We use zebrafish to study neuron-glia interactions during nervous system formation, function, disruption</p>

<p>this project?</p>	<p>and repair. We use zebrafish due to the ease with which one can generate and maintain genetically altered animals with fluorescent reporters that allow direct visualisation of molecules and cells of the nervous system in embryonic and larval stages without the need of invasive procedures, and typically without any evidence of adverse effects. We also use zebrafish because of the ease of gene editing and expression. Again, the ability to carry out transgenic manipulations and gene editing on newly fertilised eggs means that their efficiency and any potential adverse effects can be observed prior to their development to ages that are protected. This is an important refinement that reduces animal numbers used and helps reduce any potential suffering the animal might experience, were it not possible to do so.</p> <p>Our principal experimental methods involve live imaging zebafish, and we can do so at different scales. We can carry out high-resolution screens of many animals to quickly assess how gene or compound function affect biological processes of interest, and in a manner that shows no signs of causing distress to the animal. In contrast we can also carry out extensive in depth imaging over time of individual animals, either of the structure or function of their nervous system in a healthy or disease context. Time-lapse, time-course, of individual animals is a refinement, because we can gather enormous amounts of information from single animals. Such analyses provide insight into dynamic processes impossible to gain in other systems that would require the use of multiple animals if using other models.</p> <p>We continue to pursue any innovations in husbandry practices, and when trialed and deemed successful, will be applied to our protected stocks, and may significantly reduce numbers of animals used for breeding and maintenance throughout the project.</p>
<p>Why can't you use animals that are less sentient?</p>	<p>Myelinated axons are a vertebrate specific elaboration. Therefore zebrafish are the simplest standard model in which they can be studied. Zebrafish also have an early onset of myelination and so are arguably less sentient than mammals during myelination, and we make every effort to study animals at the earliest stages at which we can address the questions that underpin the aim of our studies.</p>

<p>How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?</p>	<p>REDACTED. We will implement appropriate advances through discussions with our local vets and named animal care and welfare officer.</p>
<p>How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?</p>	<p>Very few of our experimental protocols cause significant harm to animals, and are already well refined. The main source of potential adverse effects to animals comes from the generation of new genetically altered animals where all possible effects on the animal are not possible to predict. However, as noted throughout, we can assess how new genetic alterations affect animals at unprotected stages and we carefully monitor animals following the introduction of new genetic alterations. We are currently also implementing a cutting edge stock management database that will allow us to better track and monitor survival rates and any effects seen across all of our stocks.</p>
<p>What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?</p>	<p>We follow the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines issued by the National Centre for the 3Rs, and will follow the recently published guidelines put together by a group of zebrafish researchers in collaboration with animal welfare experts at the Federation of European Laboratory Animal Science Associations (FELASA). In addition, we continue to refine practice across all experimental approaches as innovations and advances are published in the literature.</p>

Project	Studying the host response to tumour and metastasis	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The relationship between tumour cells and their microenvironment has become a vanguard of cancer research. This relationship can be simply expressed by the idea that cancer cells would not be able to start and maintain tumour growth without an active commitment and changes in the surrounding host tissue. This relationship between tumour cells and their associated host tissue persistently characterizes tumour growth, from onset to metastasis and has been recently correlated with resistance to chemotherapy. We aim to study the interaction between cancer and the host to address the with the following questions:	

	<p>1. Identify novel essential cell components and signals within the metastatic and tumourigenic organ environment essential for tumour growth. This will allow us to identify novel target for anti-cancer therapies.</p> <p>2. Characterize the activity of certain cells normally involved in the protection of our body from infections during cancer. As these cells are reported to act both as pro-tumourigenic and anti-tumourigenic, differentiate what make this switch could allow us to use them against the disease.</p> <p>3. Explore what kind of inflammatory stress trigger disseminated dormant cancer cell reactivation and relapses in cancer patients that were “clinically cured” more then 5 years before. This understanding will greatly help the management of cured cancer patients.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The immediate benefit of the project will be the discovery of novel players and mechanism driving cancer onset and progression and in longer period to identify pharmaceutical strategies to interfere with those mechanism and design better anti-cancer treatments.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use mice only. Based on the work carried out in the past 5 years by my laboratory under our previous licence, the number of mice expected to be used over the 5-year period will be approximately 50000 mice bred 15000 mice used under the various experimental protocols.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The studies performed under this project licence will be tumour studies. In addition to the close monitoring of the mice under an experimental protocol, the use of known and as controllable as possible models or protocols to induce the cancer disease will be a strategy to limit the adverse effect. The expected level of severity for all the experiments is either mild or moderate. The majority of the mice will undergo schedule 1 killing at the end of the procedures. On more rare occasion terminal anaesthesia will be required.</p>

Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>In contrast to other tumour studies, here, a tumour is analysed in the context of its local microenvironment as well as the systemic changes induced in the host organism affected by the disease. Therefore this study must mainly be performed <i>in vivo</i> as the complexity of these changes and the number of players involved cannot be modelled <i>in vitro</i>.</p> <p>However, targeted <i>in vitro</i> assays will be designed accordingly with the gained information used to reduce mouse workload. For instance, in order to assess specific interactions between tumour cells and certain components of the microenvironment or of the immune system. This approach will also have the advantage of identifying the impact of these components on each other in a “clean” system where all the players are known.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We employ several strategies to try to limit the number of mice in the study. Firstly, we will always aim to maximise the amount of data we get from each mouse and when possible, we will use it for the study of both primary tumour and metastasis. Also, we will limit the use of genetic models (that often require many generations breeding) using orthotopic transplants of labelled cells and treating the mice with chemical agents either to block immune-system components or to generate tumours. We also use the minimal amount of mice needed for statistical significance when testing the experimental hypothesis. Finally, by careful monitoring of our transgenic mouse colonies we try to breed as few mice as possible.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The scientific question to be address with this project is the understanding of the complex interaction of the cancer disease with the entire host organism, therefore the closest model to the human disease need to be used.</p> <p>The reasons why mice are the best choice as cancer experimental models, can be</p>

summarized as follow:

- the physiology of cancer in mice is consistent with the human disease,
- the need for working with genetic modification (knock out, transgenic models). In mice, many models are available as well as well defined techniques for de novo production,
- they are economic, easy to handle, they produce multiple offspring and they have a very short gestation period as well as a functional survival time.

When working with animal models it is essential to minimise any possible adverse effects of the experimental procedure. We closely monitor the animal's reaction to specific experimental procedures and pay attention to any sign of sufferance.

Some of the genetic alterations , for instance, mice carrying genetic predisposition to tumour, may show a higher risk of mortality especially after 7-8 months of age. The colony of these mice will be kept as young as possible and monitored closely for the expected potential risks.

When implementing surgical procedures standardized by other laboratories, they are always further refined to the best possible standard. For instance, the size required for the insertion of the scaffold in protocol 15 was reduced to the minimum required. Similarly the position of the incision was changed to avoid contact with the upper metal grid or plastic in the cage.

Project	Studying the origins of cancers from stem cells	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our main objective is to understand the earliest events that drive normal stem cells to become malignant (cancer initiation). Better understanding of this process could lead to new approaches for early cancer detection and diagnosis, as well as novel therapies. Importantly, understanding how these are similar or differ among cancers may also provide fundamental insights into the origins and basis of different cancers. Additionally, we would like to understand the fundamental biological differences between neonatal and adult stem cells as there is an impact on cancer risk between children and adults. We have shown that neonatal mice are more resistant to getting cancer. We aim to	

	<p>understand these differences by understanding the biology of young and old stem cells in normal mice as well as in mice that have been subjected to various organ damaging agents. The overarching aim of this is to be able to introduce biological mechanisms that protect neonates in adult stem cells and reduce cancer risk and incidence in adults.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Our project holds great promise to make fundamental and much needed progress in advancing understanding of the origins, biology and treatment of adult and paediatric epithelial cancers. The benefits of this project are numerous and include, but are not restricted to: (i) advancing the knowledge of a variety of epithelial cancers (ii) provide insight into difference of neonatal and adult stem cells that will significantly change the way that we understand the biology of aging stem cells and their impact on cancer (iii) Pave the way for new cancer therapeutics that will shift the direction of cancer treatment not only to understand how we can treat people living with cancer, but strategies that attempt to completely prevent the formation of cancer in the first place.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will be working with mice and expect to use around 13848 mice over the licence period of 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>For our experiments animals undergo injections into the abdomen with drugs. These drugs can result in a fluorescent protein being expressed or can cause mutations in the DNA resulting in cancer in multiple organs in the mice. This injection itself has minimal effects and animals will recover quickly. We are also performing experiments where specific organs are damaged using chemicals that have known targets, such as how smoking damages the lung, or alcohol damages the liver. These damaging chemicals are also given in the animals diet, or by inhalation into the lungs or by injection into the abdomen. The animals may suffer from laboured breathing or abdominal pain after exposure to these</p>

	<p>chemicals, however they will be monitored closely for signs of suffering including losing weight. At the first sign of pain, animals will be treated with drugs that relieve pain. Animals are expected to recover from these treatments. After injection or chemical damage the animals are left to age normally in their housing and are monitored for any signs of tumour development or organ damage. Animals are expected to live a normal life and as the animals age, they are likely to develop tumours in multiple organs within their body. Signs that tumours are growing include; weight loss, general under conditioning and sometimes growths can be felt under the skin of the animal. At the end of the experiment animals can be given another injection into their abdomen to mark dividing cells. This injection has limited effects and the animal will not suffer. In some instances, we may be interested in imaging the mice using sophisticated techniques such as MRI scans in order to understand how changes are taking place in the body and exactly how tumours are behaving inside the mouse. For imaging, mice will be anaesthetised in order to be able to be imaged correctly. During anaesthesia, there are no expected further adverse effects on the health of the mouse. After imaging has taken place, if mice are to be kept for further experimentation, then mice will recover from anaesthesia with no further adverse effects expected as a result of the use of the anaesthetic. At the end of the experiment, animals will be killed by a humane method and tissues taken for analysis after death.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Because our approach requires the use of specific cancer-susceptible cell types at specific points in development, this is currently only possible by using live animals that fully recapitulate the complexities and cell populations present in development.</p> <p>Non-animal models cannot imitate the complex human or animal body. The advancement of knowledge and development of concepts to</p>

	improve human and animal health and well-being requires the use of living animals.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We use in vitro methods where possible to limit the number of animals required for the in-vivo investigation stage. Additionally, where multiple organs need to be assessed, as many organs as possible will be used from one animal thus minimising the number of animals used in total to study the biological mechanisms of multiple organs.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the ideal species for our experiments: their lifespan (approx. 2 years) allows us to investigate tumour development over time and the scientific community has a range of techniques to manipulate the mouse genome allowing us to answer questions regarding tumourigenesis and tumour biology.</p> <p>We will minimize the animal suffering by monitoring the tumour growth and ensure it does not extend beyond the maximum permitted size/load. The surgeries will be performed under published best practice guidelines, or where we have modified these to reduce suffering further.</p>

Project	Subclinical disease resistance and resilience in ruminants
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research <input checked="" type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Drug resistance hampers disease control in livestock and we need alternatives to control disease. We have previously shown that nutritional strategies can ameliorate the effects of parasitic disease. In this project we aim to investigate how the nutrition, the genetics and the behaviour of the host can interact with the parasites, in order to optimise the use of alternative strategies for parasite control in sheep
What are the potential benefits likely to derive from this project (how science could be advanced or	The knowledge generated from this project will result in original scientific publications and presentation to scientific and lay audiences to

<p>humans or animals could benefit from the project)?</p>	<p>inform how feeding strategies, host genetics and host behaviour can be used to achieve worm control with minimal drug use. The information will benefit both conventional and organic farmers, to manage their livestock in a sustainable way. The long term benefits will also reach human parasite control strategies in developing countries, where infection and malnutrition often go hand in hand.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Sheep is the livestock species of choice in this project; drug resistance is particularly relevant for sheep and as a consequence the alternative approaches for parasite control have been studied in more detail in sheep compared to other livestock species. Total of up to 300 ewes and up to 600 weaned lambs will be used in the 5 year duration of the project. Through lab-based studies and statistical tests we will identify the minimum number of sheep required to observe significant effects between the groups throughout the project</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Sheep of different breeds will be given parasites orally at levels that will not make them sick but but will result in poor growth or weight loss. Infection protocols are sufficiently refined to only expect very occasionally mild adverse effects, such as mild diarrhoea. Parasitised sheep will then be offered diets that will contain anti-parasitic compounds or diets that are supplemented with extra nutrients that can reduce the level of parasitism. Their behaviour will monitored with the use of activity loggers to test whether changes in behaviour can be used as an early indicator of disease. Blood samples will be collected to investigate how dietary interventions affect host's immune responses to the parasites. Sheep are expected to recover quickly from all sampling procedures, such as faecal and blood sampling. At the end of the experiments animals will either be killed by a humane method for tissue recovery and parasite quantification or will be returned to stock and enter the food chain if appropriate</p>

Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The investigation of the interactions between nutrition, pathogens and host responses to subclinical disease requires animal use, due to the complexity of the biological processes involved, such as nutrition and digestion, immunity, and genetics. In relevant cases, extensive in vitro studies, which test the antiparasitic activity of plant extracts in isolation, will be used prior to in vivo testing. These assays have been developed previously by us and others to support the screening of large number of extracts. From all those extracts tested in vitro only the most active one will be tested in animals, resulting in lower numbers of sheep used in animal experiments</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>To ensure the minimum number of animals required to test our hypothesis, all our experimental work will receive statistical input from experienced biostatistician(s) familiar with this type of studies. In addition, our previous experience with this type of work has enabled us to quantify the variation between groups and as a consequence, using mathematical formulas we are able to estimate the minimum numbers of animal required to demonstrate significant differences between groups</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The work is investigating the interactions between nutrition, behaviour and genetics to subclinical parasitism in sheep and as a consequence sheep is the animal of choice. Although complimentary work will take place in rodent models, particularly in relation to investigating the effects of nutrition non immune responses, sheep are ruminants and as such their metabolism is distinct from that of monogastric animals (such as rodent models). In this project, sheep will be offered lower levels of protein nutrition; we have already refined the levels of nutrition sheep will be offered to reflect the often observed protein scarcity under commercial conditions. Sheep will also be offered extracts that contain active compounds against parasites. Having had experience from such studies, we have refined</p>

	feeding protocols e.g. how often to offer these extracts and for how long, to ensure no detrimental effects are observed in these animals. Close monitoring of animals and early intervention should this be required will minimise any adverse effects arising
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Project	Super High Affinity Sheep Antibody Creation	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To create super-high affinity sheep monoclonal antibodies for use in clinical diagnostics (ie. blood tests, urine tests, serum tests, etc.). These tests might take place in hospital laboratories or at “point-of-care”.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our high affinity sheep antibodies have been proven to give rise to more sensitive & accurate diagnostic tests, enabling clinicians to better diagnose, monitor & therefore treat patients. Many diagnostic tests still use polyclonal antibodies (derived from repeated immunisation & blood sampling of animals) which require constant use of animals over time. Monoclonal antibodies (derived from animal cells grown in the laboratory) only require animals to be used once during their production. Therefore, by replacing the polyclonal	

	antibody element of a diagnostic test with a monoclonal antibody, fewer animals will be used in total.
What species and approximate numbers of animals do you expect to use over what period of time?	Sheep aged between 0.5 and 4 years. We expect to use approximately 250 (maximum 500) over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All methods used are classified as “mild” severity by Home Office classifications. A relatively low number of animals experience localised inflammation/abcessation as a result of the procedure but do not display any signs of discomfort. All animals are euthanased humanely by a vet following procedures, in line with Home Office requirements.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal alternatives do not produce antibodies of sufficiently high sensitivity/affinity. Antibodies are large proteins with complex structures; it is not currently possible to create correctly formed artificial antibodies, let alone those with high affinity.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Extensive experience in immunising sheep to give rise to high-affinity antibodies and constant data monitoring ensures the number of animals used per project is sufficient but not excessive. We are working on an in vitro (non-animal) technology that can be used as an adjunct to this process which will enable us to reduce numbers still further.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the	Large mammals have a larger diversity of white blood cells than rodents (mice are most commonly used for antibody production). This allows us to make antibodies that others cannot. Of large animals, sheep are suitable because of: <ul style="list-style-type: none"> • Ease of immunisation and blood sampling procedures – usually remain placid during procedures. Whilst sheep do not like to be singled out, they remain calm so long as they are within sight of other sheep and are in no visible pain. Procedures are completed very quickly (usually within a couple of minutes), so any stress of being restrained is transient.

animals.	<ul style="list-style-type: none">● Ease of housing – can live a “natural” life on a farm.● Availability of a cell line in our laboratory that allows us to make the sheep’s white blood cells (which produce the antibody) “immortal”. <p>In work done in previous projects, refinements to the precise location of the immunisation site and the technique used have minimised inflammation.</p> <p>Monitoring sheep for general welfare and for any adverse reactions is regularly conducted.</p>
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Project	Survival of commercially caught and released marine fish
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X Basic research
	Translational and applied research
	Regulatory use and routine production
	X Protection of the natural environment in the interests of the health or welfare of humans or animals
	X Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To increase our understanding of the survival of marine fish after they have been caught by fishing vessels and released back to the sea. This knowledge will be used to advise UK and international government, stakeholders and NGOs and support sustainable fisheries management.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit)	There are two main benefits; i) fishermen are permitted to release unwanted fish catches back to the sea only when it can be shown that these fish survive when returned to the sea. Without this evidence, many unwanted fish will be

<p>from the project)?</p>	<p>brought ashore and will die, which could reduce the sustainability of fishing; ii) in setting the permitted amount of fish catches, it is important to know how many fish are killed by commercial fishing. Currently, it is assumed that unwanted fish catches that are returned to the sea do not survive. Information on the amounts of fish that do survive, will improve the data used in setting sustainable fishing levels.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Adult, sub-adult and juvenile marine fish. Up to 4000 animals would be used over the 5-year period of the work.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>There are two procedures proposed, i) attaching tags to fish and releasing them back to the sea; ii) using holding facilities to observe and monitor fish that would have been released back to the sea. i) The tagging is assessed as 'Mild' severity. Possible adverse effects are pain during tagging, scarring and/or infection of the tagging location. Where needed, pain relief will be used on the tagging location. Adverse effects will be minimised by using practiced tag attachment methods. Aseptic precautions will minimise the risk of infection. After the tag has been attached, tagged fish will be discharged from the Act and returned to the sea. ii) The observation method is assessed as 'Severe'. The aim of the work is to estimate how many fish survive after release, and it is possible that some fish will die during the monitoring period. We will introduce humane end points at the earliest point of the monitoring period. Fish will be killed if they display a health condition that is linked with a low chance of survival or they stop feeding. Therefore, the procedures will evolve as we learn more about how the health of fish relates to its chance of survival. At the end of the monitoring period, the fish will be killed by an approved method.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-</p>	<p>The project will provide knowledge on the survival levels of commercially caught and released fish. This information does not exist, and the survival levels are likely to be different</p>

<p>animal alternatives</p>	<p>depending on the species of the fish, and on how the fish are caught. It is currently not possible to find a non-protected animal alternative. The project will seek, review and incorporate alternatives throughout the project duration.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The methods and numbers of animals used will be based on experience and previous research. There will be input from a statistical team and the Animal Welfare and Ethical Review Body, so that the minimum number of animals are used.</p> <p>We will find out how the health of fish, at the time they would be released from the fishing vessel, relates to their survival chances. If a relationship can be found, it will be possible to assess the fish at the time they are released, to predict their survival levels, and there will be no need to tag or observe fish.</p> <p>This method will also be used within studies. The tagged and observed fish will show how health condition relates to chances of survival. A larger number of fish will have their health assessed at the point they would be returned to the sea. The health-survival relationship will be applied to all of these assessed fish to give an overall survival estimate. This reduces the number of fish needed for tagging and observation.</p> <p>Where possible, data and knowledge from other research will be used instead of using animals. For example, sharing of knowledge and data will continue at the International Council for the Exploration of the Seas Working Group on Methods for Estimating Discard Survival. Opportunities to reduce the number of animals used will be assessed throughout the project.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to</p>	<p>The project will provide new knowledge on the survival levels of commercially caught and released fish. This information does not exist, and the survival levels are likely to be different depending on the species of the fish, and on how the fish are caught. It is not possible to find a non-protected animal alternative. A range of commercial fish species will be studied.</p>

<p>the animals.</p>	<p>Where survival chances are shown to be high, this work will support requests to allow fish to be released back to the sea. Therefore, the species and fisheries selected for study will be those where the potential for survival is highest.</p> <p>The methods proposed are based on direct experience of fish tagging and husbandry techniques developed over 20 years. The tag attachment method will be one that minimises pain and suffering. Tagged fish will be checked that they are fit enough to be released to the wild, and those assessed not to be fit, will be killed by an approved method.</p> <p>For the observation method, the holding facilities and husbandry techniques will be chosen to minimise suffering to ensure reliable results. Humane end-points will be used at the earliest point in the monitoring time for fish that are unlikely to survive. This will be continuously evaluated so that any suffering is minimised and the results are useful. The monitoring periods will be as short as possible while still producing robust results.</p> <p>Refinements will be reviewed throughout the project; information will be sought from other relevant research and from NVS/NACWO.</p>
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Project	Synaptic organisation of neuronal circuits for perception and behaviour	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim to improve our understanding of the organization of the neuronal circuits in the brain that are responsible for representing sensory information. In particular, we wish to find out how nerve cells in sensory areas of the brain communicate in order to represent and integrate information from our different senses to guide behaviour, for example, during the selection of relevant targets. We also wish to find out how these representations are altered when visual or auditory perception improves as a result from learning.	

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Improving our understanding of the neuronal basis for perception and behaviour is in the first instance a matter of considerable fundamental scientific interest. By generating fundamental new knowledge about the structure and function of specific brain areas we will not only advance our knowledge of brain mechanisms in health, but also help understand what may go wrong in neurological disorders which are currently poorly understood and represent a heavy burden in society. Indeed, the estimated annual cost to the UK of mental disorders is £100 billion. The difficulty in designing better treatments for neurological disorders has highlighted the urgent need to improve basic knowledge of neuronal circuits. The data collected during this research program will also be used to build, refine and test biologically accurate models of brain circuits. A lasting benefit of this animal research will be to provide computer models that can be used by other scientists in their future research. Such models can generate new hypotheses through predictions and enable neuroscientists to a better understanding of complex neural systems.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>In order to gain understanding and test causality, complex systems need to be dismantled and probed invasively. These experiments can thus only be done in reduced model systems. Our system of choice is mice and we will use approximately ~6500 mice over 5 years in procedures other than simple breeding and maintenance. We may breed and/or maintain up to 5000 mice, some of which will be the same ones as in the additional procedures. Due to the statistical nature of genetics, around half of the animals bred under this protocol will not undergo other regulated procedures but will be reintroduced to the breeding stock or terminated humanely.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at</p>	<p>Part of the project will involve the raising of genetically altered mice to allow us to study the functions of particular molecules in sensory processing. These animals are expected to be no different in the way they behave from wild-</p>

the end?	<p>type controls. The behavioural testing procedures we will use to measure sensory abilities in head fixed or freely moving animals are painless. In some cases, it will be necessary to motivate the animals to perform these tasks by rationing their food or water during testing. This may result in temporary weight loss, but this will always be monitored carefully and extra food or water provided if this occurs. The ability of modern techniques to monitoring or altering neuronal activity in particular regions of the brain make it possible to carry out almost all of this work in a manner that should cause only temporary pain or discomfort to the animals under study. For example, surgical operations for implantation of ultrafine microelectrodes or for inserting genes into the brain will be carried out under general anaesthesia, in aseptic conditions, and with appropriate post-operative care. Adverse effects may occur, but the incidence is likely to be low and methods of control (e.g. analgesia) and the most refined experimental techniques will always be used to mitigate them. Chronic implants for recording neural activity or for delivering flashes of light for the purpose of altering that activity are small and light- weight and do not materially affect the animal's quality of life. Animals will be killed humanely at the end of the experiment.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Because this project investigates the neuronal circuit organization for multisensory integration and behaviour and how this changes during learning, it can only be carried out using <i>in vivo</i> approaches. Moreover, a key aim of this project is to try to account for changes in sensory perception at microscopic level in terms of the underlying circuitry. This requires the use of post-mortem histological measurements, which would not be ethical or practical to carry out in humans. Finally, computer modelling does form an important component of our work, but this relies on the information provided by the animal studies and cannot replace them.</p>
2. Reduction	<p>Calculations are carried out to determine the necessary number of animals for each</p>

<p>Explain how you will assure the use of minimum numbers of animals</p>	<p>experiment, ensuring significance of our results but also minimizing the number of animals used. We are additionally able to keep animal numbers to a minimum by using cutting edge methods that yield large amounts of data and experimental designs that allow multiple measurements to be made from each animal.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We will use mice for this study because they are particularly suitable for cellular imaging studies and the availability of transgenic animals expressing genetically-encoded fluorescent proteins in particular neurons enables long-term imaging of their structure and function. Besides their neuronal circuit organization shares many similarities with that of humans. Previous research in rodents also provides a platform to build on. State-of-the-art recording and analytical methods will be used to interrogate neuronal function. We will constantly monitor international and local developments in refining surgical and experimental procedures.</p> <p>The data obtained from these experiments will be used to refine computer models of the brain that will help guide subsequent experiments and contribute to a reduction in the number of animals needed.</p>

Project	Synaptic plasticity in the formation and disintegration of neural circuits	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Throughout life the brain continually adjusts itself to understand the ever-changing world around it. This process is known as plasticity. We know that plasticity is triggered by our senses and involve alterations within the synapses, neurons and circuits inside the brain. In this project, we aim to explain how these internal changes bring about changes in brain function, and how they go wrong in neurological diseases like autism and dementia.	
What are the potential benefits likely to derive from this project	Identifying and understanding the mechanisms that drive brain plasticity then means that we can	

(how science could be advanced or humans or animals could benefit from the project)?	aim to manipulate them. Successful manipulations could be used to optimise brain plasticity and provide the basis for new therapies designed to fix plasticity that goes wrong in neurological diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice (max 3650) and rats (max 450) over a 5-year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Approximately half of the animals we will use are genetically modified to express genes that model human diseases, or genes that enable experimental recordings. A minority of the disease-related animals may show some unusual behaviours, but we do not anticipate any more than Mild effects on their wellbeing. Most of our experiments involve making recordings of brain function. This is usually achieved by surgically implanting a recording device on the skull under anaesthesia and pain relief. For many experiments, the implant is sealed, and the animals wear it for the remainder of the experiment, even when they return to their home cage. The vast majority of animals show no ill effects of the implant, which is kept small compared to the animals' head. Following recordings under anaesthesia, animals may lose a small amount of weight, but we will limit the amount of time and frequency ensuring that animals are able to quickly and fully recover. During awake recordings, any animal that becomes stressed by the recording environment and equipment will be taken out of that experiment. These procedures are classed as Moderate severity. On infrequent occasions, an implant comes loose or is damaged – in these cases, the animal would be humanely killed to avoid any adverse impact on the animal. At the end of experiments, all animals will be humanely killed by a trained researcher.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-	The link between the sensory environment and the brain circuits that are used to understand it is essential to the brain plasticity we will study. There are no experimental alternatives that

animal alternatives	model this link, meaning that use of mammals is essential.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We design our studies so that we can make many measurements from individual animals before, during and after they experience brain plasticity. This means we can track the fate of neurons and synapses across time. That gives us much more insight into the plasticity mechanisms because we can avoid the variability between animals. Ultimately that means we get better data from fewer animals.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the best model for the vast majority of our studies because their brains have similar basic structure to humans. Importantly, they are known to undergo brain plasticity in response to changes in their sensory world. This means we study plasticity and relate it to basic neural structure and function that is relevant for higher mammals. Also, because mice can be altered genetically, we are able to model human disease by altering their genes.</p> <p>Animals are closely monitored during and after experiments to ensure they are not showing signs of suffering. Because our aim is to study how sensory experience changes brain function, it is vital that animal well-being is maintained so they do not interact with their environment differently. As such, all our techniques are designed to minimise any ill effects on the animals. This includes the use of anaesthesia and painkillers if there is any risk of pain.</p>

Project	T lymphocyte-dependent immunity	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	An important feature of the immune system is to generate killer T cells that can clear infections and repair damaged tissues as quickly as possible. However, cancers are able to stop killer T cells from entering cancers and avoid being destroyed by the immune system. The aim of this project is to understand what signals pass between T lymphocytes and blood vessels that allow entry into infected tissues but not into tumours.	
What are the potential benefits likely to derive from this project (how science could be advanced or	A major breakthrough in cancer therapy has been the use of checkpoint blockade inhibitors that boost the patient's own T lymphocytes to	

<p>humans or animals could benefit from the project)?</p>	<p>kill cancers. However, this therapy only works in a minority of patients and other strategies are required. There are currently no anti-viral drugs available to treat the vast majority of viruses that cause a wide range of illnesses in humans and animals and vaccines for influenza need to be re-formulated every season. Therefore, new ways of stimulating protective immune responses against viruses are required. The idea of boosting the immunity to infections or cancers by increasing T cell entry to tissues is unexplored. The results of this research will benefit academics and biotechnology industries interested in how immune responses are regulated by T cell migration in and out of tissues and provide training for postgraduate and postdoctoral science and medical students. It also has educational benefit to undergraduate and school students in understanding the complex interplay between the immune system and cancer. There is currently a lot of interest in the use of immunotherapy to control cancers and this research will be of general interest to the public. This research will contribute to improving the health of humans and animals by combating chronic debilitating diseases such as cancer, autoimmunity and chronic infection.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>It is estimated that approximately 7000 mice will be used during the 5-year course of this proposal.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The project will use mice to find out whether immune responses to viruses or cancers can be improved by changing the migration of T lymphocytes around the body. Mice will be infected with viruses such as influenza via the nose which is the natural route in humans. Cancers will be grown in the skin where their growth can be easily measured. Mice will be injected with therapeutic cells or substances that change T lymphocyte migration and the growth of the virus or the cancer measured. A moderate level of severity will be associated with these procedures. Mice suffer a temporary drop in weight and may become lethargic. This will be monitored by checking the health and weighing the mice daily and effects on animal welfare will be minimised by supplementing the</p>

	<p>cage with wet mashed up food on the cage floor that is easy to eat and digest. Mice will be sedated during virus infections to avoid stress associated with holding the mice still during the procedure. Injections and blood samples will be done without sedation, as these are so quick that sedation and short drowsiness would likely induce more stress. Virus-infected mice recover from a temporary loss in weight within 7 days and do not experience any other adverse effects. Mice with cancers recover from a temporary loss in weight within 14 days. Cancers are located on the back or side of the mouse's body so that as it grows, it does not interfere with its' ability to move, feed or groom. Cancer-bearing mice will be checked routinely for health and welfare. Exceptionally, cancers may ulcerate or bleed and if this happens, mice will be humanely killed. All mice will be killed at the end of a specific set of procedures. Breeding of mice for these studies will be mild except for ~10% of GA mice which will be moderate due to their genetic status. Viruses will be grown in chicken embryos at a time when their nervous system is poorly developed to minimise pain.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Some aspects of T lymphocyte migration can be studied in the lab such as looking at the interaction of blood cells in an incubator. However, the 3-dimensional organisation of tissues and blood vessels cannot be mimicked in a tissue culture dish or by computer modelling. Therefore, animal models need to be used for the generation of definitive data. The animal model to be used is the mouse as, over the past few decades, a large body of information has been gathered about the mouse immune system. Much of this information has shown that immune responses in mice closely parallel those in humans. Mice are well defined immunologically, allowing us to reduce the number of unknown factors in any given experiment and increasing our chances of obtaining interpretable and meaningful data. In addition, genetically altered mice expressing mutations in cell adhesion and signalling</p>

	<p>molecules known to regulate leucocyte migration are available and provide an ideal opportunity to analyse their roles in regulating the integrated function of the immune system.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The experimental models are well established and individual experiments will be designed with the aid of appropriate statistical analyses to ensure that no more animals are used than required for statistical validity. The project will use of different imaging techniques for measuring tumours that are not visible to the naked eye. This will allow us to carry our longitudinal monitoring of tumour growth in individual mice and reduce the numbers of mice needed to assess the effect of a given immunotherapy on tumour growth over time.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The overall architecture and distribution of lymphoid tissues is very similar between mouse and man. Importantly, many of the homing associated molecules that guide T lymphocyte entry into tissue are highly conserved between mouse and man and hence experimental data gained about the role of these molecules in mouse T lymphocytes are very likely to apply to humans and other animals. There exist in the mouse defined genetic altered (GA) mutants lacking expression of specific homing proteins. There also exists the potential to generate transgenic mice expressing specific homing proteins which provide an ideal opportunity to perform detailed analyses of their roles in regulating immunological function. Mice are well defined immunologically, allowing us to reduce the number of unknown factors in any given experiment and increasing our chances of obtaining interpretable and meaningful data about leucocyte homing and immunity. There is a potential to cause harm due to the impact of viral infection and tumour burden. The potentials harms are minimised by using minimal doses of virus and tumour cells required to establish an infection or a cancer. Animal suffering is minimised during administration of substances by anaesthesia and by resting mice in their home cage between multiple procedures carried out on a single day. On the rare occasion that cancers need to be</p>

	<p>removed by surgery, pain and infection due to surgery will be minimised using perioperative analgesia and aseptic techniques. To further minimise harms, animal welfare during the course of viral infection and tumour growth will be monitored daily using multiple parameters including appearance, weight, behaviour and clinical signs and animals treated accordingly. Animals will be promptly killed at the end of experiments to ensure suffering is minimised at all times.</p>
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Project	Targeted mouse models for pre-clinical studies in cancer	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cancer is the leading cause of death of children aged 1-15 in the UK. There have been improvements in survival rates for children with leukaemia and lymphomas, however, there is still a great need for progress in treatment of solid tumours. Neuroblastoma is the most common childhood “non-brain” solid tumour and medulloblastoma the most common malignant brain tumour in children. A significant proportion of children with these tumours are considered high risk, that is, despite intensive treatment they have a very poor prognosis. In addition, many of those children that do survive suffer a number of late effects that are severe or life limiting. There is a definite need to improve treatment options for	

	<p>these children.</p> <p>The current treatment for medulloblastoma involves tumour removal, chemotherapy and or radiotherapy. When this fails there is currently no further treatment option available. By mimicking this treatment plan we wish to understand how these tumours respond to current treatments and determine how new treatment approaches could be implemented to improve outcomes</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Developments in molecular screening technologies have meant that it has been possible to identify more of the defects associated with these high-risk tumours. Using this information, we can develop animal models of high risk disease and use these to develop improved and more targeted treatments. We will use preventative experiments using animals prior to tumour onset to assess whether therapy can delay or prevent tumour development. We will carry out intervention trials using animals bearing tumours of a specified size dependent upon the tumour type and location. These are performed to assess the effect upon tumour growth and/or survival of the animal. This includes assessing when tumours become resistant to treatment drugs. Combination experiments will be designed based on current clinical practise whereby up to three drugs are administered together cyclically and may be followed by a fourth treatment with a different drug. We are proposing these experiments in order to identify treatments that are more effective and less toxic to children with these tumours. We anticipate that our work will lead to the development of treatments that will be more specific for an individual's particular cancer type and therefore giving the doctors treating them a better choice of more effective medicines.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use genetically engineered mice that are pre-disposed to develop tumours that represent the high-risk disease seen in children. We anticipate breeding approximately 35,000 – 40,000 mice over the</p>

	<p>next 5 years. Of this number there will be a number of animals that are not the correct genetic make-up to produce tumours and these will be used for future breeding or killed by humane methods.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Neuroblastoma tumours normally arise in the abdominal cavity and occasionally in the thoracic cavity, the primary adverse effect is weight loss, if the animals lose 15-20% body weight that doesn't respond to diet supplements they are humanely killed. The primary symptoms of medulloblastoma, a tumour in the back of the brain, are either circling behaviour or domed heads, when they show these signs they are humanely killed, where possible we use non-invasive imaging to detect tumours before the onset of these symptoms. Using our existing models, we have a great deal of knowledge as to the expected symptoms and time of onset and thus are able to prevent progression of tumours to the stage that they cause ill health. With our new models we will monitor the health of the animals carefully and where possible use non-invasive imaging techniques prior to onset of symptoms. We will test methods of treatments such as surgery and drugs. This could cause pain and weight loss. Pain relief and diet supplement will be given and if they don't respond they will be humanely killed.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The development of tumours depends upon a number of interactions between the tumour cell and the local tissue environment. Therefore, we are currently not able to replicate this environment in a laboratory setting. There are a number of factors vital for drug testing that are not possible to address in the laboratory, such as does the drug get into the tumour? Does it require modification by some other process in the body? Are there any toxic side effects that can only be assessed in a live animal? The mouse is the species of choice due to the amount of genetic mapping information available and the ease in which</p>

	they can be genetically modelled.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>When breeding our genetically engineered mouse models we will design good breeding programmes to prevent over breeding and to keep numbers to a minimum. We have a workflow process whereby we test the validity and relevance of our models and use this to make decisions as to how to proceed with models. When testing drugs, we follow a workflow process to determine the effectiveness and use this to determine how far to proceed with a particular drug. Experiments are designed to use the minimum number of mice whilst providing statistically significant results.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are widely used for <i>in vivo</i> drug development and there is a lot of information available for comparison. Genetically engineered mouse modelling technology is well established, and we have previously demonstrated that we are able to produce and utilise our models to advance clinical trials. Using information from our current models as to potential symptoms we will closely monitor all animals that are expected to develop tumours. Any new drug regimens will be discussed with Named Persons, i.e. the vet and animal care and welfare officers, in advance. We will use refined techniques such as clinical score sheets, regular observations, combining drugs to reduce the number of injections and good handling techniques. We will use analgesia and anaesthesia as recommended to minimise any pain from surgery.</p>

Project	Targeted Prevention and Therapy of Cancer	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The prevention and therapy for most human cancers is still a huge challenge in medicine today, Although substantial progress has been made through years of research. Recent advances in understanding molecular mechanisms of tumour development have made it possible to target specific molecules for cancer prevention and therapy. We have identified a specific pathway ([REDACTED-INTELLECTUAL PROPERTY signalling pathway) involved in many different types of human cancer and especially the kidney. Current agents available for treating tumours associated with faulty activation of this pathway are very limited in terms of their success. Therefore throughout this project, we aim</p>	

	to identify more effective targeting agents and strategies for tumour therapy.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Anti-tumour agents and strategies identified in this project will help design clinical trials to prevent and treat cancer. Information obtained from this work will also advance our understanding of mechanisms underlying drug activity, which will help us and others to be able to design more effective drugs. Understanding the mechanisms underlying drug activity will also help to understand why some tumours are resistant to therapy.
What species and approximate numbers of animals do you expect to use over what period of time?	About 4000 mice are estimated to be used within next 5 years. The mouse is one of the most suitable small mammals for human tumour research. Both genetically altered mouse models and models with transplanted tumours have been very useful in tumour drug discovery. The genetically altered models are particularly useful because they develop tumours in an intact immune system and use the natural tissue environment. The models with transplanted tumours are also useful because they provide an easy and quick approach to test drug effects and also to test how tumour cells grow and form a tumour mass.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The procedures include breeding and maintenance of genetically modified mice, transplantation of tumours to mice and testing of anti-tumour agents. The major expected adverse effect to the animals is the production of tumours, this may happen spontaneously or after a transplantation of tumour cells. Another potential adverse effect may be toxicity caused by anti-tumour agents as this may make animals feel sick. We have a detailed score sheet that will be used to regularly monitor the clinical signs expressed by the animals and the sizes of their tumours. All animals will be humanely killed at the end of the experiments.
Application of the 3Rs	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We will use alternative cell systems in the lab to achieve our research objectives wherever possible. However live animals are needed to confirm the pharmacological activity of anti-tumour agents for various reasons. First of all, the pharmacological effect of an anti-tumour agent on cultured cells may be different from that of live animal tumours, although this cell testing is useful to rapidly identify candidate therapeutic agents. In addition, the effect of agents on cell expansion and growth rate can be assessed in an incubator but animal models are needed to test whether the agents can cause tumour shrinkage or eradicate the tumours. Furthermore, it is necessary to assess potential adverse effects of therapeutic agents in animals.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>To reduce the animal numbers, extensive testing of therapeutic agents in cultured cells will be performed before any live animal work. Pilot studies involving a very small number of animals will be performed in the case of new agents to be tested. Studies will be properly designed and analysed with the assistance of a trained medical statistician to ensure that the maximum data output can be obtained by using the fewest animals. The use of live imaging will also help reduce animal numbers.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We will choose mouse models that have defined genetic changes with characterised pathological features. These models will be most suitable to test anti-tumour agents and strategies with accurate assessment of tumour burden in the kidneys.</p> <p>To minimise pain, suffering, distress or lasting harm, the least invasive procedures will be used for the minimum amount of time. The animals' condition will be frequently monitored including weight loss and tumour burden by trained staff. If any signs indicate that pain, stress, suffering or lasting harm is caused or significant weight loss or tumours reaches an unacceptable size, the animals will be humanely killed. Substances administered should have little or no detrimental effect on the health of the animals. In some cases,</p>

	<p>effective doses have been described in detail in the literature. On occasions when new agents are to be tested, stepwise tests will be used, starting with a low dose and using no more than two animals per step.</p> <p>To minimise pain or distress caused by tumour growth, animals will be carefully monitored daily by qualified and experienced technicians. Tumour burden will be limited to the minimum required for a valid scientific outcome. Humane end points are well defined according to tumour burden as well as the general condition of animals together with specific clinical signs caused by anti-tumour treatment.</p>
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Project	Targeted therapeutic interventions for liver disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Clinical Need : In common with much of the developed world, the UK is currently experiencing a rapid and dramatic increase in mortality from liver disease. Some of this reflects increased alcohol consumption and obesity-related conditions but viral infections and immune diseases are also causing liver damage. Mortality from liver disease in the under 65's has risen 500% since the 1970's, with 80% of these cases presenting as an emergency, either because of alcohol-related liver damage or decompensated cirrhosis. Although disease is on the increase, we still lack effective therapies for many</p>	

	<p>conditions. We also lack good non-invasive tools that can be used to assess whether someone has liver disease, and if so, how severe it is. This means that increased numbers of patients are dying from liver disease in the UK.</p> <p>Our solution : We wish to apply our knowledge of the molecular pathways that cause liver</p> <p>Injury and fibrosis in response a variety of different damaging insults to design and test new diagnostic tools and therapies for patients. Our overall aim is to gain a wider understanding of the cellular mechanisms that cause acute and chronic liver injury with a view to informing design and validation of new diagnostic or prognostic tools and therapies. Thus we wish to use mouse models of disease to address the following specific aims</p> <ol style="list-style-type: none"> 1. To understand whether disease burden is modified in animals that are genetically deficient or transgenic for key inflammatory, metabolic or fibrotic markers. 2. To test if chemical therapeutic tools reduce disease burden 3. To test whether using cell-based therapies modify disease burden <p>We work closely with doctors who treat liver disease and their patients, so our laboratory performs human cell based experiments alongside the animal studies we propose here. This means that our studies are informed by prior identification of candidate molecules in both human and murine mouse models.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This work will significantly enrich the knowledge base in our field of expertise as it is directly intended to identify and test novel molecular interactions with the potential to translate to clinical treatments using new compounds or new targets for existing drugs. Our mechanistic knowledge will be important for the scientific, medical and pharmaceutical communities. We also hope to identify new treatments that we can use in patients with acute and chronic liver disease. This is important for patients because</p>

	<p>not all will respond to current treatment options and for some acute presentations and those with extensive fibrosis there are currently no licenced therapies. We are primed to move rapidly into early phase clinical trials through the [REDACTED – Place] with the partnership of the pharmaceutical industry. Our pioneering studies have already illustrated common mechanistic regulators of disease in several organs and extension of these studies has the potential to not only identify new therapeutic targets but also to extend the licensed use of pre-existing therapeutics. Thus our data is thus likely to be used by basic scientists and clinical scientists to inform the design and outputs of their own experiments. As required by our funding partners, data originating from these studies will be published in high impact scientific journals confirming with the ARRIVE guidelines provided by NC3Rs, and presented at national and international symposia and conferences. Thus benefits from our work include transfer of knowledge, training opportunities for future clinicians and academic scientists as well as improvements in treatment for UK patients and the healthcare industry.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use mouse models of liver injury as our main models. Dietary and chemical models of liver injury will be used to simulate different aspects of human liver disease. The planned work will be conducted over five years and we will use a maximum of 10000 animals</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>As we are modelling liver disease, on occasion our animals may demonstrate symptoms related to liver disease. This will vary depending on whether we are studying acute (sudden) liver injury or chronic (progressive) disease. For example, similar to patients who experience liver injury due to paracetamol or drug toxicity, some of our mice may experience moderate severity symptoms including sluggish behaviour and reduced feeding after exposure. On our prolonged injury protocols, some mice may exhibit weight loss, altered grooming behaviour, reduced mobility and jaundice. These resemble the symptoms of human chronic liver disease such as liver fibrosis and fatty liver disease, and</p>

	<p>tend to be mild in nature. In some situations, animals will experience interventions such as injections using intraperitoneal or intravenous routes, or blood sampling from a peripheral vein. Here animals might experience temporary pain due to the injections but will return to normal behaviour rapidly. For animals that undergo intraperitoneal or intravenous injections, extra caution will be taken when performing injections to prevent injury to other organs or haemorrhage. All animals are culled by schedule 1 methods at the end of our experiments.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Liver disease in humans is a complex, multi-staged process involving many different cell types interacting with each other over many months. The molecules and cell types represented within the disease liver are not reproduced in non-vertebrate species. Human patients tend to realise that they have liver disease late on in the process, by which time the liver damage is significant. This means it is impossible to model such complexity and test the use of new therapies without using animal models.</p> <p>Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace in vivo animal use.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>Our experiments are designed based upon evidence we generate using human cells and tissues in vitro. Where possible we generate as much data as possible using samples derived from patients with liver disease. This is used to identify important mechanisms that we then test in our mouse disease models. It is possible to calculate the numbers of animals required for experimentation based on data from previous experiments and results. In all cases we ensure that we have calculated the minimum number of animals required for the experiment to give us useful data. This approach reduces the animal numbers required, and also reduces the likelihood that the animal experiment would have</p>

	<p>to be repeated. Importantly we also share material and data from our experimental models with researchers at other institutions nationally and internationally to ensure that other groups do not needlessly repeat experiments.</p> <p>We will use the NC3R's experimental design tool to aid experimental design and consult trained statisticians before using any new protocols. All staff performing animal experiments will attend appropriate training on key aspects of experimental design. We will publish in open access journals that support the ARRIVE guidelines for reporting.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>As we are recreating diseases that affect the liver and cause complex systemic effects, we need to use animals to test the effects of any new therapies and to understand the cells and molecules involved in the liver damage. Mice represent the smallest vertebrate species for these studies as they share liver physiology, key biomarkers and immune system function with humans. Importantly genetically altered mouse model are widely available which helps us assess the function of key molecules in a way that is not possible in humans. We look to our experience and that of our scientific peers to refine the disease models we use to reduce animal symptoms and to improve the effectiveness of our models. In general, we recreate the disease causing stimuli (eg paracetamol toxicity or obesity induced fatty liver disease) in a way that recreates the appearance of human patients as faithfully as possible. We also continuously refine our protocols. This could include replacing male genetically modified animals with female mice on liver injury protocols to maximise disease phenotype without recourse to modified animals with a damaging phenotype. We may also refine dosing strategies to minimise handling and maximise benefit. We have also modified to dietary routes of administration for some disease-causing agents to recreate injury without the need for gavage or injection. In addition, we have optimised dosing strategies tailored to individual animal weight and physiological signs to minimise harm and maximise generation of valid experimental data</p>

	<p>for a group of animals. The behaviour of animals and signs of discomfort are monitored throughout our protocols. We constantly seek improvements on our current protocols by seeking knowledge in the published literature, at scientific meetings and by exchanging knowledge with researchers within the field to reduce discomfort and establish models that are the most relevant to human chronic liver disease.</p> <p>We will also systemically review each experiment on completion to see what lessons can be learned from the study in terms of endpoints (scientific and humane) and any animal welfare issues that may have arisen during the experiment that could then guide any subsequent experiments.</p>
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Project	Targeted therapies to modulate inflammation in alcohol-induced injury	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Clinical Need : In common with much of the developed world, the UK is currently experiencing a rapid and dramatic increase in mortality from liver disease. Mortality from liver disease in the under 65's has risen 500% since the 1970's, with 80% of these cases presenting as an emergency, either because of alcohol-related liver damage or decompensated cirrhosis. This means that the cost to the NHS linked to alcohol-related liver disease is estimated at £3.5 billion per annum.</p> <p>The most severe form of alcohol induced liver disease is alcoholic hepatitis (AH), characterised by a rapid onset of jaundice and/or ascites following alcohol</p>	

	<p>consumption. This is particularly challenging to treat and up to 65% of patients will die within 1 month. Importantly, the current therapeutic gold standards, namely administration of corticosteroids and pentoxifylline have recently been shown to give NO improvement in three-month or one year mortality in a large multi-centre trial. For many, the only option is transplantation, which is ethically sensitive in actively drinking individuals. Thus we are a population with rising alcohol consumption and very little in the way of non-transplant therapy to treat those who succumb to liver damage. This is important because there is currently no proven effective therapy for treating AH</p> <p>Our solution : We wish to apply our knowledge of the molecular pathways that cause liver inflammation in response to alcohol consumption to gain a wider understanding of alcoholic liver disease and design new therapies for patients.</p> <p>The overall purpose of our project is to understand whether it is possible to target the processes of inflammation in order to treat alcohol-induced liver disease.</p> <p>Thus we wish to use a mouse model of alcohol-induced liver injury to address the following specific aims</p> <ul style="list-style-type: none"> i) To understand the contribution of platelets to development of and recovery from alcoholic liver injury ii) To understand the contribution of white blood cell populations to development of and recovery from alcoholic liver injury iii) To test whether inflammation and liver damage following administration of LDC/ethanol can be modified by administration of therapeutic agents <p>These studies will be informed by our prior identification of candidate molecules in both human and murine mouse models.</p> <p>We also regularly review the scientific literature to ensure that we are using the most refined animal models and so that we can respond to new developments in model design, particularly where newly emerging <i>in vitro</i> techniques could replace animal use.</p>
What are the potential	This work will significantly enrich the knowledge base

<p>benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>in our field of expertise as it is directly intended for testing novel molecular interactions with the potential to translate to clinical treatments using novel compounds or new targets for existing drugs. Our mechanistic knowledge will be important for the scientific, medical and pharmaceutical communities. We also hope to identify new treatments that we can use in patients with alcohol-related disease. This is important for patients because not all will respond to current treatment options. We are primed to move rapidly into early phase clinical trials through the [REDACTED – Place] with the partnership of the pharmaceutical industry. Our pioneering studies have already illustrated common mechanistic regulators of disease in several organs and extension of these studies has the potential to not only identify new therapeutic targets but also to extend the licensed use of pre-existing therapeutics. Thus our data is thus likely to be used by basic scientists and clinical scientists to inform the design and outputs of their own experiments. As required by our funding partners, data originating from these studies will be published in high impact scientific journals confirming with the ARRIVE guidelines provided by NC3Rs, and presented at national and international symposia and conferences. Thus benefits from our work include transfer of knowledge, training opportunities for future clinicians and academic scientists as well as improvements in treatment for UK patients and the healthcare industry.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use mice for our experiments and expect to use up to 800 over the five year term of the licence.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>This model is classed as severe because it is necessary to induce liver injury and inflammation in our mice. We would expect all of the mice in the untreated injury groups to exhibit some degree of weightloss (<15%) and deterioration in condition (ruffled coat and reduced mobility) for a transitory period after alcohol administration. All animals are humanely killed at the end of the experiment</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p>	<p>The complex disease pathways we are interested in involve the interaction of several cell types and</p>

<p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>regulatory signals that are hard to recreate <i>in vitro</i>. We also do not have access to samples from humans in all stages of alcohol-induced injury. Mice share the main components of their immune systems with humans, and established alcohol injury models recreate the patterns of disease seen in humans. A wide range of genetically manipulated strains and therapeutic reagents are available for mice and thus they are the best model for us.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We have prior experience using the model, which will inform the design of experiments in this study. Importantly we have noted inter-individual variation in response and so our experiments are powered to take this into account and in conjunction with our local facility we have devised a flexible dosing approach based on clinical scoring to maximise our outputs and minimise animal loss. We have built in checks in our workflow to ensure that experiments do not progress if statistically significant results are not evident upon an intervention. Similarly experiments run serially with outcomes from initial animal groups informing the design of subsequent experiments. For all experiments the scientific team meet regularly to discuss data and seek advice from local statisticians and clinical staff.</p> <p>. Importantly our experimental design strategy is informed by use of the NC3R's experimental design assistant (EDA : http://www.nc3rs.org.uk/experimental-design-assistant-eda) and in conjunction with adherence to the ARRIVE guidelines, to ensure the minimal numbers of animals are utilised in order to gain valid experimental outputs.</p> <p>Many of the molecular pathways we investigate operate in more than one organ. Therefore to maximise the useful information we can collect from each animal, we will collect blood, liver and other solid organs. These samples can later be used to investigate the wider significance of our pathway or therapeutic intervention. We work closely with collaborators at other institutes and have a policy to ensure tissue is shared with our colleagues so that maximal use is gained of each individual animal and that new knowledge generation is facilitated.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s)</p>	<p>Although many alcohol injury models are used worldwide, few both recreate the histological picture seen in humans AND meet the strict welfare conditions we adhere to in the UK. We have chosen a model that</p>

<p>you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>is quick to perform, recreates human alcoholic hepatitis, and has been refined by our past experiments. This means we individually tailor our monitoring and alcohol exposure to ensure weightloss and loss of condition are minimised</p>
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Project	Targeting cellular signalling and calcium handling in models of cardiovascular disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Heart failure remains a leading cause of death worldwide. Statistics are not helped by the fact that current medication for heart disease, although effective in the short-term, can have detrimental effects in the longer-term due to the non-specific nature of the drugs involved. There is therefore a pressing need for better medication in order to improve patient outcome. Heart disease and ultimately heart failure occurs as a result of physical or chemical stress being placed on the vessels that carry the blood around the body and on the heart itself. Sources of stress	

	<p>include (i) underlying disease in the vessels and heart, (ii) ageing of the vessels and heart or (iii) toxicity of certain types of medication to the heart e.g. medication used to treat cancer. We still do not fully understand the process by which these sources of stress result in the cells of the blood vessels and heart adapting and behaving abnormally. In order to improve heart medication in the longer term, we need to have confidence that drugs will specifically target the culprit(s) within cells of the heart and blood vessels that contribute most significantly to development of heart failure. Importantly, we can use animal models of cardiovascular disease as well as cardiotoxicity to very effectively introduce these same stressors and investigate in detail the changes that occur in the blood vessels and heart. Previous work by our group has identified a protein [REDACTED – Intellectual property] as one that is dramatically altered in the heart during disease. We now require evidence of what the actions of this protein are in different cell types of the heart and blood vessels and, importantly, whether functional changes in this protein may be a common denominator in how the heart responds to different sources of stress. The key aims of this project are (i) to identify changes in specific protein function that may occur in parallel across different cells of the blood vessels and heart following disease or ageing and demonstrate how this impacts upon heart function (ii) to identify key proteins (e.g. CaMKIIδ) in cells of the blood vessels and heart that contribute to the toxic effects of drugs (e.g. anti-cancer drugs) on cardiovascular function and (iii) to examine whether disrupting key proteins (e.g. CaMKIIδ) at a functional level can reduce disease progression or toxic effects on the heart.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>In the short-term this project will advance understanding across the cardiovascular and oncology community of scientists and clinicians. In improving our understanding of common mechanisms that become defective in the heart and blood vessels following different types of stress, we can advance drug design towards more selective targeting across different groups of patients. This could have implications for patients suffering from cardiovascular disease as a result of progressive atherosclerosis and/or</p>

	hypertension as well as patients who may have developed heart problems as a result of taking a particular medication e.g. medication for cancer. Ultimately, this will result in improved patient care for those suffering from heart disease as well as cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats will be used. Maximum numbers would total 1700 (~1200 mice and 500 rats) over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Adverse effects on the animals in the models of heart disease we propose to use will be rare. From experience, any adverse effects are likely to occur during surgery when there is a risk of bleeding (<20%), and only very rarely (<1%) following surgery. The number of animals undergoing surgery will be limited. Animals could exhibit breathing difficulties while under anaesthesia for ultrasound however since we use inhalation anaesthesia, this can be well controlled. The level of severity for these procedures is moderate. For animals that are genetically manipulated the level of severity is mild. All animals will be euthanised at the end of the procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	For studies investigating cardiovascular disease, it is imperative that we use adult animals to recapitulate what happens in the adult human. We cannot use neonatal cardiac cells or cell lines for this work since they are completely different to the cells that exist in the adult heart and blood vessels. In order to understand how certain stresses affect the heart, we need to be able to monitor effects on the cardiovascular system in the whole animal as well as at a cellular level. This is particularly important since ultimately, we wish to investigate how interfering with particular targets in the cell may impact upon heart function in the live animal.
2. Reduction Explain how you will assure the use of minimum numbers of	We will ensure wherever possible that one animal is used for both heart and blood vessel work so that multiple outputs can be achieved without the use of multiple animals. For cell-based work, we

<p>animals</p>	<p>can maintain cells in culture over a period of several weeks. This will allow for repeat experiments without the need for repeat animal use. For tissue-based work where possible we will process one heart or vessel in multiple ways to ensure we can use the tissue from one animal for multiple types of assay. Importantly, by sharing animals through the ShARM initiative or indeed in-house, one animal can be used for multiple research purposes. Where possible, and in particular for research on ageing, we will aim to use the heart and/or aorta from animals that are already being used by other research groups. This ensures that optimal use is being made of one animal with little waste.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice will be used for both surgical and pharmacological models of disease. We already have a significant amount of data from a mouse model of disease so it makes sense that future work in this area is continued using the same species. This also ensures that future work on genetically-modified animals can be performed. Both models of disease are minimally invasive to the animals but allow a significant degree of heart remodelling to occur to allow us to investigate early stages of heart dysfunction. Ultimately, as indicated previously, we would plan to refine our approach for inducing cardiac remodelling by switching from a surgical approach to a pharmacological approach. We will use rats for studies on ageing and cardiotoxicity because we can gain more material from rat hearts and blood vessels and this will allow more cost-effective projects using fewer animals. All animals will be housed in our [REDACTED- Place] and will be individually housed when required to provide optimum care (e.g. when recovering from surgical intervention). They will be closely monitored by staff and students within our research group and by staff within the unit.</p>

Project	Targeting IL-17 driven pathology	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to determine whether a molecular pathway known to have a key role in inflammation (IL-17 pathway) can be effectively targeted and reduced through the use of small molecule inhibitors. Our initial experiments will use mouse models which genetically delete molecules in the pathway, providing a very robust system to understand exactly what these molecules do and what will potentially happen when targeted with inhibitors. These studies will then inform experiments where small molecule inhibitors targeting these transcription factors are assessed trying to recapitulate results from the genetic targeting. These small molecule inhibitors will be selected as those already	

	<p>being proposed for therapeutic use and at the pre-clinical stage of development, using existing data on efficacy and toxicology.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The work proposed here will generate fundamental knowledge on the molecules that control IL-17 responses. IL-17 responses are key drivers of inflammation and targeting this pathway has substantial therapeutic potential particularly for inflammatory conditions such as inflammatory bowel disease. New recent data from our lab indicates that targeting specific pathways offers new hope for controlling inflammation through blocking damaging IL-17 responses produced by T cells, whilst leaving protective responses from other cells intact. We have established genetically modified mice that enable us to test this hypothesis and these will define the requirements of the two main IL-17-producing cell types for certain molecules in controlling the IL-17 response. Using a series of mouse infection models we will determine the requirements of the different cells. Informed by these experiments, we will then use small molecule inhibitors already at the pre-clinical stage of development (through industrial collaborations) to test the ability of such inhibitors to block the IL-17 responses. These experiments will provide key data demonstrating the efficacy of the pre-clinical reagents. Importantly, the IL-17 models developed will provide crucial mechanistic data into their effects on cells producing IL-17. We aim to demonstrate how T cell driven production of IL-17 can be blocked to limit inflammation. We will identify the consequence of this inhibition on the T cell response. The benefit of this work will be the translation of our initial basic studies showing that targeting transcription factors in the IL-17 pathway can block inflammation and thus offer therapeutic benefit in the clinic. The knowledge gained here will reveal the effects of specific small molecule inhibitors on immune cells in vivo. This data is needed to enable the subsequent testing of these molecules in patients and the generation of new therapeutic approaches in the clinic.</p>

<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Approximately 6,500 mice will be required to perform the planned experiments over the five year time period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In the course of these experiments, animals will necessarily be subjected to injections, oral dosing, blood sampling and/or modification of their diet. In many of our experimental models the mice will only experience a transient discomfort from an injection in for example the tail vein or by oral dosing for compounds given through the mouth. We have established model infections where the response is induced by bacteria normally found living in the tissue without causing clinical signs. In these experiments, the mice will suffer only the discomfort of introducing the bacteria to this site, for example inoculation into the nose. In some experiments the mice will suffer more significant effects such as local skin inflammation, typically on the ears, which will become slightly inflamed and thickened. In our infection studies the animals will likely suffer discomfort associated with the site of infection, for example inflammation in the mouth due to oral candida infection. Where intestinal infections are used, mice will suffer intestinal discomfort due to local inflammation here which may result in diarrhoea. In all cases adverse effects will be minimised by the use of the most refined techniques by skilled staff, and humane endpoints have been predefined. Infection studies will be conducted for the minimal period of time to address the scientific question and the minimal dose to elicit the desired response. In systemic responses mice are likely to experience fever-like symptoms. Mice are expected to show some weight loss. Careful observation of animals throughout the infection and the use of scoring systems will ensure humane end points are identified and used as appropriate. All mice will be killed humanely at the end of the protocol or should clinical end points be reached, then prior to the end of the protocol</p>
<p>Application of the 3Rs</p>	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>This project requires animals as immune responses must be analysed within live animals rather than in a test tube to accurately model the complex dynamics of what actually happens in the body. This work is required to provide clear basic information on the ability of potential therapeutic agents to work and as such it is the first step in translating this scientific work. There are not reliable non-animal alternatives for modelling how these compounds would affect cellular responses and the use of such compounds in patients absolutely requires testing in animal models to really assess how they work and to try to rule out possible adverse effects.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We have developed sophisticated models of responses where a high degree of reproducibility between mice reduces mouse to mouse variation enabling smaller group sizes. We will only use compounds for which established toxicity data exists sparing the need for screening experiments that use large numbers of mice. Small scale pilot experiments will quickly identify dosing for infectious agents using the minimal number of mice required to do this. Experiments will be designed following ARRIVE guidelines and using power calculations and previous advice from in-house statisticians. I have been publishing the results of my work on immune responses for over 10years in top immunology journals reflecting the expertise we have in the appropriate design of this type of experimental work. Should further assistance be required we will reach out to statisticians locally or the local NC3Rs advisor. We constantly re-evaluate our experiments in light of new data in order to ensure optimal design and minimal animal useage to ensure robust scientific data</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise</p>	<p>Mice are an excellent model for the human immune system and have been extensively characterised and validated. These animals provide the best means for analysis given the wealth of reagents available and the wide range of genetically modified mice that enable precise mechanisms to be tested, facilitating</p>

welfare costs (harms) to the animals.	<p>the development of therapies for human use. Many of the methods described are established in the lab and all have been selected as models providing robust data without causing overt clinical signs where possible.</p> <p>General approach:</p> <ul style="list-style-type: none"> ● In general our approach has been to ensure responses can be measured without overt disease, thus minimising adverse effects to the mice and every effort has been made to develop refined techniques causing minimal clinical side effects. ● All infection studies will be established through initial pilot experiments identifying the minimal dose and duration required. ● The infection models we have chosen are those that enable precise assessment of the IL-17 response, whilst minimising adverse effects. ● We have and will continue to collaborate with others researchers with experience in using some of the animal models we use to avoid unnecessary animal use and reducing adverse effect to the minimum possible. <p>Specific examples of refinements include:</p> <ul style="list-style-type: none"> ● We analyse responses of host cells to bacteria that naturally colonise the intestine. We now understand that these responses which do not show any clinical signs, are informative of other responses. ● We use studies of immune cell populations naturally occurring within the mice rather than transferring certain cells into our mice at the beginning of the experiment. This reduces the experimental procedures experienced by the mice and also enhances the quality of the resulting data since our experiments are more physiological.
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	<p>Rather than provide tamoxifen (a drug used in some of the mouse models to induce gene expression) by repeated injection, food containing tamoxifen is used. Where this is not possible, oral administration rather than via an injection into the peritoneal cavity will be used.</p> <ul style="list-style-type: none">● Only small molecule inhibitors for which clear toxicity data exists will be used, acquired through industrial collaboration. This will focus our studies to proven compounds with significant therapeutic potential. We will not screen unknown compounds which may have adverse effects.
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Project	Targeting inflammation in cardiovascular disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our research interests focus on the regulation of the cardiovascular system in health and disease in an attempt to provide a better understanding of the mechanisms involved, and for the design of novel therapeutics. In particular, we are interested in the role of the endothelium, which lines the inside of blood vessels, in controlling cardiovascular function. Many substances produced by the endothelium, including nitric oxide (NO), prostaglandins and the kinins, are all important in the control of the cardiovascular system and manipulation of these naturally occurring substances form the basis of drug treatment for heart disease.	

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Since inflammation is believed to be an important step in the pathogenesis of a number of cardiovascular disease including heart attacks, high blood pressure and shock a better understanding of the actions of these substances might explain some of the abnormalities of blood vessels that are seen in these disease, and suggest new treatments.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>All studies will be conducted in rodents, the vast majority in mice to exploit transgenic ('knockout') technology. This integrated programme of work will run for 5 years and will utilise approximately 1000 rats & mice per year.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The protocols described in this licence allow us to model various aspects of inflammation in the cardiovascular system and to investigate the efficacy of newly developed compounds in the inhibition of this inflammation. Animals will undergo exposure to procedures that cause cardiovascular disease in humans. For example, some will have their coronary arteries blocked to cause a heart attack or heart failure; others will be exposed to an environment mimicking high altitude which causes pulmonary hypertension; some techniques involve administering inflammatory substances to local sites (e.g. the foot) to evaluate how this might be modified by drug treatment. In all cases, protocols are designed to cause the least discomfort (e.g. by the use of anaesthesia and analgesics) and interventions used are always the minimum consistent with the scientific objective. At the end of all studies, animals will be euthanised humanely.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We plan to employ a number of cell-based techniques prior to and during studies involving regulated procedures to guide and minimise animal usage in addition to replacement wherever possible (including the use of human tissue). However, whilst this reductionist approach can aid in the understanding of new biological mechanisms, and thereby the</p>

	<p>development of new classes of drugs for the treatment of cardiovascular disease, because of the dynamic interactions that typify the course of the disease, to facilitate the identification and subsequent development of therapeutic agents it is also necessary to use animal models. Whilst there are few animal models that faithfully reproduce all the pathology of the analogous human condition, it is possible to identify fundamental processes that may either reveal new targets or act as screens for drug testing. Thus, provided one is aware of the limitations of animal models they play a valuable role in the development of novel and more efficacious medicines.</p> <p>This project licence includes the breeding of animals in which nitric oxide, prostaglandins and kinins (and other related substances) have been 'knocked-out' (i.e. artificial deletion of the genes) for use in our research. This is necessary since selective inhibitors of these substances either have yet to be identified or have proved inconclusive in studies of whole animals and humans. Whilst cell culture has helped enormously in our basic understanding of the biochemical processes involved in the production of these substances, the impact upon disease of altering the production of these natural substances can only be assessed in the whole animal. This application seeks to gain permission to use these 'knockout' breeding colonies. Mice are used for these studies since the technology of genetic manipulation has been advanced in this species particularly and indeed all of the genes that we are particularly interested in have been 'knocked out' in mice only. The use of such 'knockout' mice will enable us to determine the roles and relative importance of the above substances in physiology and in various cardiovascular disorders.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We will utilise cells and tissues initially and concomitantly with animal models to guide and optimise the latter thereby minimising animal usage. Transgenic ('knockout') mouse colonies will be used extensively in this programme of work (since specific pharmacological tools are lacking) and we will use the minimum numbers of breeding pairs to provide offspring to</p>

	<p>enable efficient <i>in vitro</i> and <i>in vivo</i> experimentation. We also have considerable experience and expertise in the experimental approaches outlined in this application, and therefore a huge bank of historical data upon which to draw to determine with good accuracy the minimum number of animals studies to conduct to establish a meaningful effect. For example, in the pulmonary hypertension models the average SD for RVSP is 5%. Thus, to detect a 5mmHg change in RVSP with a power of 80%, 8 animals per group are needed. For heart failure models, the average SD for HW/BW ratios is ~11%, and therefore to detect a 20% decrease in hypertrophy, 10 animals per group are necessary. Finally, the average SD for MABP as measured by telemetric probes is 5%. Thus, to detect a 5mmHg change in MABP with a power of 80%, 8 animals per group are needed. Experimental design is also optimised in consultation with statisticians at the [REDACTED – Place] Research Institute.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>All of the models described in this licence are mild to moderate; there are no models that fall into the severe category. Through many years of experience and utilisation of the animal models described in this application, and through collaboration with other academic experts in the field, we have constantly refined the procedures. Each is well-validated and widely-used for investigation of the mechanisms underpinning cardiovascular disease, and gleans robust & informative with minimal animal suffering; analgesics and anaesthetics are used whenever necessary, and close monitoring of all animals undergoing licensed procedures is undertaken. Any animal deemed to be experiencing unnecessary suffering by the investigator or veterinarian will be culled immediately by a Schedule 1 method. The work will be conducted exclusively in rodents, with the vast majority in mice to enable exploitation of transgenic ('knockout') technology. Individuals working under the auspices of this licence will regularly attend NC3R or similar meetings to keep abreast of new developments.</p>

Project	Teleost models of haematopoietic diseases	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The goal of this project is to develop new models of blood diseases (Diamond-Blackfan anaemia (DBA), myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL)), to further our understanding of these conditions and rapidly identify much needed new treatments. Most children born with DBA require life-long treatment and have an increased likelihood of developing cancer. Blood cancers we are studying in this project affect more than 5000 people in the UK each year and the mortality in adults remains extremely high (around 50% will die within 5 years). Furthermore in children, the treatment currently available result in life long toxicity. Better	

understanding and new treatments for these conditions are desperately needed.

To achieve the goals of this project we will use fish, zebrafish and/or killifish models that develop diseases that resemble the disease in people with DBA, MDS and leukaemia. We will use these fish to study their developing blood system and the onset of any tumours they develop. We will also use these models to identify novel treatments for DBA, MDS or leukaemia by exposing the zebrafish to libraries of different types of chemicals.

We have chosen to use zebrafish because we can utilise zebrafish embryos to screen for chemicals that may be of therapeutic benefit in children and adults with DBA, MDS or leukaemia and this is not achievable in any other living organism . Each zebrafish pair produces up to 300 embryos per mating, and they have fully functional blood system by 5 days of life. They are also transparent allowing us to observe the effects of many compounds in the live animals that model these diseases before they reach 5 days. There is no suitable cell culture system that would allow this type of analysis in vitro. We will also use another fish, killifish to model leukaemia's and blood cancers that occur in older people. We think the killifish is the best model in which do study these conditions because this fish naturally ages very rapidly and therefore provides a more natural environment in which to study cancers that occur in older individuals. Cells from patients with these conditions or volunteers will be used to confirm findings that we identify in zebrafish.

This project will provide valuable new zebrafish models allowing us to perform in vivo screens to identify new treatments of DBA, MDS and leukaemia. Drugs and mechanisms of disease identified from this project will improve our basic understanding of disease biology and may even identify new therapeutic treatments which could be used to treat patients in the foreseeable future. This is truly a realistic goal to aim towards since chemical screens in zebrafish have already led to a clinical trial of new drugs in patients receiving bone marrow transplants within 5 years of undertaking a screen such as that described in

	this project.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Developing effective animal models for specific blood disorders is crucial to understanding the disease. Better understanding of the genes that lead to blood diseases and progress in our ability to alter and track changes caused by loss of these genes permit us to study disease in a more targeted way. In the recent past, trialling new therapeutic molecules on zebrafish has sped up the process of taking drugs to clinical trials considerably. The nature of the zebrafish as a model organism, particularly in regard to its rapid development and quantity of embryos produced per breeding pair, allows for a large cohort of drugs to be trialled in a short space of time compared to other models.
What species and approximate numbers of animals do you expect to use over what period of time?	All the studies described in this project are undertaken teleost fish (zebrafish or killifish). . The majority of studies will be undertaken on animals before the onset of independent feeding during which time we do not believe they are able to experience discomfort. These studies account for 8000 embryos per year. We expect that around 2200 adults per year will undergo procedures, however the vast majority of these are breeding procedures of animals that carry genetic modifications. Only 200 adult animals per year will undergo procedures that may result in moderate harm to the animal.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of scientific procedures to be carried out on zebrafish are expected to have no or mild effects on the animals because they are predominantly conducted prior to the onset of independent feeding or under anaesthesia. Our goal is to develop fish that develop diseases akin to those we see in humans. This is the maximum amount of suffering we expect an animal to endure in this project is moderate severity, this accounts for 200 adult fish in this project per year. However this is the maximum amount of suffering we would anticipate. The actual number of animals experiencing moderate suffering in the context of what we are doing is around 10% of the animals undergoing these procedures (i.e 20 fish per year). An example of this would be that a fish may develop leukaemia as a result of loss of a

	<p>leukaemia gene and we may then treat that animal with a drug to assess whether this improved the disease in that fish. Both the leukaemia and the drug may result in some discomfort to the fish. Therefore they may experience adverse moderate effects from this such as difficulty with energy levels/swimming (due to anaemia or leukaemia). In the unlikely event that any animals show evidence that they have experienced moderate harm they will immediately be killed using a licensed technique that has been approved as being the most humane.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Where possible non-protected fish under 5 days (3 weeks for killifish, or longer pre-hatching if maintained in diapause) or primary samples from patients will be used in the described project. We have consulted web-based source to determine possible alternative strategies. Cell lines are unsuitable for the work described because they carry a large number of mutations and thus are likely to provide unreliable data.</p> <p>We have also pre-screened substances where possible to show that small molecules are unlikely to cause harm to fish before we use them.</p> <p>Some of the conditions we are modelling are caused by an abnormality in a single gene (e.g. Diamond-Blackfan Anaemia, DBA) . Importantly in this instance it is extremely difficult to use patient material for experiments as most of them are children, and the cells do not grow in the laboratory.</p> <p>For our leukaemia studies our goal is to assess the biology and the effects of drugs on fish carrying genetic alterations that predispose to or "drive" the development of leukaemia. This is important because although a leukaemia may have many mutations, they remain dependent of the presence of one or two specific "driver" mutations that we are trying to target directly for new therapeutics. Therefore using this very targeted screening approach that assesses the effects of drugs in the whole animal provides an extremely rapid and specific method to find new</p>

	drugs.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The choice of teleost is because it is very easy to follow the effects of disease and treatment with minimally invasive protocols. The animals are transparent during development permitting us to visualise effects on blood development prior to the onset of independent feeding. In order to study the development of leukaemias we have a large number of genetically distinct lines of fish permitting us to study the effects of individual genes and the interactions of several leukaemia causing genes in combinations that are observed in patients. This means that our research involves the maintenance of large numbers of adult fish. The majority of these fish are maintained solely for the use in natural matings to produce eggs for our research projects. Several factors determine the number of adult fish we need to maintain. The first is that all of the genetically distinct lines of fish need to be maintained as separate stocks. The different lines may contain mutations in different genes that are being analysed or may contain transgenes (such as green fluorescent protein) that allow us to track cells or manipulate gene function.</p> <p>The second is to allow us to maintain fertility in this short-lived species. The third factor determining the numbers of adult fish that we maintain is the research demands placed on the specific line. E.g. those used for screening will need to be bred more often to obtain sufficient eggs and therefore more adults will be required.</p> <p>To reduce numbers of animals used, our fish stocks are made available to other scientific personnel in order that only the necessary number of animals are maintained for use in procedures at [REDACTED – Place] fish facility (providing experiments are covered by appropriate personal and project licenses). In keeping with this, great efforts are invested in maintaining all adult stocks in peak breeding condition. This ensures that we can keep the minimum numbers of fish required for embryo production. To facilitate this, all stocks are well documented on our databases with periodic assessment each month and detailed stocktaking performed by all personal licence holders every three months. These procedures</p>

	<p>help to ensure that we only maintain those fish required for our experimental research. In addition some projects may be more active than others at certain times and we regularly assess the need for maintaining lines and where possible store sperm for genetic mutations that we are not actively using fish from.</p> <p>This project also utilises a new way of rapidly generating animals with mutations that minimises animal numbers by testing non-protected fish under 5 days of age post-fertilisation.</p> <p>We will also reduce animal numbers by employing transplantation methods to test the effects of small molecules on leukaemia. In this way we will reduce the need to generate transgenic or mutant animals that may or may not develop leukaemia and may be more difficult to assess outcome of drug treatment.</p> <p>Finally the work we have done validating human disease models in zebrafish will continue to reduce the number of mammalian models needed to validate biological findings or drugs.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Zebrafish are the ideal choice of species for this project because our aim is to utilise zebrafish non-protected embryos prior to 5 days post fertilisation to screen for chemicals that may be of therapeutic benefit in children and adults with DBA, MDS or leukaemia is not achievable in any other vertebrate. A further refinement to this is the use of killifish which show rapid features of aging, that are known to influence the development of haematological cancers. This newer model system may provide a more faithful model that will permit higher throughput of studies refining our use of teleosts for MDS and AML studies.</p> <p>Furthermore, while in some instances mice are preferred because they more closely resemble human disease, this is not the case for Diamond-blackfan anaemia and leukaemia also reflect the diseases seen in humans. We have refined the way in which we house single fish that need to be isolated to track the development of disease by utilising new equipment that permits single fish to be housed and fed while remaining on constant water flow of our water system. In addition to continual water replenishment these transparent</p>

	chambers permit fish to visualise many other fish in the system simulating social interactions that are preferred by this species.
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Project	Tendon homeostasis, injury and repair	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Tendon injury is common in both athletes and the general populations. Injured tendon heals poorly and is prone to re-injury. Currently, there are no effective treatments for tendon injury; this is largely because we do not understand what happens within the tendon during the early stages of injury or how the cells resident within the tendon respond to injury. We know that there are several different cell populations within tendon; in the project we will establish how these different cells maintain healthy tendon structure and respond to injury and potential treatments for injury. We will also determine the role of microorganisms (e.g. bacteria) and the immune	

	system on tendon inflammation and healing.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Understanding the roles of specific cells within tendon in health and with injury will provide information that will allow the development of more effective treatments for tendon injury, targeted at developing methods to stimulate the resident tendon cells to fully repair the tendon after injury and resolve chronic inflammation. Not only with this benefit humans, but findings will also be of benefit to animals that suffer from naturally occurring tendon injuries, such as horses and dogs.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use rats and mice in all the planned experiments. We anticipate we will use approximately 600 rats and 150 mice over 5 years. In preliminary work to develop and refine techniques, we will use rat cadavers obtained as waste material from other unrelated experiments, which will reduce the number of rats used. All experiments will be carefully planned and we will perform calculations to ensure that we use the minimum number of animals required to obtain statistical significance. Animal numbers will be further reduced by using one hind limb as a control, and performing experiments on the other hind limb.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will use a minimally invasive procedure to label proteins within the tendon by injecting heavy labelled water into the abdominal cavity and mixing into the rats' drinking water. This substance is non-toxic and has no side effects, and therefore this is classed as a mild procedure. After varying periods of time, we will cull the rats and analyse the tendons to determine the rate at which proteins within the tendon are being synthesised and degraded. We will develop 2 models of tendon injury – one will be induced by creating a small wound in the tendon with a needle, and the other will be induced by loading the hindlimb while the rat is anaesthetised. Both these procedures are moderate – they involve anaesthesia and may result in short term pain which will be reduced by giving the animals painkillers before and after surgery. All animals will be culled at the end of the experiments so we can analyse their

	<p>tendons within the laboratory to assess how the cells within the tendon respond to loading and where within the tendon injury initiates. We also intend to suppress the immune system in some of the rats so we can perform a bone marrow transplant prior to tendon injury to assess how bone marrow stem cells respond to tendon injury. For the majority of rats, this is classed as a moderate procedure, however if the bone marrow transplant fails there is a high risk that the rats will die. This is expected to occur in less than 10% of rats, such that the overall severity limit for this procedure is classed as severe. We will also perform experiments using germ-free mice to establish the effect of microorganisms on the development and progression of needle-induced tendon injury. This is classed as a moderate procedure, as germ-free conditions can affect the intestines, which may result in dehydration and diarrhoea, therefore these mice will be monitored closely and will be culled if showing signs of distress.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Developing new treatments for tendon injury is currently limited by a very poor understanding of the roles of the cell populations within tendon during health and disease.</p> <p>Little is known about the initiation of tendon injury as samples from naturally occurring injuries in humans and other species can only be obtained in the late stages of injury.</p> <p>In our previous experiments, performed without the use of live animals, we have started to understand what happens when tendon injury starts. However, these studies have raised important questions that need to be answered before we can develop effective treatments for tendon injuries. These questions can only be answered by performing experiments on animals, as current models of tendon injury in the laboratory are not able to replicate the complex structure and loading environment within tendon, and are not suitable for longer term experiments. The experiments we have planned will allow us to identify which cells are responsible for repairing the tendon. We can</p>

	<p>then isolate these cells and perform experiments on them in the laboratory which will allow us to develop methods to activate these cells and promote tendon repair.</p> <p>Moreover, it is not possible to determine the role of the microbiome in tendinopathy in other models or other animals, as currently only mice are available as animals raised in a germ-free environment.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We always aim to reduce the number of animals we use. We have performed calculations to ensure that we use the minimum number of animals required to obtain statistical significance in each experiment, and will use randomisation and blinding approaches to reduce any bias. Animal numbers will be further reduced by using one hind limb as a control, and performing experiments on the other hind limb. We will source rodent cadavers from other unrelated experiments to use in pilot experiments to refine techniques, reducing the number of animals that will be culled for this project.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse and rat are well-established models for investigating tendon injuries and the effect of mechanical loading. We have chosen a needle-injury model, as this creates a small, highly reproducible injury which will not result in excessive pain or lameness. We will also induce tendon damage by loading the hind limb under anaesthesia. This allows us to apply well defined and controlled loading protocols, leading to similar levels of damage between animals, decreasing the variability and increasing the sensitivity of the experiments.</p> <p>Animal suffering will be limited in our studies by our strict monitoring of actual severity and severity limits. Our protocols are also designed not to produce excessive trauma or suffering, and painkillers will be administered before, during and after any procedure that is expected to caused pain. Animals will be killed if they approach the limit of severity.</p>

Project	Testing new <i>Clostridium difficile</i> directed therapies and vaccines.	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<i>Clostridium difficile</i> associated disease (CDAD) is a significant cause of morbidity and mortality in humans worldwide. Vaccines to prevent infection and the choice of antibiotic therapies is limited and may be further compromised by the emergence of resistance. The aim of this project is to investigate novel and emerging therapies for the treatment and prevention of <i>Clostridium difficile</i> infection.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or	The animal models will allow testing of the efficacy of new therapies generated by ourselves or collaborators. This could lead to more effective treatments for human disease.	

animals could benefit from the project)?	
What species and approximate numbers of animals do you expect to use over what period of time?	Hamsters – 3680 Used over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals will show symptoms of <i>Clostridium difficile</i> infection (including wet tail, diarrhoea, and hunched posture). With an expected level of moderate severity. Once the animals are showing non-recoverable symptoms of <i>C. difficile</i> infection they will be humanly killed using a Schedule 1 method.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The combination of the requirements for an anaerobic environment and the gut specificity of <i>C. difficile</i> has meant that suitable non-animal alternatives have not been identified.</p> <p>The information that can be obtained from established tissue culture assays, is limited as the infection process involves complex dynamic interactions between host and bacterium which are impossible to model effectively in non-animal alternatives at present.</p> <p>Testing of vaccines involves the generation of immune responses and delivery of drugs to the appropriate tissues which also requires living animals to determine clinical response.</p> <p>We have considered the use of <i>Galleria mellonella</i> (wax moth larvae) as an invertebrate model to replace the use of animals. So far we have found that this organism is not susceptible to <i>C. difficile</i> toxins when injected however oral inoculation with <i>C. difficile</i> results in a degree of infection. However since the intestinal tract of the wax worm differs significantly from that of higher vertebrate organisms, the full spectrum of disease symptoms is not observed in the <i>Galleria</i> model. We are continuing to develop this model however to determine its relevance as a colonisation model.</p>

<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We aim to reduce the number of animals used by carrying out power calculations and implementing a block design for each experiment, initially using groups of 3-4 animals per treatment group and common control groups, with all experiments being blinded. Once the initial experiment has been completed and the data analysed, additional groups of animals of an appropriate size (up to the number obtained from the power calculations and to a maximum of 15 animals per group) will be used and the data combined for statistical analysis.</p> <p>To date we have typically used combined group sizes of between 8-15 animals. Where possible the data generated from the control animals will be used across a number of studies.</p> <p>In order to ensure that high quality, reliable and valid data is extracted from the minimum number of experiments, the ARRIVE guidelines (Kilkenny, 2010) will be followed (http://www.nc3rs.org.uk/page.asp?id=1357). The following websites will also be utilised to provide additional information on experimental design and statistics; The NC3Rs experimental design assistant http://eda.nc3rs.org.uk/ and the 3Rs-Reduction.co.uk site http://www.3rs-reduction.co.uk</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Hamsters are the lowest vertebrates in which the pathology of infection by <i>C. difficile</i> resembles that manifest in humans. This includes the germination of spores and colonisation of resultant vegetative cells in the intestine, inflammation due to toxin release and transmission of spores in faeces. The hamster is particularly susceptible to CDAD and develops wet tail; diarrhoea being a classic symptom of CDAD in humans, and rapidly progresses to the defined end point. The hamster is therefore invaluable in quickly assessing the efficacy of interventions in preventing disease onset or delaying the time from infection to the defined end point.</p> <p>The scientific objective is to administer therapies or vaccines to the hamsters and determine if they are efficacious in treating or preventing CDAD. The condition of the infected animal, degree of colonisation of <i>C. difficile</i> (as determined by the degree of shedding of this bacterium) and extent of pathology in the gut (as determined by tissue histological studies) of treated animals versus control animals will provide an indication of the success of the treatment. Ultimately the scientific end point is to achieve complete prevention of CDAD.</p>

The procedures proposed to be conducted in hamsters were previously optimised by the applicant. The practice of these methods has been closely observed by the NVS, NACWO and Home Office Inspector with minor modifications implemented upon recommendations. An existing monitoring scheme established and widely used for hamsters will be strictly adhered to ensuring that appropriate non-lethal end points are achieved. Based on this scheme, a specific scoring sheet will be used to assess a number of parameters. A score will be given between 0-3, and any animal scoring 15 or more will be euthanized using a Schedule 1 method.

Animals will be handled by trained competent staff at all times and will be returned to their home cages as soon as possible following any procedure in order to minimise stress. LASA guidelines will be followed for dosing and blood sampling.

Animals will be administered a maximum dose of 20ml/kg of sodium bicarbonate by the oral route to neutralize the stomach acid an hour before administration of therapy or vaccine. Topical local anaesthetic cream will be applied 30 minutes prior to any blood sampling to prevent any pain from the venepuncture. The volume of blood withdrawn will not exceed 10% of the blood volume of the animal as recommended by the LASA guidelines. Immune response markers will also be sampled from faecal samples and if a direct correlation can be established between the levels seen in blood serum and faecal samples, blood sampling will no longer be conducted.

Hamsters will be group-housed until oral administration of Clindamycin after which time they will be individually housed. As hamsters are typically solitary animals, single housing should not adversely affect their normal behaviour and is required for the experiments as the monitoring of infection requires analysis of faecal samples collected from individual animals. In addition, single housing is required to ensure that any infection observed is as a result of the planned infection and not as a result of animal interactions.

All animals will be provided with paper nesting material, cardboard tubes and wooden chew blocks in the cages. Hamsters will also be provided with wheels as a way of providing stimulation and environmental enrichment.

Animals will be given free access to food and drinking water at all times, unless withdrawal of food is required to minimise stomach acidity for the experiment. If the removal of food is required, it will be returned after the shortest time

	period possible typically 2 to 4 hours.
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Project	The ability of biomaterials to form ectopic bone
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	In this study we are going to screen the ability of a range of non-toxic biomaterials, as particles or scaffolds, with/without stem cells, to encourage bone formation. This will be done by placing the biomaterials in pouches under the skin (subcutaneously) of a rat. This will allow us to compare the amount of new bone formation and also blood vessels that each type of material causes over a set period of time and then select the best performing material to use in a large animal bone defect model.
What are the potential benefits likely to derive from this project (how	Large skeletal defects resulting from trauma, tumour resection and disease remains a largely

science could be advanced or humans or animals could benefit from the project)?	unresolved clinical problem, requiring a bone tissue engineering solution. Therefore there is an unmet clinical need to develop new therapeutic approaches for bone regeneration and vascularisation in bone defects. It is hoped that by screening a range of non-toxic and biodegradable materials we will be able select one capable of supporting bone growth, cell migration and the formation of blood vessels within a defect site, and could benefit humans. This in turn should reduce the number of surgeries and infections associated with bone grafting and provide a material which encourages better bone fill to a defect site.
What species and approximate numbers of animals do you expect to use over what period of time?	278 male rats over 250g over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The expected severity of the study is moderate. We expect that the animals will show signs of mild pain and discomfort for less than 12 hours after surgery has been carried out, but this will be closely monitored and the animals will all receive analgesia both pre- and post-operatively. The animals will be humanely killed at the end of the study and the implants will be analysed using micro-computed tomography and histology to look for new bone and blood vessels.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	<i>In vitro</i> work was initially carried out to ensure that the implants were not toxic to cells, but <i>in vitro</i> assays do not allow us to assess the physiological response to an implant and whether it will be capable of stimulating new bone formation. For this reason we need to carry out this work in a living animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will insert up to 2 implants per rat of a maximum size of 11mm wide by 15 mm high or 2 chambers of a maximum size of 15 mm wide by 5 mm high, each in an individual pouch under the loose skin on the back of a rat, which will reduce the number of rats we need to use. To achieve statistical significance we will be

	<p>using 6 implants per group (3 rats).</p> <p>Data will be analysed using a suitable statistical package and statistical tests, for example one way analysis of variance and post-hoc testing. All experiments will be conducted in a manner that will allow high quality publication.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The rat is commonly used for subcutaneous implantation as they are relatively large and have loose skin to accommodate a moderately sized implant without much discomfort to the rat. Rats will be group housed and acclimatised for at least 1 week before surgery. All surgery is carried out aseptically in dedicated facilities with experienced staff. All animals will receive pain relief during and after surgery. Antibiotics will be given if required. Once fully recovered from the anaesthesia they will be returned to group housing with behavioural enrichment. From previous studies carried out at this facility we do not expect the animals to show signs of pain and distress for a significant period of time, but if they are not showing signs of improvement they will be killed to prevent any suffering. A scoring system will be used to monitor the animal's wellbeing after surgery.</p>

Project	The aetiology of metabolic disease																
Key Words (max. 5 words)																	
Expected duration of the project (yrs)	5 Years 0 Months																
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<table border="1"> <tr> <td data-bbox="734 593 756 651"><input checked="" type="checkbox"/></td> <td data-bbox="756 593 1394 678">Basic research</td> </tr> <tr> <td data-bbox="734 678 756 763"><input type="checkbox"/></td> <td data-bbox="756 678 1394 763">Translational and applied research</td> </tr> <tr> <td data-bbox="734 763 756 857"><input type="checkbox"/></td> <td data-bbox="756 763 1394 857">Regulatory use and routine production</td> </tr> <tr> <td data-bbox="734 857 756 1023"><input type="checkbox"/></td> <td data-bbox="756 857 1394 1023">Protection of the natural environment in the interests of the health or welfare of humans or animals</td> </tr> <tr> <td data-bbox="734 1023 756 1117"><input type="checkbox"/></td> <td data-bbox="756 1023 1394 1117">Preservation of species</td> </tr> <tr> <td data-bbox="734 1117 756 1211"><input type="checkbox"/></td> <td data-bbox="756 1117 1394 1211">Higher education or training</td> </tr> <tr> <td data-bbox="734 1211 756 1305"><input type="checkbox"/></td> <td data-bbox="756 1211 1394 1305">Forensic enquiries</td> </tr> <tr> <td data-bbox="734 1305 756 1417"><input type="checkbox"/></td> <td data-bbox="756 1305 1394 1417">Maintenance of colonies of genetically altered animals</td> </tr> </table>	<input checked="" type="checkbox"/>	Basic research	<input type="checkbox"/>	Translational and applied research	<input type="checkbox"/>	Regulatory use and routine production	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals	<input type="checkbox"/>	Preservation of species	<input type="checkbox"/>	Higher education or training	<input type="checkbox"/>	Forensic enquiries	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
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Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Diabetes has reached epidemic proportions in Western societies: it affects 450 million people worldwide (5-10% of the adult UK population) and numbers continue to climb. It also costs the NHS £1.5 million an hour. The disease is characterised by increased blood glucose levels, caused by insufficient insulin secretion from the beta-cells of the pancreas. Chronic elevation of blood glucose (hyperglycaemia) has adverse effects on many tissues including the heart, kidney, eyes and pancreatic islet cells, resulting in secondary complications. Knowledge of how insulin secretion is controlled, and how and why it is impaired in diabetes is therefore of fundamental importance. However, there are large gaps in</p>																

	<p>our understanding of these processes.</p> <p>The aim of this project is to define the molecular mechanisms that result in impaired insulin release in type 2 diabetes (the most common form of the disease) and neonatal diabetes (a rare inherited form of diabetes that manifests soon after birth). We also wish to understand why some patients with neonatal diabetes have neurological problems.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This project will provide novel information about the molecular basis of both type 2 diabetes and neonatal diabetes. It should help us understand why type 2 diabetes is a progressive disorder, why it can sometimes be reversed (e.g. by weight loss), and why this is not always the case. The results are likely to be of clinical value and we will strive to ensure that, where possible, they will be rapidly translated into clinical practice.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice, ~30,000 over 5 years Xenopus, 150 over 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We will study mice carrying a genetic mutation that causes neonatal diabetes in humans. Mice in which the gene is turned on in the pancreatic beta-cells will develop diabetes. In our experience they cope with this very well and do not appear to suffer any harm, apart from urinating more frequently (so they will be kept on highly absorbent bedding and carefully monitored). We will also study mice carrying mutations that affect glucose metabolism (breakdown), as our studies suggest metabolism is impaired in type 2 diabetes. A small number of mice will be given tests similar to those used to study blood glucose regulation in humans (e.g. we will inject a small amount of glucose and then take a 3-4 blood samples to measure the change in blood glucose concentration over time). Most mice will not undergo these tests. Instead, they will be humanely killed and their tissues isolated after death so that we can study how diabetes affects the structure and function of the different types of pancreatic islet cells, and the</p>

	<p>tissues associated with the secondary complications of diabetes in humans (heart, kidney etc). We will also investigate the role of the mutant gene in brain cells, to understand its normal functional role and why it impairs brain function when it is mutated (as in some patients with neonatal diabetes). Some mice may undergo recovery surgery, for example to implant a pill that controls their diabetes. In these cases, perioperative analgesia will be used. We will also humanely kill some <i>Xenopus</i> (frogs) (by an overdose of anaesthetic) and isolate their eggs. These will be used to (i) study the effects of human mutations on the function of the gene that causes neonatal diabetes in humans, and (ii) to determine if novel mutations cause a patient's diabetes, which will help inform the choice of therapy. All animals will be humanely killed at the end of the experiments. The expected severity level of these studies is mild to moderate.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Where possible, we will perform experiments on human islets isolated from organ donors. However, islets from patients with type 2 diabetes can only be obtained rarely and this is impossible from patients with neonatal diabetes</p> <p>Although cell lines will be used for some experiments, unfortunately they do not respond in the same way as freshly isolated beta-cells, and culturing them at high glucose does not reproduce all the effects found in diabetes.</p> <p>The use of animals is also essential to understand the basis of human disease at the systems and whole organism level, and to provide a link between <i>in vitro</i> studies and clinical disease. For a multi-organ disease, like diabetes, there is simply no substitute for animal studies. It would be neither permissible nor ethical to carry out the necessary procedures in humans, and simulations cannot provide answers to the questions we seek to address. Studies of genetically modified mice are of considerable value in this respect. For</p>

	<p>example, mice carrying a mutation that causes neonatal diabetes in humans should help us understand precisely what causes the human phenotype.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Appropriate calculations will be performed to ensure that the maximum amount of scientific information is obtained from each individual animal and the results are statistically significant. Where animals have only been subjected to minimally disruptive procedures, they will subsequently be used for other procedures. When animals used in procedures are sacrificed, their tissues will be used for cell and isolated tissue studies. This should help keep animal use to a minimum.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse is the lowest vertebrate with enough aspects of its genetics, anatomy, physiology and embryonic development shared with humans to generate biologically relevant data that ultimately can be extended to our understanding of diabetes in humans</p> <p>The mutant mouse models we will use will be those that are relevant to (i) understanding human diabetes and that display phenotypic and pathological features similar to those seen in humans; (ii) understanding the effects of human maternal diabetes and obesity on embryonic development; and (iii) that can be used to address basic biological questions about the normal regulation of glucose homeostasis. For example, we will use a mouse model that mimics human neonatal diabetes.</p> <p>Animals will be closely monitored and if any become unwell they will be killed and an examination performed to identify the cause of death and inform subsequent experiments. If animals exhibit diabetes, measures will be undertaken to minimise the consequences of this, such as using ultra-absorbent bedding, frequent refilling of the water bottles and reducing the numbers of animals housed per cage. All terminal procedures will be carried out under appropriate levels of anaesthesia. Whenever an animal has surgery it will receive pre- and/or post-operative analgesia as</p>

	appropriate.
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Project	The assembly and function of neuronal connections in health and disorders of the brain	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim of this project is to understand the mechanisms that control the development of neuronal connections and circuits, describe how these connections and circuits are altered in disorders of brain development such as autism, intellectual disability or schizophrenia, and begin to dissect how changes to these mechanisms may lead to such alterations. We will also begin to test new therapeutic interventions that may be able to modulate these mechanisms to improve eventual developmental outcomes. We	

	<p>will do this by genetically manipulating the proteins that are important for different aspects of neuronal activity and examining how this affects the development of synaptic connections between neurons. We will also use genetic models of disorders, by deleting or changing genes that we know to be affected in individuals affected by these disorders. Finally, we will try to model the effects of environmental risk factors for these disorders. We will assess the impact of these experiments on synapse formation, connectivity in the brain and behaviour. This will allow us to test whether new treatments can rescue any of the changes we see, as well as design new therapeutic targets based on our findings.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This will lead to a better understanding of how genetic and/or environmental abnormalities actually lead to changes in how the brain wires up during development. In the long-term, this will allow more rational design of new treatments for these disorders.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Rodents (predominantly mice, some rats), approximately 5,000 over the 5 year project</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The rodents will mainly be used to breed genetically altered animals (mild severity only at most) and humanely killed to obtain tissue for research. Much smaller numbers will undergo surgical procedures which are not expected to have any serious adverse effects and every effort will be made to ensure minimal suffering (good anaesthesia and post-operative pain relief). Animals will be humanely killed at the end.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>To do the experiments required to improve our understanding of the underlying mechanisms for these diseases, we cannot ethically perform these in humans. Although we will also use non-animal alternatives, such as cells taken from humans, these have significant limitations and so many experiments can only be conducted in</p>

	animals.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Firstly we will maximise the data from each animal by doing many experiments from multiple different cells and/or tissues after humane killing. We will use tissue from genetically altered animals of both sexes and all genotypes after humane killing, meaning we will generate far more information without any additional numbers of animals or suffering. Also, we will use the optimum experimental design and statistical tests to minimise animal numbers.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We will do these experiments in rodents as these offer the best compromise between relevance to humans and sentience. The regions of the brain that are known to be important for neurodevelopmental disorders are relatively similar in rodents, and it is possible to measure behaviours relevant to these disorders. Also, mice are ideal due to the number of transgenic (genetically modified) mice available including disease-relevant mutations as well as reporter lines, and increasingly transgenic rats will be available as well. Working with rodents also builds on the wealth of knowledge and research already available and minimises unnecessary repetition.</p> <p>To minimise animal suffering, the vast majority of animals will only undergo a single procedure, and much of the work will be done in fixed tissue or 'in vitro' (ie. not in the live animal) using tissue. All animals undergoing surgery will have effective anaesthesia and be given additional pain relief to minimise suffering. The system we will use to deliver genes to animal tissue has been shown to result in optimum survival and minimal tissue damage. Also, many of our preliminary experiments will be done in cell culture or tissue taken from wild type rodents which will enable us to plan experiments and minimise animal usage and suffering.</p>

Project	The biological function of RNA modifications	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our immune system is the name for the cells in our bodies which protect us against infections including bacteria, viruses and fungi. The immune system also plays an important role in killing cancer cells. This project focuses on a specific set of cells in the immune system called "T cells". T cells live in the blood and various organs of the body. When T cells meet a cell which is infected or meet a cancer cell they undergo some dramatic changes. The T cells become much larger and start dividing rapidly to make many more T cells. A few days after meeting an infected cell or cancer cell, the T cells either make proteins which kill the infected cell or cancer cell or make proteins which help	

	<p>the other immune cells fight infections or cancer. These changes that happen to T cells after they meet an infection cell or cancer cell are not well understood by scientists, but they are vital for protecting the body from cancer and infections. The aim of this project is to determine how T cells respond to meeting an infected cell by getting larger, dividing to make more cells and producing proteins which fight the infection.</p> <p>We believe that many of the changes that happen to a T cell when it meets an infected cell or cancer cell occur due to changes in how DNA is read. DNA is the substance in every cell which has the information for making proteins. In the cell, DNA is used as instructions to make a substance called RNA, and RNA is used as instructions to make proteins. We study a part of RNA called the “cap” which is very important for RNA to work properly to make proteins.</p> <p>Our experiments have revealed that formation of the cap on RNA is an important means by which T cells change in response to meeting an infected cell. The RNA cap has many different parts to it. In this project we will use mice which cannot make the different parts of the RNA cap properly. This will allow us to see how those parts of the cap are used by T cells to respond and protect the body when they have seen an infected cell or cancer cell. Specifically, we will look at how removal of a parts of the RNA cap changes how cells grow and divide and make more protein. We will also look at how the RNA cap helps T cells respond to an infection in the mouse. The ultimate aim of this project is to discover if the RNA cap should be investigated as a part of the cell through which infections and cancer could be treated.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>T cells are critically important for fighting infections and for killing cancer cells. T cells are also important for preventing the immune system from becoming hyperactive which results in the immune cells damaging healthy cells. Major human health problems results from T cells not functioning properly. The mouse immune system is very similar to the human immune system and therefore in this project we will perform experiments with mice to investigate how T cells</p>

	<p>function. Specifically, we will ask how T cells respond to infection by growing, dividing and making proteins which fight infection. This project will provide information to pharmaceutical companies and medical doctors on how the immune system makes the proteins which fight infection and kill cancer cells. Potentially the work we are doing in this project could identify new approaches to the treatment of infectious diseases, cancers and inflammatory disorders.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This application will support the work of 5 scientists over 5 years. We propose to use up to 17000 mice including genetically modified animals.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Over 95% of the procedures which will be performed will be sub-threshold in terms of severity (so less unpleasant for the animal than getting a single injection) and will involve only the breeding and maintenance of mice who have had their genes altered in some way, but with no outward welfare issues. Mice will be killed humanely and tissues will be analysed in the laboratory. When we understand in detail how the enzymes which we study are likely to affect the ability of T cells to respond to infection some mice will be given microorganisms and viruses. This is likely to result in deviation from normal welfare in some mice, classified as moderate. We expect to see signs similar to those observed in humans with 'flu. These mice will be euthanized as early as scientifically possible. In the course of these experiments we aim to get as much information from each mouse as possible. Therefore, mice may be subject to blood sampling, administration of drugs and imaging under anaesthesia. These treatments are likely to result in only transient discomfort. However, mice will be monitored regularly for signs of weight loss and signs of distress/discomfort. Any unlikely adverse effects will be discussed with the NVS and a humane cause of action agreed. Animals will be killed humanely either at the end of the study or when the NVS advises that euthanasia should be performed due to the severity of unexpected adverse effects.</p>

Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>This project investigates how the mammalian immune system is regulated and controlled by the mRNA capping enzymes. Our model organism is the mouse. The mammalian immune system is a complex cell system in which the many different types of immune cells communicate with each other and surrounding non-immune cells via chemical messengers. Although we work with established cell lines when informative, the complexities of immune cell interaction and development cannot be modelled in tissue culture. We also work with human blood donor cells when informative, however this source of cells does not produce sufficient “naïve” T cells which we study.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We work with the minimum numbers of mice to produce statistically significant and therefore useful data. Small scale pilot experiments are performed with 3-4 mice of each type to give an indication of results. We then work with a statistician to determine the minimal numbers of mice needed to produce a statistically significant result. By performing high quality experiments, we reduce the numbers of mice needed for repeat experiments. When possible, several experiments are performed on tissues or cells harvested from the same mouse, thereby reducing the numbers of mice used. By rapid genotyping and by using a careful breeding programme we keep mouse numbers as low as possible. Mouse sperm or embryos are frozen regularly to preserve lines that are not currently needed.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Our research aims to make discoveries concerning the mammalian immune cell function with the potential to provide new therapeutic approaches to treating immune cell disorders in humans. We perform these studies in mice. The mouse immune system has been studied extensively and has found to be very similar to the human immune system. In addition mouse immune cells are amenable to the genetic manipulation and biochemistry required of this project. The mouse is the mammal of lowest</p>

sentience in which this project can be performed. Over 95% of the procedures which will be performed will be sub-threshold. Most experiments will involve mice with a mild phenotype being euthanised by schedule one method. When we measure the response of T cells to infection, we will use the most refined and defined systems possible. End points to these experiments are set as early as possible.

We will balance breeding efficiency very carefully against animal welfare to minimise welfare costs. We expect the great majority of mice to experience essentially normal welfare throughout their lives.

Project	The biology and genetics of Strongyloides nematodes	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Parasitic nematodes infect humans and other animals. The objective of this project is to understand how genes and molecules such as RNA and proteins are used by parasitic nematodes to infect their host, how these genes are regulated, and how they interact with their host.</p> <p>We will achieve this by studying the parasitic nematode Strongyloides in its natural animal host, the rat. This is a well-established system for studying these parasites, which are a closely related to a parasite of humans. Rats will be</p>	

	<p>injected with a low dose of Strongyloides nematodes which live as adults in the gastrointestinal tract. We will collect nematodes and study the DNA, RNA and protein to improve our understanding of how these parasites infect the host. We will also collect tissue from the rat to understand how the rat responds to a Strongyloides infection and to better understand how the parasite and host interact.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This work seeks to understand the fundamental biology of parasitic nematode infection at a genetic level. In the long term, the information collected about how parasitic nematodes infect their host, can be used by other researchers to develop new ways to treat and control nematode infections and the disease that they cause.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>3100 rats over five years (600 rats for the maintenance of nematode cultures; 2600 for experimental nematode infections). Genetically altered (GA) rats may have up to a maximum of three uses (with a minimum of a two month interval between uses). In total we estimate that up to 90 uses will be carried out in GA rats. The total number of uses is 3190 (3100 rats plus 90 additional uses). Additional uses of GA rats will be carried out when additional nematode material is required e.g. to study their DNA. The parasitic nematodes live for longer in some GA rats and we can therefore collect larger numbers of nematodes from these rats if we use them again.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>There are no expected overt adverse effects. The expected severity is mild for all protocols. Rats will be injected and some irritation e.g. inflammation or granuloma, may occur at the site of administration of injections. GA rats will be re-used by infecting up to three times in total (with minimum of two months between uses) and only when the previous infection has been clearer. Re-use of these animals in this way, has a low associated risk of harm and is therefore preferable to using additional rats. GA rats with an immune deficiency are more susceptible to opportunistic infections so they will be kept in a biocontained environment and provided with autoclaved water to minimise this risk. All rats</p>

	<p>will be socially housed. All animals exhibiting overt signs of suffering or (e.g. signs of ill health, pain and distress including, pain and distress including piloerection, hunched posture with reduced locomotion, sunken eyes, marked weight loss, abnormal gait, inactivity or inappetence) will be killed by a Schedule 1 method. However, based on experience the occurrence of adverse effects is very rare. All wild type rats will be humanely killed at the end of the procedure. GA rats will be maintained and will be humanely killed at the end of a maximum of three uses.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The use of rats in this work is essential. The parasitic nematodes naturally infect rats and cannot be maintained outside of an animal host. Therefore, there is not currently a non-animal alternative available.</p> <p>The use of rats in this work is essential and the objectives cannot be achieved without the use of rats. The parasitic nematodes are obligate endoparasites and cannot be maintained outside of an animal host. Therefore, there is not currently a non-animal alternative available.</p> <p>There is no <i>in vitro</i> model available. Culturing of nematodes <i>in vitro</i> can enable some life cycle stages to survive for days-weeks but under current methods these nematodes do not develop into the next stage of their life cycle and they do not reproduce. Parasitic nematodes can therefore not be maintained or studied effectively in this way. This type of nematodes only infect vertebrate host and an infection model in an invertebrate species is not possible.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We use well established methods to infect the rat with the parasitic nematode, using the optimal safe dose of nematodes so that the fewest number of rats are used. This project will use the minimum number of animals to generate the parasitic nematodes necessary to achieve the objectives of the project. These numbers have been calculated based on extensive previous experience. The minimum number of rats</p>

	<p>required to generate robust and biologically significant data will be used. Experimental designs will be implemented e.g. the use of controls to maximise the robustness of the data generated using the fewest number of animals. Where relevant advice will be sought from a statistician.</p> <p>Where appropriate GA rats are used which can maintain a nematode infection for longer periods of time. Although a slight harm is caused to the rat because it is genetically altered, the number of rats used is reduced.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use rats because they are a natural host of the parasitic nematode. Using the natural host is important because it means that we will study the natural infection process that has evolved. During infection no noticeable harm is caused to these rats and eventually the rats become immune to the nematode infection. We will use doses and routes of infection that best mimic the natural route of infection, and that cause minimal harm to the animals to achieve the scientific objectives. The parasite-rat system is well-established and collective experience from this field of research has provided well-established protocols which minimise clinical symptoms. Where genetically altered nematodes are used these are expected to be less effective parasites than wild types strains. However, as a precaution we will initially infect any new strains at very low doses.</p>

Project	The Breeding, Maintenance, Genotyping and Genetic Monitoring of both Genetically Altered and Wild Type Rodents	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This service licence will facilitate the breeding and maintenance of genetically altered animal lines in a managed and controlled environment, utilising the highest standards of welfare, colony management and husbandry practices allowing animals to be kept at a high health status and for their genetic status to be correctly monitored. Subsequently the animals are supplied for research with the knowledge that the animals have been bred to a high standard making them	

	<p>suitable for scientific research.</p> <p>The use of genetically altered animals in biomedical research allows for the specific traits of certain genes to be studied in a complex physiological environment that cannot be achieved by laboratory methods, further allowing for a greater understanding of the function of genes in disease and ill health.</p> <p>As a result of naturally occurring genetic mutations, certain animal strains will display similar diseased states to that of humans e.g:</p> <ul style="list-style-type: none"> ● rats whose mutation results in hypertension, allowing for advancements in the treatment of high blood pressure. ● mice whose mutation results in reduced functionality of their immune system, thus allowing for rapid uptake of cancer cells and the resulting growth of tumours, allowing for the development of new cancer treatments. <p>Skilled animal technologists who are fully trained in caring for laboratory animals of this type will be responsible for managing colonies in accordance with the guidance outlined by various groups with expertise in this field.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The project will prevent unnecessary breeding of animals by carefully monitoring researcher's usage needs and forecasts, alongside good breeding colony management. This is in line with the 3R's (refine, replace and reduce). The management of this centralised service will provide benefit to those institutions that do not have the necessary expertise or infrastructure to produce their own animals. It also prevents duplication of colonies at multiple establishments and allows the research facilities to focus on the refinement of their experimental programs. Overall this results in a lower number of animals used in both breeding and experimental areas. The use of high quality animals in research is critical in reducing variability in the data or results obtained. It dramatically reduces the need for repeat experimental programs where results are inconsistent due to the quality of animals utilised</p>

	e.g. variability in the genetic status of animals may result in significant variation in the results obtained within a single group of animals
What species and approximate numbers of animals do you expect to use over what period of time?	Mice = 720,000 Rats = 26,000 over the life of the licence
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	This project will predominantly focus on the breeding and care of mice and rats up to 12 months of age, with a small number of animals maintained from 12-18 months of age when characterisation of disease pathology at various life stages is required. All programs will be managed and monitored within conditions according to their health status, as well as the functionality of their immune system e.g. the use of barriered (high health status) environments that reduce the risk of infection by bacterial, viral or parasitic agents within animals whose immune state is compromised by their genetic alteration. In order to establish the genetic status of animals produced, tissue samples will be taken from animals utilising the most refined method (ear punch system) that focuses on the welfare of the animals but also ensures sufficient DNA can be obtained to analyse their genetic state. Physical tests to confirm the presence of a genetic alteration that results in hypertension will involve the restraint and warming of animals within specially designed equipment for no more than 30 minutes. This allows for dilation of the blood vessels and for the subsequent accurate reading of blood pressure to ensure the procedure takes as short as time as possible for the animal. Some animals produced will display similar cognitive deficits to that of an ageing human, this may result in reduced spatial learning and memory deficits in line with symptoms of neurodegenerative disease e.g. Alzheimer's. One particular genetic alteration has been noted to result in spontaneous death within <5% of all animals carrying the genetic alteration born between 4-7 months of age, the cause of death is non-convulsive seizures brought on by physiological changes in the brain and is as a result of their genetic alteration (neurodegenerative disease model). These seizures induce a state whereby animals are not

	<p>aware and die without suffering. Procedures are in place to reduce the risk of triggering seizures e.g. reduction of noise and sudden changes in lighting. Animals produced will be supplied into the project licence authority of other establishments in the UK and bona fide establishments abroad.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Non-animal methods are not always able to model or replicate the complete array of behavioural, cellular, molecular and physiological interactions required to fully understand how genetic alterations result in normal or abnormal processes. Mice and rats bred or maintained under this project will be subject to scientific justification in the researcher's protocols demonstrating that the goals cannot be met with the use of non-animal methods.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The use of effective and stringent colony management systems will result in a reduction in the number of animals required in the breeding aspect of this project. Colonies will be planned according to the demand of end user groups and will be subject to continual review to ensure production levels are in line with the forecasted demand. Should usage reduce and remain sporadic, colonies will be closed and embryos frozen to preserve the model of interest.</p> <p>The supply of high quality animals according to client specifications will result in more effective experimental programs where variability would ultimately impact upon the results obtained. The ability to offer this as a service will also result in a reduced need for duplicate colonies at various establishments, also lowering the number of animals of a similar type needed for breeding programs.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the</p>	<p>End users will be required by the project licence holder to satisfactorily justify the need for the animals that will be bred and maintained under the authority of this service licence. The choice of species/strain is driven by end user</p>

<p>general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>requirements.</p> <p>Prior to the receipt of any animal model, stringent data collection will be performed to ensure all known traits and observations due to the genetic alteration are known, this will allow for specific refinements to be made in terms of breeding practices, husbandry, nutritional requirements, enrichment and handling. For example, animals that carry a genetic alteration which results in reduced functionality of their immune system will need to be housed within specific barriered environments to maintain their high health status and reduce the risk of infection e.g. with opportunistic bacterial agents.</p> <p>Stringent colony management systems are in place and controlled by animal technologists fully trained in the breeding, care and husbandry of specialised animal colonies, utilising their experience and expertise as well as guidance from experts in the field of genetically altered animals.</p> <p>Prior to the start of any breeding program all responsible members of the team will ensure specific details related to the animal model are known and used to set up the specific breeding and maintenance plan. The breeding plan will be subject to changes throughout the lifetime of any colony and will be in line with the forecasted usage. Breeding systems that minimise overproduction of unwanted genetic status animals will be used.</p> <p>Animals will be housed in optimal social groups, allowing for a reduction in potential aggression or overt dominance behaviours, thus reducing any associated stress.</p> <p>When determining the genetic status of both genetically altered and wild type colonies, the least invasive and most refined method, ear punch system, will be used for the retrieval of tissue, whilst the most advanced methods and technology will be used for the analysis of DNA to maximise the likelihood of success in this procedure, therefore reducing the need for re-sampling.</p>
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Project	The carotid body in the neurogenesis of hypertension	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to understand the brain's involvement in generating high blood pressure and to gain insight as to how and why this occurs so that new therapeutic strategies can be harnessed. This is particularly important since ~14% of hypertensive patients are resistant to contemporary drugs or cannot tolerate medications. The causes for high blood pressure, which affects 1 in 3 of us, are unknown in 95% of patients. Given the poorly tolerated side effects of blood pressure medications and the numbers of patients that become resistant, new therapeutic strategies are urgently required. High blood pressure causes stroke, heart failure/attacks and kidney damage costing	

	<p>significant reductions in life quality, suffering and medical expense to the state. It appears that in most patients with high blood pressure there is a central nervous system dysfunction. Whilst we understand what has changed, we do not know why this has occurred. Our primary aim is to understand the neural regulation of the circulation and to determine the changes that occur during the development and maintenance of high blood pressure in animal models of this human syndrome. We hypothesise that there are changes within areas of the nervous system controlling blood pressure that reduce or restrict blood flow and oxygenation, particularly in the kidney, triggering high blood pressure. It is this that we wish to study.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The results from these studies will provide us with fundamental new information relating to how the body receives, handles and then acts upon changes in blood pressure. It will reveal the way in which the body normally responds to these stimuli and reveal how this is altered in conditions of high blood pressure. We will obtain a better understanding of the genes in the brain that are responsible for high blood pressure and how external factors interact with them. As such, it will help in providing much needed information that will assist in the design of new medicines and/or therapies to treat people and animals who suffer from high blood pressure and related diseases. As such it is envisaged that this work will be of significant benefit to the large number of patients who are stricken with hypertension and other related diseases.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>It is expected that no more than 645 rats will be used during the course of this project (5 years). Rats are the most established animal species for understanding the central nervous control of the circulation and respiration and there are established models of hypertension in rats that share commonalities with human hypertension. We have much experience (15 years) with these animal models. For all experiments we use power calculations to ensure that the minimal number of animals are used to achieve biological significance. In all experiments, the design is carefully planned to ensure maximal data output</p>

	from each animal.
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>From previous research we know which regions of the nervous system that regulates key involuntary functions of the body (autonomic nervous system) control the heart and blood vessels. We also know from studies in humans, and from animal models of hypertension, that there are changes in the activity of nerves that control the cardiovascular system in the high blood pressure condition. Interestingly, these changes precede the onset of high blood pressure suggesting a possible causative link. Changes in autonomic control of the cardiovascular system are now used clinically as prognostic indicators of cardiovascular disease. In addition, most medicines that are routinely prescribed to patients to lower blood pressure also affect autonomic nervous activity. Hence we use a well-established animal model in which we partially restrict the blood flow to one kidney and thereby control the level and timing of blood pressure increase. In order to make measures of blood pressure that remain under the control of multiple interacting regulation systems it will be necessary to make measurements in conscious animals. We will use the most modern methods that allow remote recording of blood pressure (via radio waves) such that the animal is undisturbed, unrestrained and behaving naturally in its home cage. These experiments will permit us to look at the long term experimental perturbations on blood pressure control. This is crucial as high blood pressure is a chronic disease which develops over time. Our experiments aim to reproduce this. Animals may undergo one or two surgeries with general anaesthesia to induce hypertension, to implant a telemetry device and/or cut nerves. Furthermore animals may be caged individually, undergo drug tests and collection of blood and urine. In a few animals the effect of timed feeding or a shift in light/dark phase will be investigated. Expected adverse events and the likely/expected levels of severity: Surgeries: General anaesthesia can cause adverse effects and will be prevented by appropriate surgical practice, close monitoring, and appropriate analgesic. Hypertension could cause stroke (severe severity) and will be prevented by good surgical technique, close</p>

monitoring and by not allowing the blood pressure to exceed a specific limit (i.e. >200 mmHg). Typically the used animal model will generate hypertension that is less than this limit or are used before they reach it. However we will kill any animals that go over this limit or show any signs of stroke. Surgery can cause wound breakdown and infection (mild severity if treated) and will be minimised by good surgical technique and appropriate asepsis and if wound breakdown does occur it will be repaired under general anaesthesia. After cannulation, cannula may become dislodged (severe severity) and will be prevented by appropriate surgical practice. In the rare event (<1%) an animal is suffering as a result of haemorrhage (dislodge cannula, electrodes or flow probes) will be immediately killed. Following introduction of a catheter into a blood vessel, for the recording of blood pressure, could (<2%) cause a permanent lack of oxygen supply to the hind body (severe severity). Placement of a telemetry device in itself is at the low end of moderate severity although typically (~90%) animals will lose body weight but will regain this weight in about a week after surgery. If animals showing significant weight loss (>15%) animals will be killed. Cutting of the nerves will have no clinical after-effect although the surgery on the carotid body or artery can cause a temporarily sore neck causing impaired feeding behaviour and hence weight loss (mild severity). Post-surgery animals will be given their standard food pellets mashed in water in the cage to allow easier access to food and water until it is judged to be no longer necessary (e.g. back to normal body-weight, or increases in body-weight for consecutive days). Post-operative pain and infection will be controlled by use of analgesics and antibiotics accordingly. Any animal showing signs of more than minor distress will be killed (<10%). Caged individually: the telemetry technological requires animals to be caged individually which causes stress due to social isolation (mild severity). This will be minimised by enrichment of the animal's environment. Drug test, gas exposure and the collection of blood and urine: some animals will undergo test to see how the blood pressure responses upon some known drugs or exposure to gas with different concentrations of oxygen. These have no clinical

	<p>after-effect as long as the correct dosing and exposure time is used (lower end of mild severity). In some animals blood and 24 hour urine will be collected (mild severity). Timed feeding or a shift in light/dark phase: some animals will undergo change in feeding regime in which they have access to food only in the night or only in the day (low end of mild severity). Some animals will be exposed to a shift in light/dark phase (low end of mild severity). Experiments will be also carried out in rats under terminal anaesthesia (i.e. the animals will not wake up from the anaesthesia). In these studies, a number of measurements can be made that are not possible in conscious animals such as activity from nerve trunks or recording detailed kidney function and oxygenation. Studies under anaesthesia will include continuous physiological monitoring which will provide an online assessment of the level of anaesthesia. The level of anaesthesia will be adjusted accordingly. In the event of blood loss (hypovolaemia), animals may receive saline solution by infusion.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Since the nature of the work is to look at the regulation of physiological systems, in vivo studies on conscious and anaesthetised animals will be essential. The proposed studies can only be carried out in living animals since the aim is to study blood pressure that remain under the control of multiple interacting processes that are controlled by the central nervous system and which cannot be replicated in computer-based model systems or in isolated tissues. In other words, experiments need to be performed in an intact and interacting nervous and circulatory systems in order to understand the brain's involvement in generating high blood pressure and to gain insight as to how and why this occurs.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We have adopted modern methods of measuring blood pressure, renal sympathetic activity, renal or carotid blood flow using radio-transmitters. These are viable for 3-4 months. This increased longevity and the development to measure multiple parameters within the same rat (e.g.</p>

	<p>blood pressure and blood flow) has reduced animal numbers and avoids the need for chronic indwelling arterial catheters, which can be life threatening if they are pulled out by the animals, and cause infection which affects data interpretation.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Implanting a device that uses radio-telemetry allows the animal to live unrestrained, unstressed, unhandled in its home cage oblivious to the fact that we are recording its blood pressure and other parameters. However, this does mean they need to be housed individually which does cause social stress. Experimental time is kept to a minimum. Furthermore, the use of tail cuff plethysmography (using the same principle as the blood pressure monitor at the GP) will reduce the number of animals undergoing surgery for blood pressure radio-telemetry and the number of animals caged individually as they can be co-housed. Animals are checked at least once daily seven days a week. Any signs of lethargy or ill health are dealt with immediately. We have an excellent animal facility and staff who are always at hand to advise us on best practice. In animals that will be anaesthetised we will have access to an anaesthesiologist who can monitor the depth of anaesthesia regularly by noxious pinching and from the stability of their blood pressure, heart rate, respiration, and temperature (and if needed blood gases). The animals are therefore kept in excellent physiological condition.</p>

Project	The costs of flight	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
		Translational and applied research
		Regulatory use and routine production
	<input checked="" type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input checked="" type="checkbox"/>	Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The airspace used by birds in flight is changing. This is because wind speeds themselves are changing, and so too is the ground that the wind moves over with humans encroaching more into the aerial habitat as we build structures from turbines to skyscrapers. We therefore need to understand how birds are affected by air currents. Factors such as wind affect the costs of flight, but it is difficult to predict how, as there is still uncertainty over the energy required to fly. This project will develop new methods to estimate the energy expended during flight in still air (simulated by a wind tunnel) and in the wild, where airflows are highly dynamic. This will be achieved using miniaturised tags attached to birds' backs to quantify how often and how hard</p>	

	<p>birds flap their wings. The project will also estimate how these measurements are affected by the tag itself. Overall, this will provide completely new insight into how much energy birds expend during flight and how this changes with the weather.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>An improved understanding of how much energy is used during flight is needed for a wide range of issues that include fundamental biology (e.g. how flight costs vary across birds with different body sizes and body shapes) and applied questions, such as how much extra energy will a certain species have to spend in order to divert its flight path round a wind farm. The results from this project will feed into both, by providing new estimates of the energy expended in still air, and the means to quantify the energy expended by birds flying in the wild depending on where and when they chose to fly.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The project will involve up to ~ 20 homing pigeons (<i>Columba livia</i>), which will be flown over the duration of the project (5 years).</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>This project involves flying the pigeons in a wind tunnel. This is perfectly safe as birds will not be able to access any of the moving parts and an observer will be present during all flights to ensure that birds do not damage themselves by flying against the wall of the tunnel. While individual flights will be short (~20 mins) birds may become tired in the period when their flight muscles are increasing. Training flights will therefore be very short to begin with and increase as training progresses. Miniature loggers will be attached to birds for the flights and these loggers will be attached in a way that minimizes any possible feather damage. The loggers will be a very small percentage of the bird's mass but this may still increase flight costs. We will use tests to measure how much extra energy it requires to fly with loggers. Measurements of energy use will require measurements of the carbon dioxide, which will involve training birds to fly with a mask on for short periods (~10 minutes), and measuring carbon dioxide and oxygen levels while birds rest. Birds will continue to be housed at the</p>

	establishment after the end of the project.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The premise of the study is to provide new insight into the costs of flight. This is necessary because previous estimates have been heavily influenced by the methods used, and data gained from laboratory trials do not agree with data gathered from birds flying in the wild, as has already been confirmed by a literature review. We therefore need new experimental data and new methods to understand why this is.</p> <p>Computer modelling is the only type of non-animal alternative for this work, as it can be used to predict the energetic consequences of a bird choosing flight path a over flight path b, and opting to fly faster or slower, for instance. In order to have any confidence in these simulations, we need to compare the results with the decisions made by real birds. We will know that we have a robust framework for predicting animal movement, and how this is affected by the physical environment, when these two approaches produce the same results. Nonetheless, the literature will be continually reviewed in order to keep up with latest developments and the possibility of any further reduction in the use of animals.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The following steps will be taken to ensure the appropriate number of animals is used, commensurate with good experimental design. This will ensure that the research will be publishable according to the ARRIVE guidelines:</p> <ul style="list-style-type: none"> ● Extensive training will be undertaken to ensure that study animals are fit, accustomed to the wind tunnel and experimental procedures. This will also ensure that the signal to noise ratio is maximised. ● Preliminary work will be undertaken to establish how long birds need to be flown for. ● The final numbers of birds used in wind

	<p>tunnel trials will reflect (1) the need for statistical power. Previous studies have shown that the variation in flight costs is very low between individuals compared to the variation caused by flight speed. Most previous studies have used between 5 and 10 individuals. Comparisons of flight costs will be made in relation to speed, with statistical models controlling for mass and sex of birds. (2) The proportion of the initial flight team that take well to training. Research at other institutions indicates that around 2/3 of all birds will end up flying well in a wind tunnel after training. Around 15 birds will undergo initial training.</p> <ul style="list-style-type: none"> • Final protocols will involve the simultaneous collection of multiple data types. This will reduce the number of overall trials. <p>Project personnel include researchers with substantial statistical expertise who will be consulted to refine the experimental design and ensure that the minimum number of animals used is commensurate with the ability to achieve statistical power.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The welfare of the animals will be prioritized during all procedures and steps will be taken to enrich their living conditions, including the provision of an external aviary, where they can be outside the loft without being at risk of predation. Predator scaring measures will be considered if there is evidence that predators are being attracted to the loft.</p> <p>Flying birds in a wind tunnel is the only way of ensuring that they experience controlled conditions. This is necessary in order to provide baseline measurements of the costs of flight, before expanding the project to assess how these vary in the wild. Methods of training birds will be reviewed before training commences. Procedures that train birds with positive associations will be favoured. An observer will be present during all flights in order to (i) monitor the bird for signs of stress or exhaustion (ii) stop the trials where such signs are observed, or in</p>

	<p>the unlikely event that a bird becomes injured/ is behaving in such a way that it might make injury likely. An emergency stop button will be located within easy reach of the observer. Flight durations will be increased from a matter of seconds during the early stages of training.</p> <p>Protocols will be refined to keep handling of animals to a minimum.</p> <p>Tagging is now a widespread method of quantifying the movements of wild animals and this project will also provide valuable data on the costs of flying with tags.</p> <p>Animal models will be refined by regular review and critical appraisal of work during the course of the licence to ensure that they remain the most refined from an animal welfare point of view and to obtain the maximum scientific output for the minimum animal suffering.</p>
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Project	The development and function of ectodermal appendages	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project aims to understand how the skin of mammals and birds develops and sustains its ability to heal itself.</p> <p>The first aim is to understand how the embryonic skin produces different structures, such as hairs, feathers and scales through different signals that pass between cells. Some of these signals have been identified, but how they work at different stages of development and in different species is not known.</p> <p>The second aim is to understand which adult body parts different embryonic structures</p>	

	<p>develop into so that an understanding of the construction of the skin's different components is achieved. Some tracing of these relationships have been done, but new tools allow this to be done much more accurately and trace the boundaries between different regions in a more refined manner.</p> <p>The third aim is to understand how skin sustains and heals itself by tracing the origin of the cells involved in healing. This process has been well described for mammals, but the means by which birds heal skin wounds is little understood at a cellular level.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>From this study of the basic biology underlying the development of the skin and how it is healed, we will provide knowledge that can be used: - to understand, and perhaps design treatments for, conditions humans are born with that affect the skin. This is through study of communication between cells and the potential to mimic or block these signals to help skin develop along the normal route. -to improve breeding of farm animals, particularly the chicken, a species in which feathering has important effects on heat tolerance. If we can understand how feather number and type is controlled we can use this information to breed birds with the appropriate number of feathers for their conditions. -to improve our understanding of wound healing in birds, potentially aiding in poultry production and welfare, due to the frequency of breast skin lesions occurring in commercially produced chickens and turkeys. In particular, understanding whether feather follicles aid in healing or not will be useful to veterinarians managing and deciding treatments for birds with skin wounds.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use, in breeding and in experiments, up to 1000 mice per year, 100 rats per year and 110 chickens per year over the entire 5 year course of the project.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity?</p>	<p>A majority of the experiments will have mild effects on the animals, primarily involving either innocuous labelling of cells so that they can be detected and tracked, or altering the structure of</p>

<p>What will happen to the animals at the end?</p>	<p>the hairs, feathers, glands and possibly teeth of the animals. Creating a small (up to 1 cm) skin wound in a chicken, to determine how it heals, carries a low risk of infection, which we will minimise by use of good surgical technique. Pain relief will also be given to chickens in these studies. To understand the relationship between embryonic and adult body parts we will do some transplantations of small pieces of tissue between chick embryos at a very early stage of development. The transplanted tissue can become a normal part of the embryo, which we can then identify later. If the chick did not develop normally it would be culled humanely. All animals will be humanely culled at the end of the experiment in which they are used.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We can not use non-vertebrate animals as the vertebrate skin has a unique structure and appendages, like hairs and feathers, that simply do not form on other types of animal. The skin and its associated structures are composed of many different types of cells interacting with one another throughout development. This complex environment can not be mimicked by a culture system. However, as far as possible we will perform experiments on cultured skin collected from culled animals, rather than the intact animal itself. Also, we will complete many experiments using embryos only, rather than manipulating adults directly.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Experimental designs will be developed with advice from our institute statistician. We will reduce variation in our experiments by maintaining animals in a constant controlled environment and by maintaining, as far as possible, inbred lines of animals so that genetic variation is reduced. This will allow effects of experiments to be detected with fewer animals used compared to populations in which there is higher genetic or environment-derived variation. The number of animals required to maintain each experimental line will be kept to a minimum.</p>

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse, chicken and rat already have suitable genetic resources, that is, mutant animals or genetically modified animals in which cells can be tracked, to allow us to address the scientific questions that we have set. The breeding of these animals in our facilities is well established, as are welfare indicators for husbandry of these species.</p>
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Project	The effect of <i>H. pylori</i> infection on colitis.	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p><i>H. pylori</i> is a bacterium that causes life-long infections of the stomach by suppressing the ability of the host's immune responses to clear the infection. <i>H. pylori</i> is well known as the causative agent of stomach cancer, however, this occurs in only 1% of all infected patients. Recently, <i>H. pylori</i> has been associated with beneficial effects. Epidemiological studies suggest that people who carry <i>H. pylori</i> in their stomachs are at a lower risk of developing auto-immune disorders, such as asthma and IBD. However, the mechanism for this association is not understood. We aim to study how <i>H. pylori</i> infection in the stomach affects the diversity of bacterial species in the intestine (by analysing faeces) and intestinal inflammation (by analysing a subset of white blood cells known as T lymphocytes) during colitis to understand the mechanisms underlying this epidemiological link between</p>	

	<i>H. pylori</i> infection and protection against IBD.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Understanding the mechanism of how <i>H. pylori</i> manipulates host immune responses to prevent auto-immune disorders may provide the rationale for drug design of novel anti-inflammatory therapeutics, which can be used to tackle auto-immune disorders such as asthma and IBD. Furthermore, our human clinical studies suggest that <i>H. pylori</i> infection in the stomach has an impact on the types of bacteria found in the intestine in IBD patients. Thus, exploring this phenomenon in mice will help us to unravel the hugely complex issue of the intestinal microbiome in colitis and how <i>H. pylori</i> affects this, which may lend itself to a protective mechanism against IBD.
What species and approximate numbers of animals do you expect to use over what period of time?	All of the studies proposed in this licence will use mice as the chosen model. The numbers of mice to be used in the next 5 years will be approximately 1000
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	<i>H. pylori</i> in humans is largely asymptomatic. Only a very small percentage (<1%) of infections lead to gastric cancer. However, in nearly all infections, <i>H. pylori</i> shapes the development of our immune system and the microbiome in the stomach and potentially, in the intestine. In mice, infection with <i>H. pylori</i> is also largely asymptomatic and severe gastric disease will only be seen after 2 years of infection. In this licence, we will not be infecting mice with <i>H. pylori</i> for longer than 12 months and so the mice will not suffer from morbidity or mortality during this period. However, <i>H. pylori</i> will have more subtle effects on the immune system and microbiome of mice and we are interested to see how this manipulation affects the development of colitis. Colitis will be induced in mice using well established methods. Mice are likely to suffer adverse effects from colitis such as diarrhoea, intestinal inflammation/bleeding and weight loss. These effects are closely monitored on a daily basis using score sheets to monitor and record disease severity. These experiments are likely to lead to a moderate level of severity. With careful monitoring, mice will be humanely killed when they reach the end of the experiment or they reach the clinical end point as defined by the score sheet. Mice will be terminated via a schedule 1 method and organs harvested for further study.

Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>In vivo, adaptive immune responses to bacterial infection develop through the interaction of multiple cell types including one or more DC subsets, T cells and B cells. We, and others, have repeatedly shown that these interactions <i>in vivo</i> are not fully replicated <i>in vitro</i>. However, we will be using immortalised cell lines and both human and mouse gastric and intestinal organoids to investigate epithelial and T cell responses to <i>H. pylori</i> and other members of the gastrointestinal microbiomes. Understanding the effect of <i>H. pylori</i> on T cells <i>in vitro</i> will inform the cell types of interest in <i>in vivo</i> studies.</p> <p>In order to study the gastric mucosal immune response to the infection, and how it influences the community of bacteria in the intestine, we require intact gastrointestinal systems. Mice provide an established and verified means of studying immunity with tractable systems that allow detailed analyses of these complex environments.</p> <p>Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace <i>in vivo</i> animal use. To this end, we will use the NC3R's systematic review tool to ensure we are using the best model for our scientific objectives. This tool can be found at www.nc3rs.org.uk/camarades-nc3rs-systematic-review-facility-syrf</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Each experiment requires a written protocol giving full details of the experimental aims, a description of each group, including numbers, treatments and possible risks associated with the procedures used. This allows others to share experiment tissues etc post-mortem, reducing experimental numbers or permitting use of the same experiment to answer multiple objectives. For instance, after experiments are concluded, tissues such as the spleen are often sectioned for immunohistology and archived. This archived tissue can be revisited by other workers at a later time.</p> <p>Power calculations and group sizes</p> <p>The minimum group sizes required to obtain statistically significant differences have been calculated using power equations with a 2-sample t-test on previous experimental data. For a power of at least 80%, group</p>

	<p>sizes should be of no less than 6-8 mice when comparing <i>H. pylori</i> infected and non-infected mice. For experiments comparing colitis in <i>H. pylori</i> infected and non-infected mice, group sizes are likely to require 8-10 mice. Short-term experiments are likely to provide statistically significant data with groups of 8, as found previously. During long-term experiments, it is possible that a few animals may die prematurely from problems that could be unrelated to the regulated procedures. Therefore, to ensure that experimental data can be analysed statistically and that data from remaining animals can still be used, slightly larger group sizes will be used. In experiments longer than 6 months, groups of at least 8-10 mice will be used.</p> <p>This website will be utilised to provide additional information on statistics and experimental design http://www.3rs-reduction.co.uk/</p> <p>In order to ensure that high quality, reliable and valid data is extracted and reported from the minimum number of experiments, the ARRIVE guidelines (Kilkenny <i>et al.</i>, 2010) will be followed. http://www.nc3rs.org.uk/page.asp?id=1357</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are an appropriate model as their immune systems share many similarities to those of humans including lymphoid organization and cellular populations (e.g. lymphocytes, DC). GA mice, provided by collaborators, will be used in this project and provide a well-established means to study the immune system. For new protocols we work closely with [REDACTED – Place] to refine the techniques to minimise any suffering that might otherwise occur.</p> <p>Bacterial and host response factors are likely to have an effect on the colonisation density of <i>H. pylori</i> in the stomach of mice. The relationship between the immune response and the density of <i>H. pylori</i> must therefore be assessed in detail. Furthermore, pilot studies will inform the shortest length of time and the lowest dose of DSS or TNBS that can be used to induce sufficient intestinal inflammation, whilst limiting any adverse effects to the mice.</p> <p>Before conducting each experiment, it is discussed with named veterinary surgeon to ensure animal welfare is maintained throughout the experiment and that minimum suffering is caused to achieve the scientific endpoints. We will also review each experiment on completion to see what lessons can be learned from the study in terms</p>

	<p>of endpoints (scientific and humane) and any animal welfare issues that may have arisen during the experiment that could then guide the next experiment.</p> <p>Additionally, animals that are infected with <i>H. pylori</i> for 6-12 months and show a weight loss of greater than 5% will be weighed weekly.</p> <p>The score sheet outlined in the Appendix will be referred to for every experiment to ensure the animal welfare throughout the experiment.</p> <p>Furthermore, if the named veterinary surgeon or the NACWO on site are not familiar with a certain technique, we will visit external collaborators in order to be fully trained on new techniques prior to initiating pilot studies.</p>
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Project	The effect of peripheral homeostatic disturbance upon the brain's defences	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to investigate how the defensive mechanisms of the brain are affected by changes in the rest of the body, and to study the links between chronic ill health and development of neurological conditions such as Alzheimer's disease. This project has two arms, firstly we will compare the effects of chronic inflammatory disease, modelled using the very common dental condition periodontitis, with acute inflammation upon the two main defences of the brain, the blood-brain barrier and the immune cells called microglia. Periodontitis has been associated with an increased risk of	

	<p>developing Alzheimer's disease in humans, but little is known about how these links occur; this project will directly investigate whether periodontitis increases the vulnerability of the brain to damage.</p> <p>The second main arm of this study is to establish how changes in the microbial communities of the body can influence the brain's defences. There is substantial evidence linking a low quality diet and poor brain health, including an increased risk of Alzheimer's disease, but again the linking mechanisms are unclear. The microbial communities of the gastrointestinal tract respond to changes in diet, producing many chemical mediators that affect the rest of the body. This project will directly investigate how diet-induced changes in the gut microbes affect the brain's defences, and their importance in linking a poor diet with the risk of neurological disease.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>As the average age of the population increases, the most significant risks to health come from age-related neurological disorders, of which Alzheimer's disease is the most common. While the cause of Alzheimer's disease is still unknown, it is increasingly clear that a significant factor is the failure of the normal defensive mechanisms of the brain. In this project we aim to investigate how two common, chronic disruptions to normal physiology can affect these defences and increase the risk of developing Alzheimer's disease. The experiments to be performed in this project will allow us to assess the state of brain health after chronic physiological disruption, providing valuable insights into the mechanisms underlying the increased risk of Alzheimer's disease and other neurological disorders associated with either chronic inflammatory disease or poor diet. This will serve as an essential platform for future studies investigating ways to counteract these changes and protect the brain from disruption, hopefully reducing the risk of developing age-related disease.</p>
<p>What species and approximate</p>	<p>The majority of the animals to be used over this</p>

<p>numbers of animals do you expect to use over what period of time?</p>	<p>5 year project will be wild-type mice and we expect to use a maximum of 1800 animals in this period. We will also use selected genetically modified animals to test specific hypotheses, and we anticipate using a total of 400 such animals over the 5 year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The majority of procedures to be performed in this project are of mild severity, and we expect few adverse effects, primarily acute and self-resolving sickness from the administration of inflammatory agents. A number of mice will receive injections of inflammatory agents directly into the brain, a procedure of moderate severity given the route of administration and the central position of the brain in health. The inflammatory agents we will use however, are deliberately chosen to be relatively mild, and are not expected to cause significant defects in behaviour or health. All animals will be killed by a humane method at the end of the procedures or if they show unexpectedly severe adverse effects.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We are investigating the impact of changes to normal physiology upon the brain's defences and the risk of developing neurological disease. As such, we will be examining interacting changes in several highly complex systems, namely the immune response, the microbial communities of the body, and the brain. Currently, there are no computer models able to effectively replicate these complexities. Similarly, the immune response and nervous systems of non-protected animals are too simple to model the interactions that occur in mammals, and we would not be able to satisfactorily address our research questions using these organisms. We will investigate our research questions as far as possible using <i>in vitro</i> techniques or through analysis of human clinical samples, such that we will only move towards animal experiments when all other approaches have been exhausted.</p>

<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>All the experiments in this project have been carefully designed to use the absolute minimum number of animals, through rigorous statistical analysis of the numbers of animals required to efficiently detect biologically meaningful differences in our experiments, informed by our previous experience in the field and by the scientific literature.</p> <p>Where possible, we will make use of non-invasive imaging techniques to permit repeated analysis over time of individual animals, significantly reducing the number of animals needed.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse has been exceptionally well characterised in terms of its physiology, genetics and microbial communities, and represents the best available model system to ensure results are applicable to human health. Moreover, there are a large number of genetically modified mouse strains available, which will allow us to examine the role of specific components of the immune, nervous and vascular systems in maintaining brain health. The use of such strains will significantly enhancing the precision of our experiments, ensuring that the data we obtain has greater clarity than can be gained from studies of wild-type animals or those of other species.</p> <p>In this project, we will use minimally invasive techniques wherever possible, including for example modification of gut microbes by changing diet or including antibiotics in drinking water, both to reduce welfare costs to animals and to avoid triggering a stress reaction, which we know from previous work to modify the response of animals to inflammatory stimuli.</p>

Project	The evaluation of veterinary medicinal products																
Key Words (max. 5 words)																	
Expected duration of the project (yrs)	5 Years 0 Months																
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<table border="1"> <tr> <td data-bbox="759 584 767 674"></td> <td data-bbox="767 584 1402 674">Basic research</td> </tr> <tr> <td data-bbox="759 674 767 763">X</td> <td data-bbox="767 674 1402 763">Translational and applied research</td> </tr> <tr> <td data-bbox="759 763 767 853">X</td> <td data-bbox="767 763 1402 853">Regulatory use and routine production</td> </tr> <tr> <td data-bbox="759 853 767 1021"></td> <td data-bbox="767 853 1402 1021">Protection of the natural environment in the interests of the health or welfare of humans or animals</td> </tr> <tr> <td data-bbox="759 1021 767 1111"></td> <td data-bbox="767 1021 1402 1111">Preservation of species</td> </tr> <tr> <td data-bbox="759 1111 767 1200"></td> <td data-bbox="767 1111 1402 1200">Higher education or training</td> </tr> <tr> <td data-bbox="759 1200 767 1290"></td> <td data-bbox="767 1200 1402 1290">Forensic enquiries</td> </tr> <tr> <td data-bbox="759 1290 767 1424"></td> <td data-bbox="767 1290 1402 1424">Maintenance of colonies of genetically altered animals</td> </tr> </table>		Basic research	X	Translational and applied research	X	Regulatory use and routine production		Protection of the natural environment in the interests of the health or welfare of humans or animals		Preservation of species		Higher education or training		Forensic enquiries		Maintenance of colonies of genetically altered animals
	Basic research																
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	Preservation of species																
	Higher education or training																
	Forensic enquiries																
	Maintenance of colonies of genetically altered animals																
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To provide data for the licencing process for veterinary medicinal products, this primarily focuses on proving their safety, quality and efficacy. The work is a service licence with the main customer being veterinary medicines companies																
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The work carried out under this licence aids the development and licensing of new medicinal products and improves currently licensed products, thereby contributing to improving animal and human health, food production and the control of infectious diseases. There is also an economic benefit to the consumer and farmers through more																

	<p>efficient production. These products are fundamental in controlling disease and the spread and effects of infectious pathogens in animals and humans.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This licence is demand led, the first protocol is for work on a Bovine Viral Diarrhoea 2 (BVD2) virus vaccine which will involve 24 calves for 3 months. Second experiment is assessing safety of two poultry vaccines and requires 60 hens and last approximately 16 weeks. A third experiment is to evaluate the safety of DIVA (Differentiating Infected from Vaccinated Animals) skin test reagents which are intended to be used in the diagnosis of TB in cattle. This experiment will involve 70 calves 42 days or older (42 days is the youngest age for Skin test for TB in cattle). The experiment will also compare the safety calves that have been vaccinated with BCG (a vaccine used to protect against infection with TB) and calves that have not been vaccinated . A subsequent experiment will look at the effect of a commercial food supplement on the efficacy of salmonella vaccine. Another study will look at the efficacy of re-vaccination of late-age laying hens to reduce salmonella colonisation and will involve 66 hens. A further study will look at validating a challenge model for porcine circovirus-2 (PCV2), using 24 juvenile pigs this model will be used to test the efficacy of vaccine candidates to go on for registration for a marketing authority.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>There should be no adverse effect of the vaccine, for the unprotected control group BVD 2 should have a transitory illness with signs such as inappetence, elevated temperature, moderate depression, mild nasal discharge and loose faeces. The poultry experiment is mild, there should be no adverse effects of the vaccine but the trial is to prove safety so health and laying performance will be monitored. The chickens will be euthanized at various predetermined time points to meet regulatory requirements. Although it is expected that the DIVA skin test reagents will not cause an adverse reaction there will potentially be adverse effects after movement and mixing of</p>

	<p>these young calves which have been born on different farms to establish the experimental groups. These effects are primarily around the spread of inter-current infectious disease including, gastroenteritis and pneumonia, that some of them maybe carrying sub-clinically. The calves will have a health plan, which will include the prophylactic use of antibiotics on arrival and use of electrolyte solutions to prevent dehydration. There will be additional visits and checks by animal care staff including, during the evenings after arrival, whilst the animals are on 3 feeds a day and if there are concerns over the animal health. Providing there have been no significant issues with intercurrent infectious diseases it is intended to re-use the calves. The chickens on the food supplement effect on salmonella vaccine, should have no adverse effects due to no challenge with salmonella. The chickens will be euthanased at set time points and the gut microbiota examined to see if the vaccine has been affected by the supplement.</p> <p>For porcine circovirus-2 (PCV2), by using high health status pigs it is expected the symptoms of the experimental infections will be a low-grade fever (up to 41°C) that lasts several days, with potential reduction of appetite and increased breathing rate followed by weight loss/growth retardation and wasting. With the length of the experiment these are expected to be mild, possibly moderate but the pigs will be monitored and euthanased if these symptoms are likely to breach moderate severity.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The response to inocula such as pathogenic organisms and vaccines is a very complex process and one that cannot be replicated <i>in vitro</i>. It is not possible to generate definitive safety and efficacy data without the use of animals.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use</p>	<p>The number of animals used is often specified in the regulatory guidelines so there is limited scope for reducing the number of animals. However, scientific expertise, input from</p>

<p>of minimum numbers of animals</p>	<p>customers, discussion with regulators and statistical rigour ensures the number of animals used is minimised. Possible reduction is discussed with the Sponsor and adjusted according to the aims of the studies.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Although study designs are often prescribed by regulators, careful consideration is given to possible refinements on a case-by-case basis. For example in batch testing it may be possible to not challenge vaccinated animals, relying on the measurement of antibody response which eliminates the development of disease. In addition, data from companies and their communications with the regulatory authorities and feedback directly from the competent authorities may allow tests to be refined. All plans undergo review by the Institute's Animal Welfare and Ethical Review Body. All require close monitoring of all animals used in Studies. Detailed Clinical Monitoring Schemes with clear humane end-points and actions are prepared for all those involved. These allow treatment or euthanasia at the earliest possible time and ensure they do not exceed the severity limit of the protocol.</p>

Project	The function of the RASSF proteins and the Hippo pathway in tissue architecture and cancer.	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The “Hippo pathway” is a signal that tells our cells to stop growing and proliferating. It is among the key signals that tell our bodies to grow to the right size and shape, but not larger or smaller. Disruption of the Hippo pathway in mice and people has been shown to lead to tumour formation. Although scientists are trying to create drugs that can restore normal Hippo signalling in tumours to stop them growing, these approaches have so far been unsuccessful. Our research is aimed at gaining a better understanding of this signal during	

	animal development and tumour formation, and to identify new ways in which it can be targeted for patient benefit.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We hope our work will identify new diagnostic markers for early detection of cancers, as well as new therapeutic targets for drug discovery.
What species and approximate numbers of animals do you expect to use over what period of time?	Based on the work we have carried out in the past 5 years under our previous licence, the number of mice we expect to use over the next 5 year period will be around 6000 mice bred and around 9000 mice used under the various experimental protocols.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The primary adverse effect of this work will be the development of tumours by some of the animals. The animals will be closely monitored by skilled staff in order to identify adverse effects as early as possible. The expected level of severity for all the experiments is either mild or moderate. The mice will generally undergo schedule 1 killing at the end of the procedures though for certain methods of tissue sampling or histological processing, some will be exsanguinated or killed by perfusion with fixative under terminal general anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use other models such as fruit flies, mammalian cells in culture and computer models as much as possible to aid our research. However, the complex physiology of a tumour means that in order to gauge the likely relevance of our work on human health, we need to use mouse models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will reduce our animal usage by careful use of statistics to limit experiment size and the number of mice in our colony. Wherever possible, we will use the mice we breed to produce different types of data. We will constantly update our experimental strategy

	based on our work in flies and cultured cells.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We have chosen to use mice for these studies for a number of reasons. They are ideal model organisms to investigate mammalian development and cancer - their biology is close enough to that of humans for our findings to be relevant to human disease. Mouse embryonic development and biology are well described, so we will be able to identify and characterise abnormalities easily. Also, the processes occurring during embryonic development and the emergence of cancer are very complex. There are many components (proteins, cells, and signals that pass between cells), which interact in ways that are poorly understood, and these interactions unfold over time. Currently, no <i>in vitro</i> system exists that is capable of accurately modelling these processes, therefore we need to study them in living animals. To further understand these processes in the adult mouse, we will make use of genetic tools to delete our genes of interest in a tissue specific manner overcoming the possible adverse effects of the constitutive deletion of genes.</p> <p>This strategy enables us to strategically target our cells of interest in different organs such as the skin or the liver, in order to study how the loss of our proteins affects cell growth mechanisms and tissue regeneration processes. To challenge the cells, we will use standardised protocols of fast recovery which aim to minimize any procedure-related trauma or infection thus avoiding animal distress.</p> <p>However, once we have some idea which kinds of processes are affected by loss of our proteins, we may be able to answer further questions using tissues or cells derived from the transgenic mice, rather than performing experiments on living animals.</p> <p>All our work will be performed by skilled personnel who will prioritise animal welfare. Animals will be monitored on a regular basis to ensure minimal harm.</p>

Project	The genetic and functional basis of proteinuria and kidney disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Kidney disease is often associated with abnormal leak of protein into the urine, due to failure of the kidney's function as a selective sieve for water and waste. This abnormal protein leak is a hallmark of diabetic kidney disease, the leading cause of end stage renal disease worldwide. It is also a feature of many autoimmune kidney diseases such as kidney involvement systemic lupus erythematosus (SLE). Kidney disease affects up to half of adults with SLE and is a major contributor to ill health and premature death in this disease.	

	<p>Proteinuria above 50mg/mmol (classified as severe) is found in up to 4/1000 individuals. Despite the major health burden of chronic kidney diseases, and the knowledge that the presence of proteinuria predicts kidney disease progression, there is a lack of specific treatments. Current therapies have not changed much in the last 20 years, with angiotensin axis inhibition used to slow progression, and non-specific immunosuppression used to limited effect for nephrotic syndrome. To address this area of unmet need there is an urgent need to develop more effective treatments, but to do this we need to understand more about how damage leads to protein leak and disease. The aim of the project is to identify novel causes of renal disease, and explore how these lead to disease at the level of cellular functions. Based on genetic observations in patients, we will develop and study new models, both in cell lines and animals with the same genetic defects as the patients. These models will help us to understand how disease develops in more common complex forms of kidney disease.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Within this project we hope to identify at least two novel mechanisms of action that improve our understanding of how injury or genetic variation lead to kidney failure. By highlighting important cellular pathways we hope to provide vital information to aid in the future development of more targeted, personalised diagnostics and treatment for kidney disorders. The targets for treatment that we identify may lead to the development of new therapies that are better tolerated because they are more specific and tailored to the drivers of disease. Ultimately more effective treatments will result in better outcomes for the patients, such as slower progression or prevention of renal damage. We also expect to provide a diagnosis to individual families with novel forms of rare kidney disease identified in our research.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>All our in vivo experiments will be performed in mice. We expect to use approximately 7,500 mice over 5 years.</p>
<p>In the context of what you propose</p>	<p>The majority of the experiments will involve</p>

<p>to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>breeding mice and these will be classified as either having mild severity (50%) or moderate if they have some evidence of renal impairment (50%). Mice with genetically or chemically induced kidney disease will be used to model kidney disease and protein leak in the urine, these mice may experience some lethargy or weight loss, these experiments will be classified as moderate severity. Following breeding 30% of the animals will undergo procedures such as blood sampling or injection of a substance, and these animals will be classed as having a moderate experience. However we are focussed on the early stages of chronic kidney disease, prior to the development of symptoms, rather than the later symptomatic stages or end stage kidney disease. Some animals will be socially isolated for short periods to collect urine samples.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We are particularly interested in how the filter system of the kidney clears toxins while preventing the loss of larger proteins. Cell cultures and non animal models cannot yet model the complexity of this filtration system, and other less sentient species such as Drosophila, Nematode worms and fish have a more primitive system that doesn't represent the architecture of the human kidney. Therefore mice provide a crucial model of human kidney disease, with a renal structure that faithfully mimics the details of human anatomy. However in this project we utilise <i>in vitro</i> modelling of candidate human disease variants in immortalised human podocytes, to generate preliminary data and select the most promising candidates for in vivo study.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use appropriate group sizes that are informative for a thorough statistical analysis and logical progression to the next step. We use careful randomisation, including blocking where appropriate and use of blinding to minimise bias and gain maximum useful information. Advice is sought from experts in statistics to support these aspects of the programme. We are very careful to reduce variation by keeping the mice</p>

	<p>together and ensuring that they are genetically identical. Several of the new approaches reduce the need for as much breeding as before, for example recent advances in gene editing allow us to replicate human genetic variants precisely in mice, without the need for crossing to additional strains and additional breeding.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The laboratory mouse is the species of choice for studying kidney disease and the least sentient model with high similarity to humans at the genetic and protein level. It is the ideal mammal for genetic studies where animals need to be generated rapidly. Using mice allows us to generate models that recapitulate human genetic disease. In the assessment of new animal lines we monitor closely for the early stages of renal impairment, prior to the onset of symptoms. By focusing on early stage disease and killing the animals before the onset of late or end stage renal failure we reduce suffering and maximise the scientific yield, by studying the initial, potential modifiable drivers of disease rather than non specific scarring and irreversible chronic damage. In some cases we will use inducible models which will not show any signs of disease until induced.</p> <p>Much of the experimental work will be done on tissues and cells taken when the animals are killed. We are careful to minimise the distress to animals from the administration of drugs or other substances. Strains are chosen that require lower doses of agents to minimise toxicity and reduce variability. Where possible drugs to induce renal disease are administered as a single intravenous dose rather than multiple dosing. If multiple doses are required we may use implants to reduce the number of procedures experienced by the animal. Analgesia and anaesthetics with aseptic technique to minimise infection are used. We monitor the animals daily and more frequently if necessary. On going assessments will include urinalysis, blood sampling for assessment of renal function and blood pressure monitoring. To minimise suffering, timed urine collections will be done over 3 hours rather than 24 hours, trialling a range of cage types to allow shelter</p>

	<p>and thermoregulation. Blood pressure monitoring will incorporate acclimatisation and habituation to reduce stress and ensure variability. The mice are co-housed and provided with enrichment material. When animals need to be housed singly for collection of timed urine samples we will limit the duration to 3 hours and use techniques that improve the environment.</p>
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Project	The genetic control of development in the life cycle of the parasitic nematode <i>Strongyloides</i> spp.	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Parasitic nematode worms are very common parasites of humans, domesticated and wild animals. We want to discover what genes parasitic nematodes use to grow in different ways outside of the host, because understanding this will help explain how nematodes evolved to become parasites, something that has happened repeatedly in their evolutionary history.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits of this work are to understand the basic, fundamental biology of parasitic nematode worms. In the longer term this fundamental knowledge could be used by others who are working to discover new ways to treat nematode infections of humans or animals.
What species and approximate numbers of animals do you expect to use over what period of time?	Adult rats; 2,000 over five years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	To do this work we infect the rats with nematode worms, which we do by giving the rats an injection. The rats do not get ill from the infection that we give them. The rats naturally become immune to this infection, so that the infection is lost in about a month. The work is of 'mild' severity. At the end of the work the animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Parasitic nematode worms' natural habitat is living inside of another animal. Parasitic nematodes cannot be maintained without using laboratory animals, and so this project cannot be done without the use of laboratory animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We reduce animal use by using the highest safe dose of worm infection in each rat, so that overall fewer rats are used. We also use genetically altered rats which keep their worm infections for longer. While there is some slight harm in these rats being genetically altered, by using genetically altered rats it means that overall fewer rats are used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms)	We use rats because they are the natural host species of the parasites we study. This means that we are studying a natural, evolved host – parasite association. Wild rats are naturally infected with these species of parasites. The rats become immune to the infections we give them. We give them infections at doses

to the animals.

which do not cause noticeable harm to them.

Project	The identity and function of sensory-motor networks underlying behaviour	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to gain a fundamental understanding of how the brain uses the information it senses in its environment to make decisions.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Learning how the mouse brain is able to coordinate incoming information and use that information to make decisions is an important step in knowing how any brain performs this function as these processes are evolutionarily conserved from flies to humans. In order to be able to treat neurological disorders we need to understand how the brain normally functions	

	<p>first. No single project is going to elucidate the function of the brain, but a scientific community-wide multi-faceted effort is underway to reveal the elegant processes that make brains work. For example if you look at a brain map you will see that different parts of the brain have been designated specific tasks e.g. vision, hearing, talking etc...but in reality multiple brain areas are required to see hear or talk. We need to know how these processes work to be able to treat abnormalities at the root cause in the future and not just to treat symptoms. At this stage we don't even know how information regarding simple decisions is integrated and used to inform behaviour. This project will contribute to this knowledge and any tools, methods, and data we collect will be shared and be of interest not just to neuroscientists but to scientists in many different areas of research.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This project anticipates using approximately 12,500 mice and 3000 rats all bred for research purposes over the course of 5 years. There are 4 research groups working under this project.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The project involves surgical procedures on mice and rats, which are bred for research purposes, in order to be able to monitor and manipulate brain function. We want to manipulate specific brain areas in order to affect the decision making an animal will make for example when presented with a sensory stimulus e.g. a sound, a flash of light, an odour, or a change in balance, which are not distressing. Animals recover from the surgeries remarkably well (usually in less than 2 hours they are eating, drinking and behaving normally) and only rarely are there complications. Any animals that are showing adverse effects will be humanely killed. Recording and imaging from the brain is not expected to cause distress and we monitor the animals' welfare using physiological parameters (e.g. weight, quality and presence of faeces and urine) and observing their appearance and behaviour to make sure they are in a healthy condition. In some experiments, the animals have restricted access to food and water to motivate them to perform tasks; however, they are carefully</p>

	monitored to ensure these restrictions do not affect their welfare. At the end of the experiment all animals are humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project aims to understand how neurons in the brain control decision making, which require studying the intact brain in a live animal. It is therefore impossible to avoid the use of animals for addressing these questions, as other approaches such as neuronal cell cultures do not replicate the connectivity structure of the brain, and preclude behavioural measurements. However, computer models will be employed throughout as a replacement for subsets of experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use several state-of-the-art methods simultaneously, together with sophisticated data analyses, to maximise the amount of data and information collected from each animal. In addition, the statistical power of each experiment will be increased by using longitudinal studies, where functional, anatomical and cellular data are collected from the same animal. Also, in most procedures the experiment and control can be performed in the same animal, which further increases statistical power and reduces the number of animals used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The experiments in this project will use mice and rats. The ability to use transgene technologies established for these species, allowing highly refined experimental design and detailed analysis of neuronal networks with molecular, synaptic, cellular and circuit level resolution. To minimise harmful effects, we will use techniques that the laboratory has performed and refined over the last 15 years. We have an experienced team of licensed animal technicians to ensure that the animals are well monitored and we have procedures in place to deal with a mouse showing any signs of distress quickly thereby minimising suffering. Manipulations will be performed by targeting small regions of the brain, with microinjections and molecular specificity, thereby minimising off-target effects.

	<p>Surgical procedures will be done with appropriate anaesthesia and analgesia. Experiments in awake animals will only be performed if the animals are stress-free and experience no visible discomfort.</p>
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Project	The long-term effects of prenatal hypoxia on cardiomyocyte function	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The overall objective is to assess the long-term effects of low oxygen levels (hypoxia) during development on mouse and rat heart function. This goal will be realized by addressing the following specific objectives in fetal, juvenile and adult mice and rats previously exposed to hypoxia during development. In addition, we will run control experiments on turtles which are naturally hypoxia tolerant, providing us with a model to identify adaptive vs. pathological responses</p> <p>1. To measure heart cell contractile force and</p>	

	<p>calcium regulation</p> <p>2. To assess mitochondrial function in heart cells</p> <p>3. To characterize gene expression and modification of key proteins involved in heart cell function</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The main benefit of the study is the advancement of current understanding of the cellular and molecular mechanisms underlying the developmental origin of cardiovascular disease. We hope to identify cellular targets for drug intervention to protect people from developing cardiovascular diseases later in life. All of the findings will be published in peer reviewed leading scientific and clinical journals as appropriate to ensure wide dissemination of the research findings. The information is of direct benefit to basic scientists, physiologists and clinical cardiologists and will provide key information enabling better management of cardiovascular disease.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Wildtype mice, approximately 110 animals over 5 years - rats, approximately 640 animals over 3 years; Juvenile snapping turtles, approximately 240 over 5 years - Juvenile and adult slider turtles, approximately 80 over 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Adverse effects in rodents: 1. Maternal reduced food intake, activity and weight (mice and rats) • Severity band, mild. • Dams exposed to hypoxic environments are known to exhibit a decrease in food intake (up to 40%) and a substantial decrease in physical activity, leading to a decrease (~20%) in maternal body weight. 2. Maternal preeclampsia-like symptoms (mice) • Severity band, moderate. • Hypoxia during pregnancy can cause maternal preeclampsia-like symptoms such as hypertension, proteinuria and kidney pathology. 3. Intrauterine growth retardation (IUGR) and physiological and morphological defects associated with prenatal hypoxia (mice and rats) • Severity band: Moderate. • Prenatal hypoxia causes IUGR and a host of physiological and morphological defects, some of which persist</p>

	<p>into adulthood. 4. Disease susceptibility in offspring (mice and rats) • Severity band: Moderate. • Although we are not specifically inducing this, it is possible that offspring exposed to prenatal hypoxia will experience disease susceptibility in association with aging (i.e. cardiovascular diseases, such as heart failure) later in life. Adverse effects in turtles: 1) Loss of body weight. • Severity band: Mild. • Likelihood: Very unlikely because we are well-within the temperature and anoxia tolerance limits of turtles. 2) Reperfusion injury. • Severity band: Mild. • Likelihood: Very unlikely because we are well-within the temperature and anoxia tolerance limits of turtles. 3. Retained "consciousness" following decapitation. • Severity band: moderate. • We acknowledge that the CNS of reptiles is tolerant to hypoxia and hypotensive conditions so it cannot be assumed that decapitation causes rapid loss of consciousness. All animals will be sacrificed at the end of the protocols or transported to other collaborators' projects with authority to use animals exposed to environmental challenges (e.g. hypoxia, nutritional manipulation, endocrine disturbance) as a fetus</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p><i>Cell lines and culture:</i></p> <p>Adult cardiac myocytes are terminally differentiated and cannot be maintained in tissue culture conditions. There are no suitable cell lines that can be used to fill these purposes. Moreover, we will be studying the long-term effects of prenatal hypoxia on cardiomyocyte function, which cannot be reproduced using cell culture techniques nor can they be suitably modelled using computer simulations given the lack of understanding of the fundamental processes.</p> <p><i>Human volunteers</i></p> <p>Human tissue is; i) of limited availability, ii) rarely not already diseased and iii) nearly always subject to pharmacological interventions.</p> <p><i>Alternative species</i></p>

	<p>Since we wish our findings to be clinically relevant and translational to human diseases of the heart, the use of other less sentient species, such as lower vertebrates (reptiles, fish and amphibians), is not appropriate for the main study animal as the structure and function of lower vertebrate hearts differ significantly from mammalian hearts and mammalian hearts are known to be significantly more sensitive to hypoxia than lower vertebrates. Nevertheless, we have utilised a lower vertebrate model, the turtle, as a control species that naturally experiences developmental hypoxia to compare to the mouse findings and separate adaptive vs. pathological responses.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Experimental design has been discussed with, and approved by, our statistical advisor. In order to minimise the number of animals required, sample size has been estimated for each experiment based on existing published data and the use of power analysis (desired power of 0.8, $\alpha = 0.05$). These estimates will be updated and recalculated throughout the project as we generate new data.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Experiments concerning mice and rats:</p> <p>We are committed to using the most translationally relevant model. We have chosen the mouse and rat as our main experimental species for several important reasons:</p> <ol style="list-style-type: none"> 4. Mice and rats have a short generation time and an accelerated lifespan (2 years) which allows the long-term effects of prenatal hypoxia to be studied within a reasonable timeframe. 5. Our ability to directly manipulate the mouse and rat genome provides an incredibly powerful tool to identify and confirm molecular targets for drug intervention. 6. Due to their small size and short generation time, maintaining mice and rats requires less resources and space, and the time required to perform research is

	<p>manageable.</p> <p>7. Rats and mice have large litter sizes which allows the generation of multiple, identically reared progeny</p> <p>Steps to minimise welfare costs to animals:</p> <p>1. Basic requirements for good rodent housing and husbandry will used at all times.</p> <p>2. It is not possible to house pregnant mice and rats in groups, but once pups have been weaned, mice and rats will be housed in stable, compatible groups.</p> <p>3. The following parameters will be measured during the protocol to ensure animals remain within the outlined severity limits: Body weight, body condition scoring (BCS), food and water intake and cardiovascular status. Control animals not subjected to any procedures will be used as a benchmark for normal changes in these parameters.</p> <p>4. Oxygen levels will never be reduced lower than 9% in mice and 13% in rats</p> <p>5. When mice are first put into the chamber, oxygen levels will be normal (21%) for 24 hours and then reduced slowly (over another 24 hour period) to avoid shock.</p> <p>Experiments concerning turtles;</p> <p>1. Protocol 2 and 3: Basic requirements for good reptile housing will be used throughout the procedure</p> <p>2. Protocol 2: Temperature acclimation and hypoxia/anoxia incubation levels and durations are well-within the tolerance limits for turtles</p> <p>3. Protocol 3: The head of chelonians can be exposed and extended by turning the turtle upside down allowing placement in guillotine with minimal handling</p> <p>4. Protocol 3: The head will be pithed to</p>
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	abolish brain activity
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Project	The Metabolism and Kinetics of Pharmaceuticals and Chemicals	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The objective of this project is to undertake non-clinical pharmacokinetic (how much a drug is in the blood, and for how long) and metabolism (how chemicals/drugs are broken down) studies and supporting validation/investigative studies in rodent species (rats, mice, guinea-pigs and hamsters) and non-rodent species, (dogs and rabbits) to enable and support pre-clinical and clinical safety testing programmes.</p> <p>The data will be used to review substances under development or satisfy governmental requirements necessary for approval of clinical trials (dosing in</p>	

	<p>humans or animals) or bringing products to market.</p> <p>The types of materials we will investigate include pharmaceuticals, chemicals, agrochemicals or food additives/substances, to facilitate a review of substances under development or satisfy a regulatory requirement.</p> <p>Studies are designed to determine specific metabolic or pharmacokinetic endpoints, including how and how quickly a compound is absorbed into the system, broken down by the liver and other enzymes, distributed around the body, and excreted in the urine and faeces, for example. Some studies will simply require blood sampling to measure drug concentration over time, and some will look at how the concentration of the drug in the blood matches the level of a specific biomarker (such as a type of cell).</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The public expects that substances we are exposed to are safe or their hazards are well understood. The main benefit of this project is the provision of high quality data that will allow both scientists and regulators to decide whether a test material is safe, or has the desired properties to make a drug in humans. This may mean the discovery of better drugs, with better pharmacokinetic and metabolic profiles, which will be available to treat a wide range of diseases which may or may not be life threatening. It may also mean better plant protection products that are less harmful to the environment or wildlife. Similarly work under this Licence will also show which compounds are not suitable to move forward into patients due to them not having the desired metabolic and pharmacokinetic profiles, or they are not safe to go into the environment or humans, for example.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The species and anticipated usage over the lifetime of the Licence (5 years) are below: Rat: 22000 Mouse: 9400 Dog: 1410 Rabbit: 980 Hamster: 380 Guinea pig: 300 However, it is unlikely that these numbers will all be reached in some or any of the species listed as these are highest-case estimates based upon potential</p>

	future needs.
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Most animals will undergo procedures that we would describe as mild. This may include dosing with test materials (as described earlier) by a variety of different routes. Most routes would involve one or more injections (a bit like when a patient receives a flu vaccination), but may involve confinement in tubes for dosing by inhalation (breathing). In studies requiring prolonged inhalation dosing, we would get our animals used to the tubes, so they know what to expect when the actual study starts.</p> <p>Most investigations will require collections of blood (like a blood sample you would give at a doctors) on more than one occasion during a study, or we would collect other bodily fluids (like urine, faeces, milk) which may involve the animals being confined to special cages on their own, so their excreta isn't mixed up. However, this would be for the shortest time possible to allow us to get the data we need (as we would always do) to minimise any suffering. Studies vary in duration from a single dose to daily dosing for up to 13 weeks, depending on the intended use or likely exposure to each substance under study. Additionally where multiple compounds need to be tested, each with a similar mode of action or therapeutic theme, these maybe administered in a series of experiments within a single study lasting up to 2 years. Most dosing periods, will however, be short term ones (< 4 weeks).</p> <p>In some studies, animals need to undergo surgery for specific measurements to be made. They would receive the same level of treatment as a patient would having an operation in hospital. Pain relief and antibiotics would be administered under the supervision of a vet. They would be carefully watched until they had recovered. If it was clear that they were not going to recover due to complications, they would be humanely killed as soon as possible. Most animals will be expected to recover from surgery and undergo their procedures. Where possible we will carry out study procedures under anaesthesia were the animals won't be allowed to recover consciousness after being anaesthetised. This will occur when we need to collect specific organs and tissues, and will</p>

	<p>mean the animals suffer little or no pain. Most animals will be humanely killed at the end of procedures. However, when we can, and where the study type allows it, (such as blood sampling for pharmacokinetics or metabolic assessment) we would try and re-use animals (particularly dogs) in future studies. This would need a vet to see the animal to check it was fit and healthy for re-use, before any further procedures are carried out.</p> <p>If an animal is suitable for rehoming as a companion animal, after being considered suitable by a Vet, this may be allowed.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Presently, there are no validated alternatives that are scientifically, ethically or legally acceptable to replace pharmacokinetic and metabolism studies in whole living organisms outlined in this project.</p> <p>For meaningful and reliable evaluation of compound disposition, the target physiological systems must be intact, with a complete nervous system and hormonal control of bodily function any responses. Test tube systems in isolation are helpful predictors of function (such as absorption through a cell layer is used as a screen) but they remain inadequate for comprehensive determination of ADME data. Although non-animal (lab bench or computer based) studies can provide useful supporting data, meaningful and reliable evaluation of whole body exposure and distribution of a compound within the body, where it is converted in the body and to what, and how quickly its passage and/or conversion through the body occurs (metabolic disposition and pharmacokinetics /dynamics) can only be comprehensively achieved in studies using intact animals where all the organs and systems are intact, interacting with each other (for examples, using the nervous system and hormonal control of bodily function) and interacting naturally with the compound, yielding a naturally complex interdependent system. For this reason, Test tube systems in isolation are helpful predictors of function (such as absorption through a cell layer is used as a screen) in isolation remain inadequate</p>

	alone.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>All experiments will be designed in order to achieve the scientific objectives using the minimum numbers of animals. For study types that are less well established and for which historical data may not be available, the literature (scientific publications) will normally be consulted to help decide the group size. We can also consult with our statisticians, particularly where the study type is not routine and/or complex, as they can use calculations to estimate the correct number of animals needed to get a meaningful result.</p> <p>Where possible, common control groups are used in order to minimise the numbers of animals used, or control animals are not used at all, where the doing so would not benefit the study design.</p> <p>The re-use of animals, (see above) under carefully controlled conditions, under the supervision of a vet, also means the overall number of animals we use is reduced.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Regulatory evaluation of drugs, agrochemicals or chemicals generally requires investigation in two relevant mammalian species; usually one rodent and one non-rodent. This project predominately uses rats, mice and rarely hamsters or guinea pig as rodent test species.</p> <p>For the non-rodent species, rabbits and dogs are used in this project. Selection of which is most suitable is considered according to the physical, physiological and behavioural requirements of the study, biochemical or metabolic similarities with target species (such as man), similarities of action and response to the compound of study, temperament and robustness in response to blood sampling.</p> <p>The rabbit shares many characteristics with rodents but are considered a non-rodent choice when it comes to selection of a second test species to meet regulatory requirements. Where the rabbit is not appropriate, dogs are selected.</p> <p>All animals are monitored for signs of any adverse effects on their health or wellbeing, and to prevent</p>

	<p>unnecessary suffering, early humane end-points are applied under appropriate veterinary guidance (such as modification/withdrawal of treatment with the test substance, provision of palliative or therapeutic treatments, or humane killing of affected animals).</p> <p>Highly trained staff use a rigid framework of welfare assessment to allow early detection of animals showing signs of discomfort or distress. We use pain relief as standard with anaesthesia and after procedures where relevant such as surgically implanted models. We sometimes, although rarely use animals that have had their genetic material altered such as such that they are predisposed to developing a condition or if a particular gene is important say in a specific component of the ADME process in which we have interest in.</p> <p>Good Surgical Practice, including aseptic technique (LASA 2017 Guidelines) will be observed for any animal undergoing surgical procedures, as well as the provision of pain relief and antibiotics, when needed. Environmental enrichments appropriate to the species are used within the animal facilities, such as play areas/toys, chew-items etc and except where the scientific objectives of a procedure or animal welfare considerations prevent it, all animals are group housed, so that they can interact and behave socially wherever possible.</p> <p>Study designs are reviewed, and new methods considered as technology best practice and standards improve and advances become adopted and approved by international regulatory agencies.</p> <p>Wherever possible, experimental samples are collected under anaesthesia or post mortem to minimise any potential suffering. In some circumstances safety markers will also be collected from the animals maximizing the data from individual studies. Maximising data decreases use of further animals and collecting samples post mortem or from terminally anaesthetised animals, minimises suffering.</p>
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Project	The nature and control of cortical activity	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<ul style="list-style-type: none"> ● We will study how epileptic seizures develop and what effects these have on the brain. Epilepsy is one of the most serious neurological conditions, and many people (estimated at 200,000 in the UK alone) do not respond to currently available treatments. ● There are many things we still do not understand about epilepsy: we do not know how seizures start or how they spontaneously end (which most seizures do, naturally). ● We also have only limited understanding 	

	<p>of how seizures affect normal brain function between times, which is highly relevant to why epilepsy is commonly associated with other brain disorders.</p> <ul style="list-style-type: none"> • We will apply new technologies for manipulating brain activity, to attempt to modify or even stop seizures. • The most important of these technologies is optogenetics, which involves getting neurons to make special proteins that can be activated by light. These are normally only found in certain kinds of bacteria and algae, but when they are introduced into neurons, it allows for very precise experimental control over neuronal behaviour. It is possible to activate or inhibit a single cell, or millions simultaneously, just using light. • Our ultimate aim is to develop this new technology for use in humans, as a brand new way to treat epilepsy.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<ul style="list-style-type: none"> • We anticipate advances in our understanding of the brain's own natural protective mechanisms, which prevent seizures from starting or spreading, and how seizures usually stop by themselves. • Our studies will also help diagnosis and management of epilepsy, by indicating new ways to identify and localise the source of seizures in the brain. This is particularly important for severe cases that may be treated surgically. • Our work will also extend our expertise in recording and manipulating brain activity using new technologies such as optogenetics. These new technologies offer powerful and sophisticated new surgical approaches for treating neurological conditions.
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Up to 4000 mice, and 300 rats over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the</p>	<ul style="list-style-type: none"> • Some animals will have epilepsy (genetic epilepsy, or induced experimentally, by

<p>expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>administering certain “epileptogenic” drugs). Once an animal starts to experience seizures, they are defined as being “epileptic” and can then be studied for our research. We do not require that animals are epileptic for long periods for our studies, and all epileptic animals will be used within the first 4 months after developing the condition. • Most animals will only have a small number of seizures that affect a small part of their brain (moderate). These often are manifest only as a subtle pause in the animal’s behaviour, and may be obvious only by recording nerve activity with electrodes in the brain. This is not painful because the brain itself has no pain receptors, and indeed similar recordings are done on human patients. • Other animals will have seizures that spread to the motor parts of the brain and will manifest as motor seizures (behavioural twitches; moderate to severe). • Seizures, by analogy with human reports, are not considered to be painful, but if very frequent, may impact on the animal welfare in other ways. We will conduct behavioural tests while recording from the brain, to investigate how seizures affect brain activity both during the seizure itself, and afterwards (“interictal” periods). Animals will be monitored for evidence of marked deterioration in health arising from their epilepsy • The majority of animals will have no neurological phenotype. Some will be carrying genes whose function is ordinarily latent, but which allow the nervous system to be manipulated (e.g. optogenetic genes). These are rated as “mild”. • Some animals will have genes introduced into the brain, by injecting DNA, packaged either in viral vectors, or as plasmids. Our investigations involve recording or imaging brain activity, and using newly developed tools to manipulate brain activity. These recordings will be one of the following: periods of awake behaving recording using wire-less EEG or tethered systems (moderate); imaging experiments of awake, but head-restrained animals (moderate); terminal recordings under anaesthesia; humane killing followed by brain slice recordings (in vitro studies). • We will record the brain activity of some animals under terminal anaesthesia (“mild”). • Some animals will be trained to tolerate head restraint,</p>
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	to facilitate imaging / electrophysiological recordings (“moderate” severity) while they are awake. This will help us understand the nature of naturally occurring seizures, in an intact, non-sedated brain, the way that seizures occur in humans. • Other animals will be killed humanely for preparation of brain slices for electrophysiology or anatomical studies (“mild” severity).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Epileptic seizures arise from the combined activity of large populations of neurons and the pattern of activity depends on how these neurons are connected. It is critical therefore to study this activity in its natural environment, in an intact brain. This information can be supplemented by studies of neuronal cultures, computer simulations and of human recordings, and we will use all these other methodologies wherever possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Many of our studies will examine brain activity in the whole animal, recorded either continuously over an extended period of time (EEG), or in repeated recording sessions after training an animal to sit under a special microscope. We will further optimise animal use by preparing post mortem brain slices, relating our findings back to the whole animal recordings in each individual case. Data obtained from these experiments will be used to develop computer models of seizures, which will help to design future experiments, and simulate treatment regimes.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to	<ul style="list-style-type: none"> • We will primarily use mice, because they allow simple genetic manipulations, either to create genetic models of epilepsy, or to introduce genes which allow experimental control of neurons (eg. optogenetics). • Genetic models are a highly refined way of reproducing human conditions, in

<p>the animals.</p>	<p>instances where a particular mutation has been shown to be associated with epilepsy.</p> <ul style="list-style-type: none">● Other studies will involve experimentally induced epilepsy, using two models which have been widely used in the epilepsy research community, and are considered to be refined models of epilepsy in that they reliably result in regular seizures, without excessive mortality or reduced life quality.● Finally, in other studies, we will study seizure-like activity induced in brain slices. In these experiments, suffering is minimised because epileptic activity is induced only after the animal has been humanely killed.
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Project	The neural basis of complex cognition
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	Regulatory use and routine production
	Protection of the natural environment in the interests of the health or welfare of humans or animals
	Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The overall aim is to identify how chemicals in different areas of the brain work together to generate the thoughts and behaviours that are commonly referred to as 'executive functions'. These are functions such as planning, goal-directed thinking, attention, expectation and anticipation. These functions are compromised to varying degrees in many neurological and psychiatric disorders as well as in the course of normal and pathological aging. Rats also have executive functions, albeit not as well developed as in humans, but there are many similarities. By testing rats, we can learn about the similarities and differences in the brains of different animals, and this will improve our understanding of the impact of</p>

	human diseases and aging on these functions.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	An expectation is that short-term benefits will arise from improvement(s) of pre-clinical models, with validation by cognitive assessment – allowing reduction of the numbers of rodents used, and possible replacement with lower order animals such as fish or even insects. We work closely with scientists in drug companies with the expectation that we can improve research techniques for preclinical testing of new drugs. We hope that this in turn might enable the medium-term benefit of progression of a new drug to clinical trials for the treatment of psychiatric illness, or at least in the capacity to increase the speeds at which novel compounds' efficacy can be established. A resulting long-term benefit might then be the establishment of a novel treatment for one or more human psychiatric illnesses.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year project, we will use up to 500 rats, bred for the purpose.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	A typical experiment involves measuring the behaviour of rats as they perform a particular tasks, which might be spontaneous behaviour (such as foraging for food in a maze or arena) or trained behaviour (such as pressing a lever for food). We measure changes in the animals' behaviour as a result of interventions, such as permanently, or temporarily, reducing activity of different brain circuits (for example, using brain lesions, neurochemical depletion, or drugs), which mimic the effects of clinical pathology, or using drugs to temporarily increase activity in some brain areas to mimic of clinical treatment. Some of these interventions (e.g., those involving surgery) are up to a 'moderate' level of severity, assumed to cause transient pain or distress. As for human surgery, anaesthetics (general and local) and painkillers are administered to reduce post-operative pain. During behavioural testing, the effects of procedures are sub-threshold or, at most, mild. We limit access to food prior to testing so that the rat is

	<p>sufficiently motivated to perform a task to get food treats, but they are maintained at a healthy weight and always fed a normal quantity of food daily regardless of whether they perform a task for food. At the conclusion of testing, the animals are humanely killed and their brain tissue may be taken for analysis post-mortem.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We are interested in the brain processes underlying behaviour. It is not possible to study behaviour in anything other than an awake behaving animal, which includes humans. However, we cannot investigate the brain processes underlying behaviour in humans because it is not possible to systematically manipulate brain function in the same controlled manner that is possible in other animals.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>To ensure the minimum number of animals we try to obtain as much behavioural data from one animal as possible, for example, by testing them multiple times in the same task to improve confidence in the accuracy of measurements, and under multiple conditions (for example, before manipulations ('baseline') and after, to measure change in behaviour as a result of a manipulation. Because the behaviours we use are initiated by the animal and 'self-paced', we can use 'rate of work' to indicate effort and willing. This provides an important 'check' on welfare: the animal stops when it wants to. Although we use food reward to motivate the animals, the rewards are 'treats' (e.g., sucrose pellets or cereal pieces) and it is not necessary to deprive the animal of food to make them work. We control access to laboratory chow by feeding them after testing, so that they are hungry but never starving. We collaborate with a statistics advisor who offers support and advice in design and analysis and supports continual professional development, particularly in statistics. We are currently working with him to develop a novel approach to our data analysis using Bayesian inference. This will enable us</p>

	<p>to gain more information from the data, so potentially increasing statistical power and enabling a reduction in numbers.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rats are the most suitable animals for this project because they are inquisitive and learn readily. Because there are brain circuits that have been conserved during evolution, the differences, as well as the similarities, between animals (e.g., humans and rats) provides important information about how behaviour is organised in the brains of different species and how this gives rise to different, species-typical, behaviour.</p> <p>Our objectives - which rely on being able to measure behaviour - can only be achieved by minimising animal suffering as the rat will not perform the behavioural testing if it is overly anxious or in distress.</p> <p>In humans, psychiatric symptoms (for example, hallucinations or depressed state) are themselves distressing. It is not possible to know the experience of a rat with perturbation of the systems presumed to underlie psychiatric symptoms in humans. However, it is not our intention to 'model' the entirety of the psychiatric syndrome and it is unlikely that this would be possible anyway. By addressing individual symptoms or symptom clusters, rather than modelling all aspects of the psychiatric syndrome, we try to minimise the severity experienced by an individual rat.</p>

Project	The neurobiological mechanisms of pain	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Everyone experiences pain following an injury, however this is normally short lasting and protects you from further damage while the tissue repairs. There is evidence that in some diseases the pain people experience is not protective and is part of the disease. This pain doesn't serve a protective purpose and stops peoples' normal everyday life. We know that the ways in which pain is generated and detected and processed are different in different types of disease and that the treatment of these types of pain requires different drugs to the ones we take everyday for a minor acute pain. In some diseases such as arthritis people start with acute short lived, intermittent pain form the joint and overtime this becomes more regular,</p>	

	<p>more intense and more debilitating pain. This change is not just because the disease in the joint is worsening, we need to understand why this happens so that new treatments can be developed. We aim to identify the mechanisms occurring at a sub-cellular, cellular and tissue level that lead to chronic pain. We will achieve this by studying models of major clinical chronic pain problems in society, these are inflammatory pain, neuropathic pain and musculoskeletal pain.</p> <p>There is increasing evidence that people experience of pain is shaped by both events that take place in early life and interactions with anxiety. For example, newly-born term and pre-term babies are exposed to a number of tests which will activate the pain pathways, it is now clear that these events may influence the experience of pain in adulthood. We will investigate how pain in early life effects the way the nerves, spinal cord and brain responses to pain in adulthood. It is clear from clinical data that mental health, in particular anxiety, modulates responses to acute and chronic pain. Why people with higher anxiety experience greater chronic pain is an important question which can be studied using animal models. At the same time our work under this authority will be complemented by studies in clinical samples and populations as well as cell based approaches to the study of pain which we and others use.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Chronic pain is a major worldwide clinical problem that impacts hundreds of millions of people every year. Pain arises following trauma and disease or as a consequence of medical interventions like surgery or as a side effect of drug treatments. Acute (short lived) pain is a necessary survival mechanism alerting us to tissue damage, however chronic pain outlasts any tissue damage and has no beneficial purpose. Pain can negatively impact the lives of anyone regardless of their age or sex. Chronic pain states are more commonly seen in older people (in diseases such as osteoarthritis) however it is also seen in the youngest children born prematurely. This research will advance understanding of how early life pain changes the way the central nervous system matures, and why this alters pain responses for the rest of an individual's life. This new information will allow us</p>

	<p>to identify new ways to prevent these changes and hopefully prevent altered pain responses in adulthood. In later life, pain can arise following inflammation, injury to sensory nerves (neuropathic pain) or from diseases of later life such as osteoarthritis (OA). In terms of the mechanisms leading to these conditions, understanding what is similar and what is different between these different types of chronic pain will allow us to identify new ways to treat these chronic pain states. People in chronic pain are more likely to suffer from anxiety and other mental health problems such as depression. The mechanisms by which pre-existing anxiety can exacerbate chronic pain are poorly understood, understanding how these interactions occur will enable the future development of new treatments. The research in this application will shed new light on these questions and directly influence clinical research.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use rats (Sprague-Dawley, Wistar and Wistar-Kyoto strains) and some genetically modified and wild-type mice. This will be over the entire course of the licence period. We have calculated a MAXIMUM of 9200 rats and 2500 mice will be used.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We will use models of inflammatory, arthritis and neuropathic pain. Adverse effects of these models vary; the models of inflammation cause some tissue swelling at the site of injection and this doesn't spread to another site. The inflammation lasts for a few days and cause some changes in movement, but this won't alter the animals' ability to access water or food. The animals have small but biologically important changes in their pain responses to a fixed stimulus applied to the site of inflammation. The models of arthritis and neuropathic pain involve either a short surgical procedure under anaesthesia or an injection of a substance that causes damage to cells that lead to the injury of the joint or the sensory nerves. The effects of these models last longer (weeks to months) than the models of inflammation, they also lead to changes in pain responses to fixed stimulus applied to the site of injury. These models may cause some short-lived reductions in weight gain and some reduction in mobility</p>

	<p>following the model induction. Between a third, to one half, of the animals used will be the controls for the surgery or treatment to induce the model, and so the adverse effects will be less in these animals. Some of the animals receive drug treatments aimed to reduce the pain, which will also reduce the burden of severity. The maximum severity of our work will not exceed moderate. At the end of all studies animals will be humanely killed.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Pain arises due to complex interactions between cells in many different parts of the body including the peripheral and central nervous systems, the immune system, circulation and endocrine systems. These cannot be replicated in vitro. The central nervous system in particular is exceptionally complex and something that cannot be replicated in vitro, similarly in silico approaches rely on obtaining large datasets from in vivo studies before they are useful. Laboratory rodents are least sentient species in which these studies are able to be performed. They are vertebrates, like humans, and share the basic anatomical and physiological responses to pain that are seen clinically in man. Pain relies on the integration of nocuous information into complex spinal and brain systems which are not present in invertebrates.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Our previous experience of performing these types of studies in the field of pain research have provided us with valuable information which can be used to perform calculations during the design of research studies so as to ensure the minimum number of animals are used in a study. All studies have to be designed and planned well in advance by each experimenter and a written plan with appropriate power calculations presented for inspection by the licence holder and/or deputy. We are therefore confident that with this step included in our procedure we can minimise animal use.</p>
<p>3. Refinement</p> <p>Explain the choice of species and</p>	<p>To ensure clinical relevance of our studies it is important that these experiments are performed on mammalian species. Rodents have become</p>

<p>why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>the ideal choice for pre-clinical research as they have proven to be reliable models of humans in many aspects. As rats and mice have been so often used their anatomy, physiology, and welfare are extremely well understood. This facilitates and expedites research that can be done into the understanding of pain and pharmacology, all whilst being able to ensure that animal welfare is conserved. Our research aims to build upon pre-clinical work performed over the last century and so the majority of models and tests we plan to use have been extensively developed, refined, and validated. We aim to use a range of models that reveal unique insights into neurological mechanisms whilst maintaining animal welfare as much as possible. The durations of the pain models vary, in part due to the duration over which individual mechanisms act, and in part due to the nature of the injury. The shortest models are the inflammatory models which usually resolve within a week, whilst the longest are the arthritic models which we have previously studied for durations approaching 30 weeks. In all cases the length of the study will be determined based on what the particular aim of the study is; however, some models will be limited in duration to ensure animal welfare is upheld.</p> <p>We will minimise unwanted pain responses due to potential irritation following repeated dosing of drugs, or post-operative pain due to a surgical procedure. This is achieved through the limited use of repeated injections into the same site and the use of local anaesthetic cream (EMLA) at the sites of an incision for example for the generation of models of OA or neuropathic pain. In cases where a recovery surgery is performed prior to the induction of a pain model, systemic analgesics will be given to minimise any pain. In all cases we will attempt to minimise suffering by ensuring that upon completion of surgery animals are placed in a recovery cage or their home cage and will be provided with bedding, warmth, and mash to ensure that they are comfortable and that their environment is enriched. Experimenters are closely observing the animals to ensure they are recovering appropriately.</p> <p>The models of pain used are associated with changes in thresholds to painful stimuli which are measurable when stimuli are applied to freely-</p>
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behaving animals. These are the same tests as used clinically in people with chronic pain. They provide use with very useful data whilst causing minimal distress. If performed at a high frequency, however, the tests may contribute substantially to the cumulative distress of the animal. To minimise this risk frequency limitations have been laid out for each test.

The other behavioural tests are also well refined and used in many labs, these tests measure activity or weight distribution on the limbs, they are not expected to cause any harm or stress even when repeated often no limitations are necessary for the frequency of testing. With measures of anxiety we have selected tests that do not induce anxiety themselves and thus ensure welfare is maintained. Again, limitations to frequency will be adhered to in order to ensure animal welfare is not compromised.

We have incorporate best practices for ensuring the welfare of neonatal animals is maintained for a broad range of pain models. In each of the pain models the refinements that have been made for the adults will be incorporated alongside appropriate adjustments for volumes administered. Furthermore, when appropriate we aim to make specific changes that may benefit the younger animals, for example inflammatory substances will be administered to the dorsum of the hindpaw to minimise the impact that the pain model has on the pups ability to develop motor skills and compete for food.

Project	The Neuropsychopathology of Trypanosomiasis Infection	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The parasite <i>Trypanosoma brucei</i> is transmitted by the bite of the Tsetse fly and causes the fatal disease African sleeping sickness in humans and the related wasting disease Nagana in cattle. The human disease has two stages; during the first stage the parasite multiplies in the blood causing inflammation and fever, and in the second stage it invades the brain causing psychotic symptoms and perturbation of the sleep-wake cycle, followed by coma and death. Even where treatment is successful, many patients cured of the second stage experience continued sleep disturbance, difficulty walking and/or psychotic symptoms.</p> <p>This project aims to explore the origin of the psychotic symptoms and perturbation of the sleep-wake cycle in the second stage infection, and to link them to the</p>	

	biochemistry of the parasite.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will lead to better understanding of the host-parasite interaction, and the mechanism that results in the observed clinical symptoms. Understanding the origin of the clinical symptoms will help underpin the development of therapeutic interventions to alleviate these symptoms, and may lead to better fundamental understanding of brain function.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 225 mice over a five year period to enable us to detect changes in the symptoms of infection with genetically modified parasites. We use mice as there is an established mouse model of stage two African sleeping sickness, and mice are the lowest sentient species suitable for behavioral studies.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mice will be anaesthetised and injected with <i>Trypanosoma brucei</i> , and the progression of the disease monitored by taking small amounts of blood to observe and count the parasites. The activity of the animals will be observed and they will periodically perform behavioral tests. Some animals may show signs of illness during the later stages of the experiment such as poor coat condition, hunching or reduced activity and altered behavior. All animals will be humanely sacrificed if effects such as shivering or complete inactivity are observed. It is possible that some animals will die as a result of the infection, but daily monitoring of animals showing any symptoms is used to reduce this possibility. Some animals will be injected with tracer substances to allow brain imaging. At the end of all experiment the animals will be humanely sacrificed, typically under anaesthetic. Post-Mortem examination of tissues and fluids will be conducted.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have to use animal experiments to find out how the parasite interacts with its mammalian host, where the parasite goes in the brain, and the effect this has on the host's behaviour. This information cannot be obtained from cell culture or by using non-protected animal alternatives, as only the animal host is capable of displaying the changes in behaviour that are

	characteristic of the clinical infection in humans.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We use the minimum number of animals required by monitoring the behavior of each animal in several different ways, and use only enough animals to gain significant results. We make use of sensitive analytical techniques to minimise the amount of animal material required for analysis.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use the mouse model because it is are predictable, well established and have made possible much of the work already done on trypanosomiasis. Mice are the lowest sentient species suitable for behavioural studies. Whenever possible, we use tissue culture-derived parasites in our experiments and only transfer to animal work when it is needed to find out how the parasite interacts with its host.</p> <p>We minimise the suffering caused to the experimental animals by administering anaesthetic prior to injections. We monitor animals daily, and more often in the stages of infection when clinical signs are likely, and animals showing distress are humanely killed to prevent suffering.</p>

Project	The organisation, function and plasticity of sensory brain circuits	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The purpose of this project is to investigate how sensory information is processed by local and long-range circuits in the brain and how this information is stored and converted into behaviour. To this end we will study the organisation, function and plasticity of neuronal interactions involved in sensation and perception, focusing on vision as a model system.	
What are the potential benefits likely to derive from this project (how science could be advanced or	The proposed research will contribute to the better understanding of the brain's function and how our sensory perception arises from	

<p>humans or animals could benefit from the project)?</p>	<p>neuronal interactions, which is still unclear. Gaining knowledge about these processes in the healthy brain is an essential prerequisite for understanding what goes wrong in the diseased brain e.g. in autism, schizophrenia or dementia and for the development of novel treatments. Moreover, during the project we will develop new tools for data acquisition and analysis, these and the data will be made freely available and will be of interest to scientists in many different disciplines (neuroscience, mathematics, clinicians, AI and machine learning, psychology).</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The estimated numbers of mice to be used are 10,000 over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In preparation for the experimental procedures in this project, mice will undergo surgery under deep anaesthesia to provide access to the brain and to implant recording devices, fixed externally to their skull. Adverse effects after surgery may reach moderate severity levels for a short period of time, but all animals receive pain relief and are closely monitored until they recover completely. In some of the experimental steps the animals will be head-restrained in order to record brain activity and might initially experience stress from the head-restraint. But the mice will be allowed to slowly get used to the experimental conditions such that stress and discomfort will be minimized. The majority of the procedures undertaken in this project involve studying voluntary behaviour in which the animals are expected to experience no or minor adverse effects. At the end of experiments, or if mice show signs of ill health, distress or suffering that are not improved or resolved within a timeframe approved by the veterinary surgeon they will be humanely killed. The brains might be removed for further study.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-</p>	<p>The project involves the study of dynamic properties of neurons, neuronal networks and their behavioural output in response to sensory stimulation. Studying live brain tissue is</p>

<p>animal alternatives</p>	<p>therefore essential for this fundamental biological research. As explained below, new imaging and data collection approaches are already enabling us to obtain more data from individual animals. As we learn more about neurons and synapses under investigation, we will be able to use mathematical modelling more extensively but for such approaches to be useful they will need to be tightly constrained with biological measurements. Therefore, the use of animals is unavoidable for the important scientific questions we would like to address. However, computer modelling will be an integral part of this research work</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We use sophisticated data analyses to extract the maximal amount of information from a particular experiment. Novel methods will allow us to maximise the amount of data collected from each animal and to increase statistical power, by for instance recording from hundreds of neurons simultaneously, by longitudinal studies in the same animal and by being able to collect functional, anatomical and molecular data from the same animal.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Experiments will be limited to mice. The mouse visual system is similar to the human visual system, allowing us to address many fundamental issues of function and dysfunction in the visual cortex without having to make use of higher mammals. Importantly, reliable transgene technologies are established for this species allowing detailed analysis of neuronal networks at the molecular, synaptic, cellular and circuit level. As more sophisticated genetic targeting methods are introduced, we will use them to further refine our scientific approach in order to gather data even more efficiently. To minimize harmful effects, we will use non-invasive imaging and well-established physiological techniques. Surgical procedures will be done under aseptic conditions with appropriate anaesthesia and analgesia. We will continue to refine our experimental techniques as new, improved methods become available.</p>

Project	The pharmacology of the pulmonary circulation: new treatments for pulmonary arterial hypertension	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	3 Years 6 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Pulmonary hypertension (PH) occurs when the blood vessels in the lungs close up and die causing the right side of the heart to fail. There is a very poor survival with around half of the patients dying within three years of being diagnosed. Modern drug treatments do not improve survival. Women get PH up to 4-fold more often than men but men die quicker. Obesity is common in PH patients and can facilitate the disease process. We do not know if sex or obesity affects the development of PAH or the effectiveness of treatments. Here we will look	

	<p>at the effects that sex and obesity has on the development of PAH and the response to drugs by using the best animal models. We will also examine the effectiveness of novel drugs in these models.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Our work previously has led to new drugs going through clinical trials but we still need drugs that better reverse or treat the changes in the arteries and heart. Here we wish to find out why women get PH more frequently than men but why men die quicker. We also wish to find out why obesity contributes to the disease. We wish to discover new drugs that better treat the disease and improve survival.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use the minimum number of rats and mice to find out why women get PH more than men but men die quicker. Legally all new drugs need to be tested in animals before entering clinical trials so we will study very good rat and mouse models which have led to previous drugs successfully entering clinical trials and going on to treat the disease. In order that we ensure robust results we need to study enough to make results statistically significant. Over 5 years we could use up to 1500 rats and 5000 mice for these studies.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Some animals (we compare males with females) will be placed into hypoxic conditions (by putting them at simulated altitude) for up to 6 weeks which causes PH like that seen in patients in that blood vessels in the lungs close up slightly and put strain on the right side of the heart. This is like putting them up a mountain and doesn't cause them any distress. Some animals may be given a drug called sugen that makes the disease moderately more severe in that very small blood vessels in the lungs completely close as seen in patients. This does not normally cause adverse events. Some animals are given a drug called monocrotaline that also causes blood vessels in the lungs to close up and put moderate strain on the right side with associated inflammation of the lungs to model what happens in some patients. This does not normally cause adverse events. These animals may be treated with novel drugs, hormones, steroids, genes or</p>

gene modifiers to test their ability to either prevent the onset of PH or to reverse it. When we administer drugs these are given by the most appropriate route such as orally (via drinking water or a small tube into the mouth), intravenously (usually via a vein in the tail), directly into the lungs through the airways via a modified syringe), via small pumps that are implanted under the skin or drug releasing pellets implanted under the skin. The doses, length of dosing and routes are carefully designed such that therapeutic effects are optimal but adverse effects are not expected. Drugs given are given at known non-toxic doses. Some genes are given via special viruses and some via special fluids that enable the genes to take effect quicker. Some animals are made obese by feeding them a high fat diet for up to 30 weeks. Their teeth are checked regularly for signs of over-growth which is the only expected adverse event. As we are interested in how sex affects the development of PH, some female animals may have both ovaries removed and males have their testicles removed prior to any of the above. This is done under general anaesthesia after which the animals are allowed to recover. The very small wound is closed with clips or sutures and they are given thorough pre- and postoperative care by the vets and monitored for any signs of ill-health. This may include bleeding or wound breakdown. If so the vet will recommend treatment or repair or that the animal is put down immediately. All animals are monitored daily for any signs of ill health. If signs occur a vet is called and either the animals will be treated or put down in a human fashion. Sometimes we wish to examine how the heart is affected by the closure of the lung artery directly. To do this rats are put under general anaesthesia a ligature placed around a large lung artery. After this the wound is closed and the animal allowed to recover for up to 20 weeks. The wound is closed with clips or sutures and animals given thorough pre- and postoperative care by the vets and monitored regularly for any signs of ill-health. The surgery may have adverse effects just as in humans including bleeding, infection, weight loss and pain (this is indicated if the animal hunches up and has fur standing up). If so the vet will recommend treatment or repair or that the animal is put down immediately. Following any of the

	<p>above procedures, animals are put under general anaesthesia and catheters placed in their hearts and blood vessels to measure heart and blood vessels pressures and function. Some are placed in special scanners that image the heart to see how it is working. This will tell us if the potential new life saving drug has actually worked by reversing or preventing this terrible disease. The animals will not be allowed to recover from anaesthesia in these experiments.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>In PH patients the changes in the lungs causes the right side of the heart to work harder and eventually the patients die of right heart failure. We can only study this complex interaction between heart and lungs in a living animal. Patients are already very ill when they are diagnosed and so experiments cannot be carried out on them. Therefore, animals are essential if we are to find new drugs to save the lives of these patients.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>For every experiment we use the minimum number of animals that we need to give statistical significance. Only this data would be considered by clinicians when looking at new therapies to put through a clinical trial on patients. The number is calculated by the variability in experimental design as well as the magnitude of any change we wish to measure. Wherever possible we would never repeat experiments. For example if we can, in one study, we will look at males and females with and without drug, fat and lean in one go. Some of these animals may also have had ovaries or testicles removed or had their lung artery occluded to simulate strain on the right heart.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms)</p>	<p>The animal models selected mimic aspects of the human disease we wish to study. In PH patients the blood becomes hypoxic (low in oxygen) as thickening and loss of lung arteries means oxygen is not effectively transferred, from the air we breath into the airways into the arteries. The rat/mouse hypoxic model mimics these effects. The addition of an injection of the VEGF</p>

to the animals.

antagonist sugen prior to hypoxic exposure refines the model such that vascular occlusive lesions appear similar to those seen in patients and this infers a pulmonary pressure which is usually higher than with hypoxia alone. The monocrotaline model exhibits additional changes in lung blood vessel function related to inflammation as this is commonly seen in some patients. Transgenic mice allow the study of one gene and its influence on the development of PH. These are selected when a mutation in a gene has been reported amongst patients with PH or changes in that gene have been shown to play a role in PH. Putting a tie around a lung artery causes a strain on the right heart as seen in patients. This allows examination of events that cause the right heart to fail and the patient to pass away. Certain diet pills caused many women to die of PH in the past and so we may also examine the effect of new drugs or interventions on drug-induced PH. All animals are studied by fully trained researchers and regularly checked by vets for any signs of ill health, especially if they have had a surgical procedure. Analgesia is always given by experienced vets where needed. Any animals showing signs of distress or ill-health will either be treated accordingly but would be put down immediately at the advise of the vet. Where drugs need to be given every day, if possible we will administer these through a drug-eluting pellet as this will mean the animals are not repeatedly dosed by mouth.

Project	The Pharmacology of the Resolution of Inflammation	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Inflammation is the process by which our body responds to infection or injury. The function of inflammation is to remove the infectious material and to repair tissue to its normal function. Whilst inflammation is a life-saving response if it is not switched off, or resolved, in a timely manner, it can become pathological. This pathological inflammation underpins many diseases that affect people in western societies such as arthritis, heart disease and inflammatory bowel disease.</p> <p>Over the past two decades we have identified pathways operative during an inflammatory response that help inflammation to resolve and</p>	

	<p>tissue to repair. We are trying to understand why these pathways are not able to switch inflammation off in patients with for example arthritis or inflammatory bowel disease. We have also identified that some white blood cells produce small packages of information called vesicles that can help to repair inflamed tissue.</p> <p>The aims and objectives of this programme of work are therefore to:</p> <ol style="list-style-type: none"> 1. increase our knowledge of the inflammatory process and how it is switched off within the body to enable the development of new therapeutic strategies for chronic inflammatory diseases. 2. Test new therapeutic agents that mimic the molecules that switch inflammation off. 3. Establish the potential of vesicles to switch off inflammation and repair tissue such as that in swollen joints in arthritic patients.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Inflammatory diseases are a leading cause of death worldwide. Current medicines used to treat inflammatory diseases do not work in a significant majority of patients (for example 30% of rheumatoid arthritis patients do not respond to therapy). Our laboratory is at the forefront of research identifying pathways that resolve or switch off inflammation and how these might be exploited for therapeutic gain. We design our experiments so that they are as relevant as possible to the diseases of humans. This increases the ability to translate our findings in animals to the human condition. Our research will benefit other researchers in the field as it is still not understood why some types of inflammation are not switched off and become chronic. We also envisage that our research will benefit the pharmaceutical industry through the identification of pathways and targets for the development of new drugs.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use mice predominantly (over 95% of all experiments). Over the five years of the licence we plan to use ~ 13,000 mice and ~ 1,000 rats.</p>

<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The project will involve the use of both acute and chronic models of inflammation to understand the mechanisms that drive inflammation as well as to identify and test potential targets for drug development. The experimental protocols have been designed to mimic specific human diseases which have a large impact on Western Societies such as rheumatoid arthritis, osteoarthritis, heart disease and inflammatory bowel disease and all of the models proposed are well characterised and are established within our laboratory. The models of acute inflammation are designed to study specific aspects of the inflammatory response in models that provoke minimal suffering. For example we will inject mice with a factor that induces white blood cells to move to the site of injection as they would in an inflammatory response. We can monitor what type of blood cells move, quantify them and measure factors that tell us how well the animal's immune system is working. These models will form the majority of our studies, they are acute models, often lasting no more than 24 hours and in many of these models the inflammation resolves or subsides. In our experience the majority of these studies provoke mild to moderate discomfort. In more complex models of arthritis or inflammatory bowel disease where severity is moderate we will take additional steps to mitigate suffering. These models may result in longer lasting pain and affect the animal's mobility or ability to feed efficiently. Through regular monitoring we will adapt the animals environment as appropriate to minimise effects on welfare. Using clearly defined clinical severity scoring systems we will establish clear end-points for these models and animals will be euthanized if these points are reached to prevent unnecessary suffering. In all studies, animals will be humanely killed as soon as possible after we have obtained all the data outputs needed to complete the study.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-</p>	<p>Where possible we perform experiments using human samples from both healthy people and patients with inflammatory conditions and aim to</p>

<p>animal alternatives</p>	<p>minimise animal use through using more complex models with human cells, for example by growing different cell types together and using microscope slides that mimic blood vessels. However, it is not possible to study all aspects of the inflammatory response outside of the body and there is not a non-animal model that recapitulates the multiple aspects of the inflammatory response. We need to use animals to study how different processes within the body interact during inflammation and whilst animal models do not completely mimic recapitulate human disease, the models that we intend to use are well characterised and validated and share clinical characteristics of the disease in humans . Over the lifetime of the project we will continue to search for available non-animal alternatives throughout regular checking of replacement websites/databases and the literature. Moreover, we are developing organ-on-a-chip models with human cells and cell lines in vitro that could help address some of the scientific objectives and, over time, replace the need of animal experimentation.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>To ensure that appropriate numbers of mice are used per experimental group we will always use preliminary/pilot data where possible to inform us of the minimum numbers of animals required for our experiments to provide robust and reliable information. We design our experiments to ensure that the researcher assessing experimental outcomes cannot influence the experiment by knowing what treatments are being applied to what animals until all the results are in order. We will consult trained statisticians where appropriate and all staff performing animal experiments will attend appropriate training on key aspects of experimental design.</p> <p>Where possible we will use imaging techniques to monitor animals over the course of disease allowing disease progression to be monitored in individual animals. We will also always endeavour to assess multiple parameters in each animal to reduce use.</p>
<p>3. Refinement Explain the choice of species and</p>	<p>The mouse is the most appropriate species to conduct these studies as it is the lowest</p>

<p>why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>vertebrate species to give an inflammatory response similar to that seen in humans and their immune system is well characterised. The time course of white blood cell recruitment in mouse models of acute inflammation is comparable to that seen in man and in mouse models of chronic inflammation the same set of inflammatory mediators drive the disease process to those seen and targeted by drugs in human disease.</p> <p>The models that we use have been selected as they recapitulate specific aspects of chronic inflammatory disorders in humans and will be used to address specific questions that cannot be addressed in our experiments using human tissue/cells.</p> <p>In our experiments we will use scoring schemes with clearly defined action points and humane end points to reduce animal suffering. Clinical scoring schemes for our animal models of inflammation have been developed to allow maximum collection of scientific data outputs whilst minimising animal suffering.</p> <p>We will minimise suffering by refining our protocols so that for example injections are given by the most appropriate route, using the smallest needles possible and implementing maximal volumes for administration. Where appropriate we will use analgesia and anaesthetics to minimize pain and stress and to mimic the human condition. In models, such as those used to study rheumatoid arthritis where mobility may be compromised we will adjust the environment by providing soft bedding and nesting material and if feeding is compromised, soft gel food and longer spouts on water bottles. We will use clinical scoring sheets in all experiments to monitor animal welfare so that experiments can be stopped before the animal becomes severely ill. We will ensure that researchers keep up to date with current research on refinement of the procedures that we use and we have consulted the relevant scientific literature when writing our protocols.</p>
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Project	The phosphoinositide-network in health and disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Our aims are to understand and find new ways to treat diseases such as chronic inflammation and cancer through better understanding of cellular molecular mechanisms.</p> <p>All of life can be viewed as based on molecules and chemistry. To be able to understand and treat human health and disease we need a molecular understanding of biological processes because it is only at this level we can meaningfully and rationally attempt to use “designer molecules” as therapeutics. Our work aims to provide a “chemical” understanding of the biology involved in inflammation and tumour progression and to identify ways to treat disease with minimum</p>	

unwanted “side effects”.

Our area of focus is on the phosphoinositide signalling network, a set of molecules in cells involved in the transmission of intracellular signals controlling cell processes such as cell growth and metabolism. We study this network in cell culture models with the aim of understanding how it works and how it goes wrong in disease. To achieve these things we genetically manipulate the cells to change specific features of the phosphoinositide signalling network (eg enzymes in it) and hence reveal their roles and importance. We also collaborate with medicinal chemists to create chemicals that target the same features (eg enzymes). In our work with cells we validate the efficiency and specificity of the chemicals. To reveal whether the network operates similarly in vivo we use well described and tested methods to introduce the same specific genetic modifications into mice and apply chemicals, we have tested and validated on cells, to mice.

The primary models we use to understand the roles of the phosphoinositide signalling network in health and disease are aseptic and septic models of inflammation and models of cancer, with a particular focus on prostate cancer. These models are well validated by past work and accepted by the academic and commercial communities as relevant contexts within which to understand whether modulation of a particular target (eg enzyme) is capable of therapeutic benefit or whether there maybe medically unwanted consequences to a treatment (eg weakened immune response). Furthermore, these models are treated as benchmarks because they have been used to study many other targets and/or potential chemical treatments and hence the therapeutic benefit elicited by a genetic modification or treatment in these setting can be valued by experts in those models. In the models of inflammation, different forms of inflammation are induced by various means to allow the potential width and/or specificity and any potential unhelpful “side-effects” of an approach to be understood. This is important because each model has a variety of very individual factors that play out in its progression that can reflect different aspects of specific human conditions. The models include aseptic (sterile) inflammation as mimics of auto-immune diseases

	<p>such as arthritis and septic (live pathogens) that allow the integrity of the immune system to be tested. The cancer models we use allow us to examine the impact of potential novel therapeutics in different potential disease settings as argued above. These studies include work to establish whether those therapeutics are working “on-target”, whether they may generate potential “side-effects” and whether they may have wide or restricted applications against different types of cancer. We also aim to test approaches that might modulate the ability of the immune system to detect and/or attack tumours.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Better understanding of the molecular mechanisms underpinning health and disease. Specifically, the experiments that will be conducted under this licence will reveal the functional importance of different components of the phosphoinositide network in normal health and disease. This will be read-out by measuring the performance of the immune system in the genetic absence of those components or in the presence of selective modulators of those components under normal conditions or in response to different inflammatory challenges such as infectious microorganisms or aseptic insults. Experiments with cancer models will reveal whether there are important roles for components of the phosphoinositide network in tumour progression. Our approaches will suggest whether the roles are embedded within those of the host (eg in the immune system) in detecting and destroying tumour or are a manifestation of roles within the tumour itself. These results will identify potential new therapeutic targets. We work closely with drug companies to improve their therapeutic strategies by identifying new targets and testing tool compounds under development to see if they are selective for their target and generate therapeutically beneficial outcomes in mouse models. In the long term our work will lead to improvements in the treatment of human and animal patients. We anticipate we will publish our results in refereed, internationally-renowned journals as we have in the past. We will also present our work to both academic and commercial researchers at international meetings on a regular basis. Our lab also engages in public engagement work to increase public understanding of science (eg, Science Festivals). On these occasions,</p>

	<p>although we actually present our research and do not speak about the ethics of doing animal research as experts, we often deal with questions about animal research, what we do and its benefits. We can provide many examples of how our work has lead to the development of new anti-cancer and anti-inflammatory drugs that are either approved or in clinical trials at the moment and broadly we have had excellent feedback from members of the public about our work. The programme of work will also create new genetically altered strains of mice in which components of the phosphoinositide network have been manipulated to test their function. As we have in the past, we will freely share these lines with other academic researchers and will licence them out to commercial colleagues. We anticipate we will create about 5-7 new strains during the life time of this licence. Experience shows that by sharing mice with experts in other fields of research unexpected and important benefits regularly emerge. Furthermore, by sharing mice in this way it reduces the number of mice used in research internationally. Our lab trains many students and part of their experience in our lab, whether they work with animals or not, is to understand how mouse models can be developed and then used to better understand health and disease. This spread of knowledge improves awareness of the value of animal work and the many factors that need to be considered in doing animal research.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice, 60,000 over 5yrs.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The majority of mice we use are genetically-altered or their wild-type controls and are simply bred and maintained at a very high health status. These mice are humanely killed with a schedule 1 method and we work with cells and/or tissues quickly dissected from the dead mice. These approaches have no significant adverse effects and are of mild severity. However, this approach allows us to isolate neutrophils and macrophages (both immune cell types) in large numbers that we can study in vitro with assays of cell migration and secretion. Through this strategy we can address many of our objectives to better understand how the</p>

phosphoinositide network regulates the immune system and test and validate potential drugs without subjecting mice to any models of inflammation. In addition, we can use cells and tissues from schedule 1 killed mice to establish a variety of other primary cultured cells that grow in the lab. We use cultures of primary fibroblast-like cells to study cell growth and proliferation; that are relevant to our second major objective to understand the role of the phosphoinositide network in, and improve treatment of, cancer. A relatively small number of the same mice are used in experiments where we ask whether the discoveries we have made in vitro apply in vivo and could give us new understanding or represent new approaches to treat inflammatory diseases such as arthritis or cancer. In these models mice are given treatments that lead them to develop various diseases and then we ask whether modulation of the phosphoinositide network possibly reduced the inflammatory responses observed. None of our models leads mice to experience severe adverse effects and we expect our modulations to reduce the inflammatory responses observed. Our models of inflammation include transient application of inflammatory stimuli to the skin or peritoneum and the large majority of mice are expected to only experience mild clinical signs. In a very small number of experiments we test the impact of modulation of the phosphoinositide network on full immune responses to infectious organisms that are very relevant to important human diseases (a common fungal pathogen, a common opportunistic bacterial pathogen that is increasingly drug resistant and a bacterial pathogen that causes pneumonia). In these models the adverse effects are associated with the infection and include fever, ruffling of the fur and being subdued and are considered of moderate severity. We also use a small number of mice in models of cancer progression (including for example a model of prostate cancer) where the mice are injected with tumour cells under the skin on their backs or are genetically disposed to get cancer. The former models only last about 3 weeks before the mice are killed by a schedule 1 method and the mice experience moderate clinical signs associated with the growth of a tumour on their flank. Humane end points are set to prevent any mice experiencing greater than moderate clinical signs. The genetic model is very slow to

	<p>progress and the mice show no clinical signs until they are 10 months of age. The large majority of mice on our licence are schedule 1 killed before 6 months of age and have displayed no clinical signs. A small proportion (about 10% of mice undergoing this procedure) are allowed to progress until they show clinical signs of the emergence of prostate cancer but those mice only display moderate signs before being killed by a schedule 1 method. In all cases the treatments we apply (inflating the phosphoinositide network) are expected, or already proven, to reduce cancer progression and hence reduce the clinical signs.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We use many approaches that allow us to avoid the use of animals in research, including use of cell lines. However, to understand healthy processes and what goes wrong in disease and to devise strategies to treat disease, some use of animals, that are similar to humans in terms of their normal cellular processes and their responses to specific diseases, is necessary.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use many tactics to reduce the number of animals we need to use. By employing good statistical methods, by using modern technologies that minimize error, using techniques that allow us to study mice non-invasively (and therefore to be able to make many measurements with the same animal) where possible.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the best species to use for the objectives of this licence. They have many similarities to humans in terms of their basic cellular processes and responses to diseases and as they are very widely used in academic and pharmaceutical research, results obtained in mice are easily compared to those from other research groups. Many highly evolved and technically efficient methodologies have been optimised with mice leading to more efficient progress per animal used. We only chose to work with models that are widely accepted to be reliable and have been optimized to minimize harm and the number of animals used. We use non-invasive techniques as much as possible and attempt to remain in touch with new</p>

	<p>advances that offer further animal-welfare advantages. Our animal work is done within the framework of a limiting clinical signs approach, operated by animal technicians and vets; that is, any mice seen to be experiencing unexpected suffering are killed by a humane method.</p>
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Project	THE PHYSIOLOGY AND PHARMACOLOGY OF CHRONIC ARTHRITIC PAIN	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Osteoarthritis (OA) is the commonest form of chronic pain and affects 8.75 million people aged 45 and over in the UK. It is expected that with the ageing population and growing obesity epidemic the number of OA patients will rise sharply. Current treatments do not offer the hope of reversing the condition hence often the end stage treatment for OA is joint replacement surgery; however around 20% of patients still experience chronic pain following this procedure.</p> <p>Current standard therapies (e.g. non-steroidal anti-inflammatory drugs, NSAIDs) are often inadequate</p>	

	<p>in terms of pain relief and are problematic in long term use, potentially leading to serious digestive, cardiovascular and renal side-effects. The development of improved drugs for OA pain therefore relies upon a greater understanding of underlying mechanisms which is achieved through studies in validated clinically relevant animal models, such as the rat monosodium iodoacetate (MIA) model.</p> <p>The proposed studies aim to better understand how changes to the way the brain controls pain occur during different stages of OA. The influence of anaesthetic agents on pain mechanisms and study outcomes will be investigated. Novel and common anti-inflammatory drugs will be examined for their pain killing ability and any potential to halt changes in joint structure in OA.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>These studies will advance understanding of mechanisms behind OA pain by investigating functional changes in the nervous system in whole animals subject to a clinically relevant model. Currently a lack of consistently effective pharmacological therapies means many patients suffering from chronic OA pain are in desperate need of relief, so basic research findings are often rapidly translated into clinical experiments. The research has the capacity to provide information towards development of new drugs or new approaches to using existing licensed compounds.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Up to 750 rats will be used over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Induction of the MIA model of OA is performed under brief general anaesthesia and involves an injection into a knee joint. The result is to produce joint pathology and pain behaviour responses that mimic key features of human arthritic pain. Occasionally a small amount of swelling may be present in the inject limb for a few days following injection but overall the MIA model is relatively mild compared to other pre-clinical models in that animals do not lose weight, continue to eat, groom and show normal exploratory behaviour. Some pain and discomfort may be present but none of these</p>

	<p>symptoms will be allowed to exceed moderate severity. Pain behavioural responses of these animals will be measured by well-established tests where the animals are unrestrained and are able to move away from the applied stimulus at any point. For the purpose of drug studies in awake behaving rats, a few animals may undergo a second brief general anaesthesia should injection of a test drug into the knee be required pre- or post- model induction, otherwise drug treatments will be given by staff trained and competent in humane methods of handling, restraint and injection techniques (or orally). At the end of a study, electrophysiological recordings will be performed in terminally anaesthetized animals or animals will humanely culled.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The research will study how different parts of the nervous system interact and how this changes in OA. This requires an intact nervous system therefore could not be carried out on cultured tissues. While <i>in vitro</i> preparations have been developed in which the majority of the central nervous system remains intact, these are almost invariably confined to neonates/juvenile animals and do not usually retain any contact between the central nervous system and the rest of the body. Hence studies on spinal reflex organization, which rely on interconnections between peripheral and central nervous systems, must be studied in whole animals. Studies in cell cultures will inform pharmacological studies of potential novel pain killers, however drugs have multiple sites of action in the body which may be strongly influenced by pharmacokinetic factors that cannot be modelled <i>in vitro</i> therefore need to be studied in the whole animal.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Group sizes will be used which are the minimum necessary to achieve a statistically and biologically meaningful outcome. To ensure this, power calculations will be performed in advance of experiments using, wherever possible, data from previous studies in order to determine appropriate group sizes. In most cases behavioural, electrophysiological and drug studies will be performed on the same animal; in some cases</p>

	additional pharmacokinetic studies or anatomical studies will also be incorporated.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rodents are the least sentient species on which studies of this nature are performed and almost all pain research in animals is conducted in rats (and mice) which are very good subjects for behavioural and acute invasive electrophysiological experiments; hence there is a wealth of historic data to compare our findings to. The standard electrophysiological experiment collects data on multiple reflexes simultaneously thereby increasing the output from a single animal and reduces the risk negative findings based on a single response. The MIA model has been chosen for its representation of clinical OA pain and is one of the least detrimental to animal welfare. Following model induction under aseptic conditions, animals will be monitored for behavioural and weight changes, and any other signs of illness or discomfort. Any lameness will be monitored using a scoring system and no animal will remain in the study if exhibiting signs of severe lameness. Due to the nature of the research, post-operative analgesia will not be possible following model induction however animals will receive the highest possible standard of post-operative care from a dedicated animal husbandry and technical support team. Animals will be closely monitored and veterinary advice promptly sought if needed. Behavioural threshold tests will be used where animals are unrestrained and free to move away from the noxious stimulus at any time, thus preventing any long lasting discomfort or tissue damage.</p>

Project	The physiology of mammalian eggs and early embryos.
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X Basic research
	X Translational and applied research
	Regulatory use and routine production
	Protection of the natural environment in the interests of the health or welfare of humans or animals
	Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>We aim to understand the basic mechanisms that operate at fertilization in mammals and to establish new ways of diagnosing and treating human infertility.</p> <p>Infertility affects about 1 in 6 couples and the major form of treating human infertility is <i>in vitro</i> fertilization (IVF). However, this technique is not always successful for reasons that often remain unknown. One major potential reason why fertilization does not occur is because the egg lacks a stimulus from the sperm. It has been previously shown that this stimulus is provided by a specific protein in the sperm. Our research will investigate how a lack or deficiency in this protein could explain these currently unresolved cases of infertility. We will find ways of making this sperm protein artificially so that it could be used in future to</p>

	<p>help couples conceive. We will use mouse eggs and mouse embryos as models for human eggs and embryos.</p> <p>Another major problem with current IVF treatments is that when fertilization is successful, there may be several embryos generated. It is common practice to re-implant 2 of these embryos into the uterus of the prospective mother. However, this can often lead to twins, and it also increases the chances of triplets. If a mother has twins or triplets there is a greater risk of problems developing during the pregnancy. It would be best to only transfer one embryo but the problem then remains of how to choose which is the 'best' embryo. In our research we will also be studying the biochemical responses in the mouse egg and embryo in the first few days of development. In some cases we make use of specialist imaging methods that do not harm the embryo. At a later stage these could be developed for use in IVF clinics to select the best quality embryos.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>We will advance our knowledge of how a sperm stimulates an egg to develop into an embryo by finding the factors in the egg that allow it to respond to the sperm protein that triggers development. By making a stabilized version of this sperm protein we will provide IVF clinics with a new way of treating couples whose eggs have failed to fertilize. We will also improve our understanding of how we can assess the ability of a human embryo to undergo successful development. We will investigate how eggs use substrates such as fats for energy, and whether their metabolism is an indicative factor of developmental viability. This could eventually lead to better methods of selecting which single embryo to re-implant into a prospective mother undergoing IVF, and this will in turn help reduce the additional problems in pregnancies associated with twins and triplets.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use up to 3000 female mice and about 200 male mice over the course of 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of</p>	<p>We will inject mice with hormones so that they make more eggs than normal. The mice are injected twice, two days apart. Then after a further delay of about 15 hours (to allow ovulation) the mice are humanely killed. This procedure involves injecting the same hormones</p>

severity? What will happen to the animals at the end?	that women use as part of IVF. We will also keep some genetically modified male mice but they are not expected to have any adverse health problems. These male mice will also be humanely killed and sperm collected post-mortem.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no 'animal-free' cell culture system for making eggs that can develop into embryos. The way in which a mammalian egg develops into a healthy baby is very complex and there are many factors that can affect the formation and health of a baby that go way beyond our basic knowledge. The timing and complexity of the signals in mammalian embryos make them rather different from invertebrate embryos. Hence, the only way to study how factors affect a human embryo is to use another mammalian embryo.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The key factor in our work is the number of eggs we can obtain for experiments. We use the minimum number of mice for each experiment by using hormones to induce superovulation and hence obtain the maximum number of eggs per female.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use mice because their eggs are most similar to human eggs in terms of their metabolism and response at fertilization. With mice we also have the ability to use genetically modified mice that lack particular proteins involved in the events of fertilization. This mutation only affects the ability of the mice to reproduce. The procedure that is carried out under a licence involves injecting female mice in the abdomen with a hormone using a sharp needle, on two separate occasions. The mice rarely, if ever, suffer any side effects or other consequences as a result of this injection other than the production of more eggs than usual.

Project	The population ecology of UK seabirds
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input checked="" type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input checked="" type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of the project is to understand changes in seabird populations experiencing fluctuations in environmental conditions, food availability, pollutants such as heavy metals and parasites such as gastric worms.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This research will help understand what is the driving force behind the widespread population declines currently occurring in seabirds in the UK, and will therefore inform government policy and aid their conservation.
What species and approximate numbers of animals do you expect to	We expect to use up to 6,500 seabirds over the next 5 years, with the greatest focus on European shags, with secondary focus on

use over what period of time?	common guillemots, Atlantic puffins, razorbills and black-legged kittiwakes
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	<p>We will take samples from birds (blood, feathers, claw tips, oral swabs, cloacal swabs, preen gland swabs). We will examine them for ectoparasites using skin palpitation and feather ruffling. We will examine them for endoparasites using gastroscopy. We will obtain diet samples using water off-loading. We will treat them for ectoparasites and endoparasites applied topically, via subcutaneous or intramuscular injection or by oral administration. We will repeat these procedures up to a maximum of five times in a three month period on the same individual (and each will not occur more than once every 3-4 days). The repeating of procedures will enable us to test patterns in the effects of parasites and contaminants over the course of the breeding season. Repeated parasite removal treatment will lengthen the period of treatment, allowing us to test these seasonal changes experimentally. We will deploy electronic devices to record location and foraging activity (via GPS or geolocation methods) attached to the lower back or to leg rings. We will undertake cross-fostering experiments whereby eggs are swapped between nests to facilitate the removal of confounding genetic effects that can mask individual variation in parasite responses, and enable seasonal variation to be partitioned between seasonal environmental variation and intrinsic differences among early and late breeding birds. In all these procedures, we expect that birds will experience short-term mild discomfort, but that there will be no long-term impacts of these procedures. We therefore consider that the level of severity is mild. The birds are removed briefly from the nesting area to obtain the samples after which they are returned to close to their nest and released back in the wild.</p>
Application of the 3Rs	
1. Replacement	To understand the effects of parasites, pollutants and other factors on seabirds, the

<p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>only option is to work on wild birds living in their natural environment</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We use experience from our past studies to minimise sample size. These studies have undertaken statistical analysis that demonstrate whether our conclusions are robust, in consultation with a professional statistician. Sample sizes are continually revised on this basis.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The rationale for working on these seabird species is that they allow us to test key questions that will provide insight into the causes of the declines in UK seabird populations, and to help devise effective conservation strategies. The choice of species, and our chosen study sites, are carefully selected to ensure that we have the best opportunity to undertake high quality research while minimising adverse effects on the birds. We have refined the procedures that we undertake on the birds to ensure that these effects are minimal, including minimising the length of time a bird is held, only undertaking procedures that are central to the research objectives, ensuring that all work is undertaken by experienced researchers, and returning the bird as soon as possible so that it can revert to its normal behaviour as a wild, free-living individual.</p>

Project	The processing of mechanosensory information in the brainstem of Xenopus tadpoles.	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project aims to use frog tadpoles as a model animal to investigate how specific sensory information is processed in the brainstem and leads to different behavioural outcomes. The specific objectives are:</p> <ol style="list-style-type: none"> 1. How does the activation of some movement sensors in tadpole head skin stop ongoing swimming? 2. How does water current stimulation (likely to be of the lateral line) evoke the turning of 	

	<p>tadpole body and subsequent escape behaviour?</p> <p>3. How do ionic pump proteins in the nervous system regulate tadpole swimming?</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The way in which the nervous system controls behaviour remains an unresolved biological question. However, the study of how the neuronal circuits work in complex vertebrates including mammals and humans has been hampered by their complexity and poor accessibility. Many regions in brainstem are critical in processing sensory information and control of vertebrate motor behaviour but detailed understanding is lacking. The use of simpler model animals like <i>Xenopus</i> tadpoles allows faster progress and a deeper understanding of the common principles applying to all vertebrate motor control. Since most neural mechanisms are conserved throughout evolution, this can provide guidance for research in more complex vertebrates in the future, which may in the long-term lead to the development of cures for human motor disorders.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>A colony of about 50 males and 50 female <i>Xenopus laevis</i> will be maintained in house for the duration of this license. We use these adult frogs for collecting embryos following induced mating by injecting them with hormone. The animals are going to be re-used because the procedure is mild in nature and the animals normally recover very well. The physiology experiments will be carried out on tadpoles before they start to feed, which do not require regulation under the Animal (Scientific Procedures) Act, 1986. These tadpoles are killed humanely immediately after experiments.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>A regulated procedure is needed to inject hormone into adult <i>Xenopus</i> to induce mating so embryos can be collected. The injection procedure does not require anaesthesia and only causes mild, transient discomfort to the animals. Because HCG injections only accelerate and synchronize the natural mating behaviour and are mild, frogs can be reused for many years. Records of embryo number and quality for each pair of animals will be kept. There are two ending points for the adult frogs depending on their embryo-producing</p>

	quantity and quality. 1. Reuse for frogs producing good quality embryos. 2. Culling using a humane method for poor performing frogs and aged frogs.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Neuronal functions emerge from neuronal circuits where different types of nerve cells are connected in specific ways. Therefore, investigation of circuit functions has to be carried out <i>in situ</i> where the connectivity is intact and animal behavioural outputs can be monitored:</p> <ul style="list-style-type: none"> - Cell culture loses all the connections between the nerve cells in intact preparations. - Computer modelling needs to be based on physiology data, which are far from sufficient to reproduce many features of network activity in real animals.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We have the following ways to reduce the number of animals and protocols required:</p> <ul style="list-style-type: none"> ● Because the mild hormone injection procedure is used to induce natural mating, we can reuse the animals many times after their recovery. The number of animals can be reduced significantly by selection of good breeders for reuse over many years. ● The resulting embryos are raised at different temperatures so from one injection there are normally tadpoles available for use for up to five days. ● Spare tadpoles will be shared with colleagues
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The use of <i>Xenopus laevis</i> for neurobiological studies has a long history. <i>Xenopus laevis</i> are easily maintained in a lab environment and natural mating can be induced by hormone injections all year round. Their simple nervous system and lack of vertebrae in early development also give high access for physiological recordings.</p> <p>We use tried and tested measures to minimise</p>

	<p>animal welfare costs:</p> <ul style="list-style-type: none">- Regular handling of the same animals can reduce fear/stress.- Add artificial plants and hiding tunnels to the frog tanks to enrich their environment.- Adding diced ox heart as food supplement.
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Project	The Production of Laboratory Animal Bio-Products	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To use animals to provide blood and tissues to generate data to support the development of effective and safe medicines to treat diseases where there is currently a clinical unmet need e.g. cancer & heart disease.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Bio-products provided will contribute invaluable scientific information to support and progress potential new medicines where there is currently an unmet clinical need. Conducting investigations using blood and/or tissues taken from animals reduces the number of potential new medicines requiring evaluation in living animals and can be used to establish whether	

	<p>conducting experiments on living animals would be beneficial. The products are also used to aid the development of new medicines in man or animals when it is necessary to calibrate and validate many of the machines or testing systems used to support research. They may also be used to support other methods in research as an alternative to live animals.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Over a 5 year period: Dogs: 275 Mice: 50 Guinea pigs: 50</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>More than 85% of the animals used under this licence will be kept under general anaesthesia throughout the sampling procedures and will not be brought back to consciousness. They will be humanely killed while still under anaesthesia with an overdose of anaesthetic. Therefore these animals are not expected nor likely to experience any adverse effects. The remainder (mainly dogs) are trained to donate small volumes of fresh whole blood at weekly or monthly intervals and these donors are not expected to suffer adverse effects as a result of the project (similar to taking a blood sample from a human). These dogs will continue to be used as donors for several years until they are retired. The dogs receive full clinical health checks by a veterinarian and experienced animal technicians. They will be retired if there are signs that their, normal health state is affected by the project, their age or health issues. Training of donors is however not always possible and in the case of mice and Guinea pigs, to avoid the need for restraint of the donor and for handler safety, sedation prior to bleeding is performed. Adverse effects from repeated blood collection are not expected under this project but could (rarely) include slight bruising, anaemia or uncontrolled bleeding. Any animal with anaemia or poor clotting mechanisms will be removed from the bio-products donor pool. Adverse effects of the sedation are not expected. Any adversely affected animals will cease to be used and will be referred to the responsible veterinary surgeon who will determine the need for any treatment, consider its suitability for rehoming or</p>

	if the animal should be humanely killed.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Drug research programmes rely, in part, on biological materials obtained from human or animal sources to validate and confirm disease-associated drug targets and the mechanisms of action for potential new medicines. This programme of work supports the replacement of using living animals by enabling the supply of high quality blood and tissue samples where living cells are needed for experiments due to the lack of appropriate cells from existing alternative sources or instances where it is not possible to use cell culture techniques.</p> <p>There are a number of promising technologies in development which aim to utilise human cells to recreate the physiological functions of organs without using animals. However, these <i>in vitro</i> approaches do not yet offer an alternative to totally replace the need for research animals and authentic blood, blood products, body fluids and tissues to enable their use in all the investigations required to support the research and development of new medicines.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of animals used is minimised by using proven collection techniques including taking blood under non-recovery anaesthesia to ensure that large volume, non-clotted samples can be obtained.</p> <p>Tissue requests will be co-ordinated in order to supply multiple samples from one animal (e.g. whole blood, pancreas, femurs and liver) to a number of requesters for their individual purposes. This reduces the total number used.</p> <p>The project aims to provide blood components and tissues of the highest quality as this improves the significance of test results in studies involving animals and can often lead to improved scientific knowledge and a reduction in the overall number of animals.</p> <p>The project can reuse animals and this means that multiple samples can be obtained from a smaller number of donors thereby reducing the</p>

	<p>need to kill animals for the purpose of taking each sample.</p> <p>The use of blood products, tissues and organs that are obtained from animals that are not suitable for direct use in research reduces the total numbers of live animals required for research</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Where there is scientific need to preserve tissue integrity/architecture or obtain high volume and quality blood samples, then taking samples under appropriate and well maintained non-recovery anaesthesia is considered the most refined approach and we have refined the technique so that we will cause the minimum amount of discomfort and distress to the animal when we anaesthetise it.</p> <p>Persons taking samples are well trained in the techniques involved to ensure high quality samples are obtained quickly & effectively with minimal impact on animal welfare.</p> <p>The choice of donor species is driven by the scientific needs of research scientists.</p> <p>When it is prudent to sedate the donor prior to sampling (if the donor cannot be readily trained or if it would be hazardous for the person taking the sample), then a second drug is used to reverse the sedative and thereby speed up recovery from sedation.</p> <p>By only using donor dogs from the colonies we house, we ensure they are kept in appropriate long-term housing. The reuse of animals in a donor pool means they are used for a minimum 1 year in ferrets and several years for dogs. The animals benefit in the long term by being housed in appropriate socially enriched housing, cared for by trained staff. This housing is in the holding rooms of the general population and they therefore benefit from being in busy, familiar surroundings with social contacts of other dogs.</p>

Project	The regulation of cardiovascular development	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Purpose of the project.</p> <p>This project uses genetically altered mouse lines, to investigate processes in the embryo and foetus that are involved in assembly of the heart and which, if impaired, might predispose to, or cause, heart defects. A significant by-product of these studies is to uncover molecular pathways and cell biology in the developing heart which might be extended to the diseased adult heart to repair any injury. The work plan consists of: (i) identifying genes important in the shaping the developing heart and those specifically involved in the formation of the outer layer of the heart called the epicardium and the coronary lymphatic vessels;</p>	

	<p>(ii) investigating the embryonic and foetal processes that lead from gene function to heart assembly and, moreover, from gene defect to heart defect; (iii) identifying new methods or pathways for preventing heart defects by 'correcting' heart development in the embryo or foetus and iv) ultimately uncovering embryonic cell potential which might be reactivated in dormant adult heart cells to instrument repair following a heart attack.</p> <p>Clinical needs.</p> <p>Heart defects that manifest at birth affect 1% of all pregnancies world- wide and, adult cardiovascular disease is the biggest world-wide killer, therefore, in combination this represents an enormous burden on society. Many children with heart defects require corrective surgery, transplantation and extended (even life-long) medical care. For example, children with hole in the heart or defective blood vessels require surgery, often repeated as the child grows older. Moreover, our studies in the embryonic heart may help identify how to stimulate gene pathways and cells in the adult heart to initiate repair of damaged muscle and blood vessels following a heart attack.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Expected benefits. These include: (i) increased understanding of embryonic heart development, both normal and abnormal leading to heart defects manifesting as congenital heart disease; (ii) improved methods of genetic diagnosis and genetic counselling, which should follow from discovery of genes that cause heart defects in mice, provided the findings are confirmed in human studies; (iii) identification of new pathways that might be amenable to drug treatment (iv) identification of pathways of development that might be recapitulated in the scenario of adult heart "repair".</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Numbers of animals to be used. In this project, the majority of mice will be used for purposes of breeding to maintain colonies of genetically altered strains. It is extremely difficult to estimate the numbers required as this depends on strength of effect. We estimate that up to 3000 mice (including all ages) per year will be involved</p>

	<p>in the breeding programme, of which 1000 are used solely for breeding and genetic typing using DNA obtained from an ear punch biopsy. 50 female mice will receive hormone injections to produce large numbers of fertilised eggs (e.g. for embryo freezing to preserve valuable strains) and a further 50 females will serve as uterine foster mothers for implanted embryos (e.g. when re-deriving a strain for importation or health purposes). The remaining 1200 mice will be mated to produce pregnancies containing embryos and fetuses for the study. Mated mice will be killed to remove embryos and fetuses for the studies or recycled into the breeding colony.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Adverse effects are associated with making new genetically modified mouse lines, via generation of vasectomised males, administration of drugs to females to induce super-ovulation and embryo transfer. These procedures are anticipated to be mild in severity, but adverse events associated with pain sensation in each case will be countered by administration of analgesics. Breeding and maintenance of genetically modified lines in the vast majority of cases approximately 90% will have no outward phenotype and lie within a mild severity category. In the experimental protocols, the harvest of blood from rats, will incur transient stress associated with the induction of terminal anaesthesia. In the studies involving administration of substances to look at effects on embryonic growth and effect on heart development during pregnancy, treated adult female mice may experience adverse effects related to the substance including shallow, rapid breathing, increased heart rate, uncoordinated or slowed movement or failure to thrive. These mice will be monitored and where distress exceeds a moderate severity limit they will be humanely culled (by a schedule 1 method). Embryos will normally be studied at an early stage of development before pain or other sensations have been acquired. These embryos are killed almost the moment they are taken, so there is minimal potential for suffering in any case. Wherever possible, experiments will be done using embryos cultured in a test tube. This minimises the number of pregnant mice that need to be used, since embryos from a single female</p>

	can act as both 'experimental' and 'control' treatments. Moreover, use of culture studies minimises the number of procedures that need to be carried out on pregnant females.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Research into heart development concerns the processes by which the embryo and foetus orchestrates the specific shape and function of the heart over time. Mice have a four chambered heart which in terms of the way it develops and the genes/proteins and cells involved in its formation are very similar to humans and, therefore, findings in mice can be related not only to human development but also human birth defects. Direct genetic studies of foetal (embryonic) humans are difficult practically, and only descriptive analysis is possible, with genetic manipulation experiments ruled out on ethical grounds. Embryonic heart development is a four-dimensional process (i.e. varying in space and time), and, therefore, requires the analysis of either whole developing embryos and/or isolated embryonic hearts, which capture the full array of complex interactions of multiple cell types and tissue formation. Tissue culture systems, although they can provide useful information on certain molecular or cellular phenomena, cannot mimic the complexity of a functioning organ such as the heart, let alone the developing embryo. Computer simulations can be valuable in extending theoretical approaches to embryonic (heart) development, but cannot tell us about real biological situations, such as those occurring in the embryo.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have a high level of expertise in our laboratory to ensure that our experiments are correctly designed and conducted, and we strive to collect as much data as possible at any one time from any single animal/embryo. Many of our experiments in animals are informed by preceding cell culture and tissue explant studies, to predict outcome and determine whether a whole embryo/heart study will be informative and worthwhile. We ensure continuous analysis of the data generated from each study and as such can clearly identify at an early stage how many

	<p>samples will be required to produce a strong (statistically significant) scientific conclusion. To facilitate this, we discuss our experimental plan and predicted outcomes with local statisticians. Should these numbers exceed our expectations we will be able to promptly make adjustments to ensure the minimal number of animals are used.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We have chosen to study a mammalian species, the mouse, so that the principles emerging from our research have the greatest chance of applying to the human situation. Mice have historically been used extensively as models of organ development; not only is the developing mouse heart similar to that of the human foetal heart in terms of anatomy and function but an extensive array of genetically manipulated strains and genetic manipulation tools (gene-editing constructs) exist for the mouse that are not available for any other mammalian model system. As such, the mouse represents the most refined choice for our studies of understanding the molecular regulation of cardiovascular development. Utilisation of other models would either require the use of more animals so that the appropriate genetic tools can be generated or would not accurately reflect human heart development and hence, would reduce the clinical application of the data generated from this study.</p>

Project	The role and regulation of reactive oxygen species in development and regeneration	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The primary goal in regenerative medicine is to facilitate the replacement of aged, injured and diseased tissues with fully functional counterparts, thus extending the healthy life expectancy of our ageing population. My research group has been investigating the molecular and cellular mechanisms involved in tissue formation, repair and regeneration in <i>Xenopus</i> and zebrafish, two animals with high regenerative capacity. We have discovered that appendage regeneration and embryonic development in these animals require sustained	

	<p>production of reactive oxygen species (ROS). ROS are natural by-products of metabolism, which, when produced at high levels, have traditionally been associated with degeneration and aging. Remarkably, our findings have shown that low, but sustained levels of ROS promote regeneration. We propose to extend these findings by addressing the following questions:</p> <p>How is ROS production regulated during development and appendage regeneration, so that the right levels to promote regeneration are produced?</p> <p>How does ROS promote regeneration?</p> <p>We expect that answering these questions will pave the way towards the development of novel therapies, including the identification of novel pro-regenerative drugs, aimed at promoting tissue repair and regeneration in human patients, where regenerative potential is normally limiting.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Our aim is to identify the sources of ROS production and how they are regulated during development following tissue injury. How ROS promote regeneration will provide clues about how ROS production might be manipulated following injury or disease in humans, as a means of promoting regenerative healing in patients. We expect that, from these findings, we will identify one or more possible drugs or drug targets, which may improve tissue repair and regeneration. That can then be explored in pre-clinical and clinical trials for their potential pro-healing/pro-regenerative effects in humans, including identifying new treatments to improve recover following heart attacks by promoting heart regeneration following infarcts. The immediate beneficiaries of this work will be the fields of regenerative biology and regenerative medicine. However, the ultimate beneficiaries of these findings will be clinicians and eventually, patients who have suffered acute or chronic wounds or are suffering from degenerative diseases.</p>
<p>What species and approximate</p>	<p>All proposed studies are to be performed on</p>

<p>numbers of animals do you expect to use over what period of time?</p>	<p>embryos and larvae from two frog species, <i>Xenopus laevis</i> and <i>Xenopus tropicalis</i>, and zebrafish embryos, larvae and adults. These species have been chosen because they have remarkable abilities to repair and regenerate fully following injury, including some of their organs like their hearts. The approximate number of animals to be used under this licence will be approximately 44,200, of which most will be at the larval stages.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The vast majority of procedures proposed in this license will not lead to any expected long-term adverse effects. The most often used protocol in the licence involves a simple injection of hormones, to induce ovulation and/or mating in <i>Xenopus</i>. The second most often used protocol under this licence will be the generation and maintenance of genetically modified frogs and fish. The remaining protocols involve transplantation of cells between embryos and larvae of fish and frogs, the treatment of larvae or adult fish and frogs with substances or heat pulses to alter gene expression, and the creation of wounds in larvae or adults in fish or frogs. On very rare occasions when adverse effects occur, the animals will be humanely killed.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The main objective of this project is to investigate the role and regulation of reactive oxygen species in development and regeneration. Most of the planned studies will be conducted in embryos or on isolated cells and tissues in culture. To study the formation, repair and regeneration of complex tissues and organs, it is necessary to perform work <i>in vivo</i>, as it is not possible to recreate fully the complex environment of the developing and regenerating tissues in culture. The complex multi-tissue events that occur during tissue repair and regeneration cannot currently be replicated fully, using tissue culture techniques alone.</p>
<p>2. Reduction</p>	<p>Rigorous experimental design considerations will be employed in the conduct of all</p>

<p>Explain how you will assure the use of minimum numbers of animals</p>	<p>experiments to ensure that the minimum number of animals is used to reach meaningful conclusions. Overall numbers of animals required are based on initial sample size estimates. These numbers will be updated as more recent and relevant data becomes available.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Our recent work has shown that early embryogenesis mimics many of the same mechanisms, in respect to the role of sustained ROS production in tissue regeneration---- and replicated tissue development. This has allowed us to refine many of our experiments in order to exploit these similarities, and thus, we will focus much of our future work investigating the basic science mechanisms of ROS in cultured early embryos. This has led to both reduction and refinement in our procedures.</p> <p>In addition, frog embryos and larvae and fish larvae and adults are particularly well suited to this project because they have remarkable capacities to heal wounds quickly, without leaving scars. Complex tissues are regenerated within days or weeks following injury. This makes these organisms particularly useful in studying both the development of tissues and their repair following injury of their appendages and organs, such as the heart.</p>

Project	The role of amino acids and their transporters in the development of diabetes	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Patients with type 2 diabetes find it increasingly difficult to control their blood sugar levels due to less and less of the blood sugar lowering hormone insulin being released from their insulin producing cells. This is because these cells become sick and ultimately die leading to a worsening of the disease and an increased risk of succumbing to serious complications. Consequently, there is an urgent need to find new therapies that prevents this from happening. In order to find such therapies it is imperative to understand how and why these</p>	

	insulin secreting cells stop working. The aim of this proposal is to understand what causes the insulin secreting cells to become sick.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The knowledge gained from this study will help understand the processes leading to diabetes and identifying new drug targets. These are important steps in the development of new drugs for the treatment of diabetes. This would have a great impact on the lives of those people living with diabetes and the economy.
What species and approximate numbers of animals do you expect to use over what period of time?	Over five years we will breed up to a maximum of 1500 mice and use a maximum of 500 adult mice for experiments. The number of animals used will be kept to minimum through good husbandry and good experimental practice.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Breeding and maintaining of animals will not cause suffering, nor will putting animals on an altered diet. Procedures to measure the ability of the animals to regulate sugar levels may cause some limited degree of stress and discomfort and are thus these protocols are considered to have a moderate severity limit with regards to pain and suffering. However, all possible precautions will be taken to avoid any suffering. At the end of the procedure all animals will be humanely culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The experiments proposed here require the use of animals as it is impossible to investigate diabetes using cells grown on a bench. Mice are a good model for this as many of the features of obesity induced diabetes in man are similar to that seen in mice fed on a high fat diet.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A pilot study will be performed to determine the viability of the proposal prior to embarking on a full study thus potentially reducing the number of animals used. Statistics have been used to ensure that we gain meaningful results using the minimum number of animals. In addition, all the experimental protocols have been refined to reduce the number of animal used and the

	<p>mouse colony will be managed efficiently in order to avoid breeding excessive mice.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse is a well characterised and appropriate model system for studying diabetes as its physiology retains sufficient similarity to human physiology to provide meaningful transferrable information. Animals will be housed in stable groups in enclosures designed to cause the minimum amount of stress. All procedures will be conducted by experienced staff and all experiments have been refined to reduce any potential suffering.</p>

Project	The role of extrusion in asthma attacks and inflammation	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Asthma is a very common and deadly disease, yet current approaches only treat symptoms. We think that understanding its root cause is important to finding ways to cure asthma, rather than merely diminish its symptoms. Our basic studies on the cells that coat the airways suggested that constriction and tightening of the airways could destroy this protective coating, leading to inflammation and more infections, which can, in turn, cause more asthma attacks. Using a mouse model for asthma, we find that mimicking an asthma attack in mice indeed destroys the barrier and causes inflammation.	

	<p>our work in mice.REDACTED.</p> <p>From these studies, we discovered that asthma attacks result from too much muscle forming around the airways in mice and others have found this to be true in humans. We now are studying how this muscle builds up to cause asthma attacks. Our current data suggests that the airway destruction, in turn, causes airway muscle buildup. If this is true, our new treatment could prevent future asthma attacks, essentially curing asthma. To test this, we plan to: 1) use mouse lung slices and live mice to test if blocking airway linking destruction prevents muscle buildup. 2) develop ways to reduce airway muscle to stop attacks.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Asthma is an extremely common disease that is on the increase with no current treatment and the main approach is to manage symptoms. We have a completely new model for what causes asthma attacks and inflammation stemming from them. REDACTED.To test this model, we need to use a well-developed mouse model for asthma. While this has limitations, it is currently the best model for asthma that does not use larger animals (sheep, pigs, etc.) where we can test numbers sufficient to warrant a clinical trial in humans. Should we be successful, the relatively small numbers of mice we will need for this study will contribute to an entirely new approach that could finally treat asthma.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We should use no more than 1000 mice over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We have labelled the severity as moderate, based on our previous studies for inducing an asthma attack in mice and treatments. REDACTED. However, should we see any unexpected affects, we will immediately cease treatment and allow mice to recover or euthanise them, based on individual mouse behaviour (outlined in our protocols). We will need to euthanise all animals at the end of the study for analysis.</p>

Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We have already developed our studies in cell culture. This led to compelling finding, which we needed to test in an animal model, since the airway architecture and downstream effects of asthma attacks cannot be replicated in cultured cells. Mice are the smallest animal that we can do this testing in.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The numbers of animals required for this project have been considered in some detail, based on our own experience, as well as on information from the literature. We have already developed a way to use post-mortem tissue for most of our experiments so that we will only need to use live studies on a minimal number of mice to confirm our findings from ex vivo REDACTED.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Our REDACTED have already been incredibly useful in honing the best dose and length of treatment needed before turning to live mice. We will continue to use this approach so that we can best refine the treatments we plan to use before we take them to live mice. We will be diligent during our live animal studies to continually monitor and refine our techniques to ensure least harmful and best practices are always performed.</p>

Project	The role of genes and environment in diabetes in rodents	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our current research programme has two overarching themes: 1) determine the interplay between genetics and environment; and 2) understand how this leads to diabetes.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Type 2 diabetes currently affects ~10% of the adult UK population and is associated with a significant healthcare burden. The chronically increased blood glucose levels can lead to renal and heart disease, blindness, cancer and amputation. Benefits stemming from our studies will include: 1) the identification of novel avenues for the treatment of type 2 diabetes;	

	and 2) the production of drugs that allow better control over blood glucose levels and which display reduced side effects. The overall outcome will be the improved treatment of diabetes, leading to healthier ageing, decrease diabetes complications and reduced costs to the NHS
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse (15500) and rat (3000) over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All the protocols used in the present project are of mild to moderate severity and involve: 1) breeding of animals with specific genetic modifications associated with diabetes; 2) feeding of a high fat 'cafeteria' style diet to induce obesity and diabetes'; 3) clinical tests similar to those used in humans for the diagnosis of diabetes; and 4) removal of tissue under terminal anaesthesia for in vitro work. Expected adverse effects include mild discomfort from injection, anaesthetic complications, surgical complications, low blood glucose and fitting, weight loss and excessive urination. These will be recognised by good monitoring, daily checking and weighing. Adverse effects will be limited using the end points listed, e.g. failure to respond to treatment, failure to respond to glucose and weight loss (20% versus reference weight). In line with good practice, LASA or NC3Rs guidelines will be used for dosing (i.e. injection volume and needle size) and sampling procedures (i.e. blood and tissue sampling). At the end of the experiments, animals will be humanely killed
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The maintenance of normal blood glucose levels involves interactions between many organs (e.g. pancreas, liver, muscle and brain). Although the majority of experiments will be performed in vitro in tissue isolated postmortem, animals are sometimes required to model the complexity of glucose homeostasis, as well as determine what goes wrong during diabetes.

<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The minimum number of animals required to give a valid result has been calculated using careful statistical analysis. Moreover, animal use will be minimised by validating the majority of experiments firstly in vitro, or using computer simulations. Moreover, we develop new imaging approaches which allow measures to be gathered using fewer animals.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are amenable to genetic manipulation, are easy to house and handle, and are specifically bred to be unstressed in a lab environment. Rats are sometimes used when larger blood samples are required (e.g. for measurement of hormones). Lower vertebrates such as fish and invertebrates such as flies are not relevant here, since they regulate glucose differently to humans. All the procedures in this licence are classified as either mild or moderate and are done under local, general or terminal anaesthesia, where appropriate, to minimise stress and suffering of the animals.</p>

Project	The role of inflammation in cerebrovascular disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project aims to find out how inflammation contributes to devastating conditions of the brain that are a result of disruptions in the supply of blood or function of blood vessels, so-called cerebrovascular disease. This includes stroke as well as vascular dementia.</p> <p>We aim to find out how changes in inflammation in the brain and rest of the body are involved in the death of brain cells as well as the functional complications (cognitive decline, depression etc) seen in cerebrovascular disease. At present this is poorly understood and more research is needed.</p>	
What are the potential	Our research hopes to find new ways to treat stroke	

<p>benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>and vascular dementia, conditions that at present have no widely effective treatments.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Studies will be mainly in mice though some experiments will use rats. Over the five-year period of the project we expect to use 6900 animals in total. Approximately a third of these (2200) will be for breeding purposes and generation of transgenic animals with the rest (3900 mice/800 rats) being used in experimental procedures.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In order to mimic human stroke and vascular dementia we will use experimental procedures to reduce the blood supply to the brain in rats or mice (cerebral ischaemia). This will mainly be done by opening up the neck of animals through a small incision to reveal the carotid artery. This artery is one of the main ways that blood gets from the heart to the brain. Then, using a number of different approaches, we will interrupt or perturb this blood supply. . One way of doing this is through the injection of very small particles (or microemboli). These microemboli flow into the brain through the artery and then become stuck in blood vessels that are narrow. Alternatively, we can advance a fine filament (or suture) into the artery which will reduce the amount of blood reaching a large area of brain. We can also physically reduce the diameter of the artery that will reduce the flow of blood to the brain. Another way to disrupt blood flow to the brain is through haemorrhage (i.e. the rupture of blood vessels), and in rodents we can achieve this by directly injecting into the brain very small amounts of substances that cause minor blood vessels to burst. In addition to accessing the main arteries supplying the brain through the neck we can also do it through a small hole in the skull (a so-called craniotomy). For all the techniques described animals will be fully anaesthetised and will receive drugs (analgesics) to minimise any pain due to the surgery that is required. We expect most of the animals to fully recover from surgery and then they will usually undergo some tests of behaviour. These behavioural tests are designed to assess any problems with movement or sensation as would be seen in stroke patients, or memory problems as seen in vascular dementia, as well as other complications commonly reported by patients, including fatigue and depression. None of the behavioural tests are harmful to the</p>

	<p>animals and often just require observation for a short period in specialised apparatus. Tests can take place a few days or sometimes weeks after the initial surgery. In a few studies we will re-anaesthetise animals and use specialised imaging techniques to look at changes in how blood vessels function in the brain or the amount of brain cell loss etc. Animals may also receive simple injections or have blood samples taken. Clinically stroke, by its very nature, is a devastating disease, resulting in significant mortality and morbidity in patients. In trying to model stroke in animals a balance has to be struck therefore between establishing a valid model and in minimising pain, suffering, distress or lasting harm to the animal. However, as far as we are aware, effects of the stroke itself mainly result in discomfort to the animals, with severity kept to a minimum to ensure no lasting harm. The experimental approach to induce cerebral ischaemia is obviously specific to the experimental studies and it is inevitable that animals will suffer some level of pain due to the surgical procedures involved. At all times it will be our aim to reduce this to a minimum by the use of pain-relieving drugs. At the end of experiments animals will be killed by overdose of anaesthetic and we will take blood, brains and other organs/tissues to investigate various measures that will help us meet our overall aims.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Studying mechanisms involved in brain diseases such as stroke and vascular dementia is extremely complex. Alongside the death of cells in the brains of stroke and dementia patients, these diseases are characterised by profound changes in behaviour, which it is not possible to study in cells in isolation. The proposed animal studies are complementary to a broad programme of work on stroke/dementia using human samples, isolated cell systems and non-protected model organisms such as zebrafish embryos.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Pathological and behavioural end points proposed in this project are well established in studies of stroke/vascular dementia and experiments are planned based on our own extensive experience or previously published data. We will use the minimum number of animals that can answer the desired scientific objectives and will extract all relevant information in the data by using appropriate statistical analysis. Studies</p>

	<p>will be designed using the newly released Experimental Design Assistant (EDA) from the NC3Rs (https://www.nc3rs.org.uk/experimental-design-assistant-eda).</p> <p>We will also consult regularly with qualified statisticians with regard to experimental design and statistical analysis.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>A critical feature of this work is that it is focussed on changes in function of blood vessels in the brain and the supply of blood that result in stroke and vascular dementia – so called neurovascular function.</p> <p>Neurovascular function in rodents is comparable to humans and animals also develop post-stroke complications that are seen in humans, including depression and cognitive impairment. The proposed studies could not be undertaken in animals with a lower degree of neurophysiological sensitivity (e.g. drosophila, C.elegans) because they do not show such similarities to humans, and <i>in vitro</i> experiments do not allow the study of interactions between different body systems.</p> <p>We will use previously published methods to induced cerebrovascular disease, the choice of model being dependent on the hypothesis being tested. Models of stroke (both ischaemic and haemorrhagic) are extremely well established in many laboratories across the world and, though there is no ‘perfect’ stroke or vascular dementia model, those to be used in this project are chosen on the basis of their pathological and behavioural similarities to cerebrovascular disease in humans, which itself is extremely heterogeneous.</p> <p>All animals will be closely monitored for adverse effects and procedures put in place to minimise these, using very recent guidelines produced by the stroke research community. These guidelines draw on a wealth of experience in modelling stroke in rodents and have been produced through an NC3Rs working group that includes veterinary surgeons and other experts in animal welfare.</p> <p>Throughout the project we will continually review the literature and engage with colleagues/collaborators to learn of any new refinements to the protocols that could be implemented. REDACTED.</p>

Project	The Role of Inflammation in Efficacy and Safety Pharmacology	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The aim of this project is to determine the efficacy and safety of new treatments for human inflammatory diseases, including respiratory diseases such as Asthma, COPD (Chronic Obstructive Pulmonary Disease), Pulmonary arterial hypertension (PAH) and Pulmonary fibrosis, treatments for pathogen (e.g. bacteria/fungus) induced lung inflammation, treatments to aid the wound healing process , and treatments targeting lung injury initiated by exposure to chemicals that may cause pulmonary (lung) inflammation</p> <p>As of 2011, approximately 235 million people</p>	

worldwide were affected by asthma and approximately 250,000 people die a year from the disease. Mild to moderate asthma is well controlled with inhaled bronchodilator/corticosteroid combinations. Severe asthma, however, is poorly controlled with this treatment and maintenance and prevention of exacerbations remains an unmet clinical need. COPD is projected to become the fourth leading cause of death worldwide by 2030 and is already the third leading cause of death in the U.S. COPD is poorly controlled by current therapies and new drugs are required that prevent the progression of air flow limitation, Air flow limitation can also occur as a result of exposure to the inhalation of noxious gases e.g. chemical leaks in an industrial setting. Pulmonary fibrosis is the most prevalent disease of the lung tissue. In 2012 around 5000 people died from pulmonary fibrosis. There are currently only 2 treatment options short of lung transplant and despite these treatment options the average life expectancy following diagnosis of pulmonary fibrosis is only approximately 3 years. There is an unmet need for new treatments for pulmonary fibrosis. Pulmonary arterial hypertension is a condition where blood pressure in the lungs is significantly raised which affects both lung function and the cardiovascular system. There are currently estimated to be between 500-1000 new cases of PAH each year in the USA. There is an unmet medical need for patients of PAH as current treatments options are limited and heart and lung transplant is still required in some cases. One of the aims of this project is to aid in the discovery of novel treatments for PAH to avoid patients having to undergo invasive surgery such as heart and lung transplant. Many people are affected by pathogen induced lung inflammation conditions such as aspergillosis which is inflammation of the lungs caused by inhalation of fungal spores. The current recommended treatment for aspergillosis is Spornox which requires close monitoring to monitor potential side effects and to ensure the drug is working effectively. New treatments are required to improve the side effect profile. One of the aims of this project is to aid the discovery of new and improved treatments for diseases such as aspergillosis. Wound healing is an inflammatory process, and one of the aims of this project will be to assess the effects of test substances on the inflammatory processes associated with various models of wound

	<p>healing. Wound healing is a complex process which is not fully understood, and more effective treatments and cost-effective solutions may be developed using the results of such studies. Exposure to certain chemicals can induce pulmonary inflammation, particularly when the chemical is breathed in. For many chemicals there is no treatment to counteract the effects so as part of this licence models of exposure to certain chemicals will be developed and treatments to counteract the detrimental effects of exposure tested. Governments require and the public expects that medicines are safe and/or well-characterised. Therefore, before humans are exposed to new substances, their safety must be evaluated; this is a mandatory legal requirement. This safety assessment requires the use of animals in studies to evaluate systemic exposure/toxicity; currently, there are no scientifically, ethically or legally acceptable alternatives available that do not involve the use of animals.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The potential benefits of this project would include the discovery of new treatments for inflammatory diseases, and the confirmation of the safety of new treatments for inflammatory diseases prior to first administration in man. Work under this Licence will also show which compounds are not suitable to move forward into patients due to them not being able to moderate the clinical condition examined, or they are not safe to go into humans, for example. Work performed under this licence may identify more effective drugs for example to those already on market, with fewer side effects and that work better than existing drugs. Some of the experiments will also aid in developing treatment strategies in case of accidental exposure to chemicals, e.g. industrial, agrochemicals etc (crop spraying) or other chemicals that the public are exposed to. Some studies will be required by regulators to help them decide whether potential drugs both work in their chosen indication, and are likely to be safe in humans.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Potentially 23500 Mice, 30500 rats, 22000 Guinea pigs, 1300 Ferrets, 500 rabbits and 200 dogs may be used in the five year period of this licence. However, it is highly unlikely that these numbers will all be reached, as the work performed will be</p>

	<p>performed at one species per study per test item. Mice and rats will predominantly be used in these studies. These species are used because they respond to inflammatory agents in a similar manner to the humans and the data produced will help model inflammatory conditions that occur in humans (including inflammation cause by exposure to industrial chemicals), predict how well the potential medicines will work in humans, and predict the potential side effects of medicines in humans. In a limited number of studies, guinea pigs, rabbits, ferrets or dogs may be used, but they will only be used when the experiment required specifically requires these species.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>For the majority of experiments the induction, assessment and resulting inflammation will cause no or mild adverse effects such as slight weight loss. A small percentage of animals may show more significant adverse effects indicating moderate severity, e.g. more marked weight loss or reduced activity. To limit the animals discomfort, additional bedding, provision of moistened food within the floor of the cage, longer sipper tubes on water bottles will be provided. Specialist veterinary staff are always available to advise and assist in the welfare of the animal. Humane end-points are applied, under veterinary guidance as necessary, meaning that in the vast majority of experiments animals will be killed before they undergo anything approaching severe effects. There are a small number of experiments where to be able to accurately recreate what happens in humans when they are exposed to chemical inducers (e.g. Chlorine gas) of lung injury, it is necessary to expose animals to these same chemical inducers which may induce severe clinical signs in the animals. These animals will be closely monitored and appropriate humane endpoints applied. The severity of the adverse effects will always be kept to a minimum that is consistent with the scientific goals of the studies. Occasionally we may perform surgery to help us achieve the aims of these studies. This may include cannulation of blood vessels to administer drugs or take blood samples. We may (rarely) implant small pumps that dispense drugs over a period of time, which prevents repeated injections. we also may need to cannulate the trachea to measure lung function, and implant microchips to take temperature. These surgeries are only performed if its specifically</p>

required and the procedure means less overall suffering to the animals. Each animal will get pain relief and sometimes antibiotics under the supervision of a vet, and will get extra care after surgery much like a hospital patient would do after surgery. Various clinical conditions are modelled in our experiments, and the animals may experience similar effects as humans do, eg. tightening of the chest in asthma (the condition modelled is usually mild), as they will be exposed to agents that cause inflammation (by injection, by inhalation or by dosing orally for example). This is under very controlled conditions, and the exposure to these agents is usually minimised to allow the development of the condition, without overly harming the animals. The animals will also be dosed with putative drugs to help alleviate these inflammatory conditions by various means including injections and dosing via an oral catheter-these are techniques we are very proficient in and the minimum distress will be caused in these situations. On very rare occasions, other less standard routes including intrapleural dosing, maybe used. This will be in specific circumstances, and with full scientific justification given prior to the start of any study. Animals may also be confined for periods to allow dosing, or to measure lung function for example, and may well be introduced to the chambers needed to do this, so they are prepared before experiments start. Some surgery and a lot of the procedures performed in this project are done in animals who will not be allowed to recover from anaesthesia. This substantially reduces any potential suffering and distress animals may experience. Surgical procedures may result in some moderate effects like short term pain and weight loss, but the use of pain relief will be standard unless this is prevented by the type of study performed (not appropriate in studies testing some anti inflammatory drugs) as it will stop proper analysis of the results. Very rarely we may use mice that have been bred and altered to include either a human gene or have another genetic modification that, for example, models an inflammatory condition that is seen in humans. These mice can be used to test drugs against something very similar to the condition seen in humans, making it easier to see what a potential drug may do in the clinic in patients. This type of study is very rare. The modifications seen in these animals will not be dangerous, but may be harmful to the animals, so like all animals on

	<p>this project they will be very closely monitored under the care of experienced staff and vets. At the end of all of our experiments all of our animals will be killed in a humane way. This is required by the law which covers the use of animals for the purpose of testing drugs for humans.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Inflammation is a complex process which is not fully understood .It involves differing body systems and processes,many of which are joined and therefore for the protocols listed in this Project, there is no adequate non animal model to replace the whole animal experimental model, as the complex inflammatory and cellular mechanisms under investigation cannot be adequately modelled in non-sentient laboratory preparations e.g. in test tubes, for example.</p> <p>In many cases the protocols listed in this Project will be used some way into the developmental life cycle of a test substance and in many cases, particularly for pharmaceuticals in vitro (tests in test tubes) tests will have been conducted earlier (often by the customer) e.g.screening of potential test compounds for further development as part of the drug discovery process.</p> <p>Experimental designs are constantly reviewed and alternative cell assays considered as technology improves, however due to the complex nature of the inflammation pathways there are no current alternatives to use animals. Similarly, the regulators who decide whether potential new drugs are safe to be tested in man, will not accept tests solely using non animal methods</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>All experiments will be designed in order to achieve the scientific objectives using the minimum numbers of animals. For study types that are less well established and for which historical data may not be available, the literature (scientific publications) will normally be consulted to help decide the group size. Statisticians are often consulted particularly where the study type is not routine, as they can use calculations to estimate the correct number of animals needed to get a meaningful result.</p>

	<p>Where possible, common control groups will be used in order to minimise the numbers of animals used.</p> <p>For less established experiments, a preliminary study may be conducted in which smaller numbers of animals may be used to generate data in order to ensure that the experiment operates as we would expect and to generate some data which may be used to get a better study design. From such pilot studies, the variability of the measurements are used by statisticians to determine the required number of animals per group required to identify whether the test substance actually has an effect in a main study.</p> <p>Variables that may affect the study are kept constant wherever possible to make sure the experiments stay the same time after time. This actually means the data is more reliable and meaningful, and easier to make assumptions about.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Wherever possible, experimental tissues and samples are collected under terminal anaesthesia or at post mortem to reduce the burden on the animal used in the protocols. In some circumstances other cells may be taken from the same set of animals to give the maximum amount of data for the fewest number of animals.</p> <p>Highly trained staff use a rigid framework of welfare assessment to allow early detection of animals showing signs of discomfort or distress. We use pain relief as standard with anaesthesia and after procedures where relevant e.g. surgically implanted models. We sometimes, although rarely, use animals that have had their genetic material altered eg such that they are predisposed to developing a disease type or if a particular gene is important say in a specific component of the inflammatory process in which we have interest in.</p> <p>Although we may use various species on this project, rats and mice are the standard species used as they are of the lowest sentience. We would only use other species where their physiology means that they would be the best animal to use modelling a specific disease type. For example, Guinea Pigs would be the best animal to use when assessing cough and constriction of the lungs because guinea pigs are the species of lowest sentience that has a</p>

	<p>robust cough reflex Ferrets are the best models to use in studies with human viruses (they respond very similarly to the way humans do), and dogs would normally only be used to model dog specific illnesses for example Kennel cough</p> <p>This means we will use mice and rats unless other species will provide a better answer to the scientific questions we are asking due to their physiology being more suitable, or their reaction to a condition we are trying to induce being more like you would see in humans</p> <p>Dosing and sampling procedures will be undertaken using a combination of dose volumes, routes and frequencies that of themselves will result in no more than low levels of discomfort and no lasting harm and will be the minimum consistent with the scientific objectives. Many of the procedures carried out produce only minor levels of discomfort, due to the nature of the procedure, and the skill of the person performing it. For example, an animal having a blood sample taken would feel the same level of discomfort as a patient in a doctors surgery having a blood sample taken.</p> <p>In some protocols, animals that have previously undergone minor procedures (e.g. the use eye drops for ophthalmoscopy) may be again subject to satisfactory veterinary examination and relevant re-use criteria set out in the regulations from the government. This means that we reduce the total number of animals we use.</p> <p>Food and water withdrawal will be kept to a minimum. This is not routinely carried out and only occurs in very specific circumstances when it is an integral part of individual study requirement.</p>
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Project	The role of inflammation in neurodegeneration	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The number of people with dementia or vision loss is increasing in our ageing population and novel treatments are desperately needed. The typical memory loss, mood changes and loss of vision, are a result of dying nerves in our brain or the loss of rod and cones in our eyes, a process collectively known as 'neurodegeneration'. For many years, it was believed that neurodegeneration is a disease of the brain, but we now know it is much more complex. Research has shown that inflammation can damage nerves. The inflammation occurs in the brain as a result of ageing, but is worsened as a result of other diseases, which are commonly seen in elderly, such as infections, arthritis, gum disease or diabetes. Some of these so-called 'co-morbidities'	

	<p>are a result of our life style and our genes. The animal models proposed in this work are designed to study the inflammatory triggers that lead to the onset and/or progression of neurodegeneration and allow us to investigate how manipulation of the immune system may prevent, halt or treat age-related dementia or vision loss.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Worldwide, millions of people are affected by dementia or vision loss, for example caused by Alzheimer's Disease, Parkinson's Disease or macular degeneration. Currently there is no treatment to halt or cure these diseases. Better understanding of the biological process(es) that results in memory loss or vision, may result in novel ways to treat these devastating diseases of the brain. When we age, certain proteins form clumps in our brain or eyes, these are known as plaques. These can cause damage to the brain, but we don't know how. Inflammation has been recognised as a factor that can also cause damage to nerves. This may occur via the activation of specialised immune cells in the brain, called microglia, or, alternatively as a result of chronic infections, or lifestyle choices. Identifying the cells and proteins/molecules involved in nerve damage may result in a delay of memory loss, mood changes or vision loss. This will improve the quality of life of patients and their carer with dementia.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>To provide a better understanding of the processes of how local and systemic inflammation affect neurological disease this project will over a five year period use about 11,000 mice. We will use knowledge from our own previous research and from other scientists to calculate the number of mice required for a reliable scientific experiment.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In our programme of work we propose to model Alzheimer's and Parkinson's Disease via injection of misfolded proteins directly into the brain, which is expected to cause disease similar as seen in humans. We do not expect any adverse effects of these injection by themselves, but mice may develop dementia or motor symptoms, similar to humans. We are interested in the early stages of disease to identify novel ways to prevent or treat age-related neurodegenerative diseases and we will use behavioural tasks that can detect changes in</p>

	<p>the brain before overt clinical symptoms. This will reduce potential suffering to moderate levels. Some animals will be exposed to inflammation, which will activate, or inhibit, their immune system and some animals will be aged to study how normal aging contributes to brain dysfunction. This may induce sickness symptoms and reduced activity, reduced vision, and/or reduced motor strength. To model age related vision loss as a result of macular degeneration, we will use a laser to injure the retina; this procedure is painless in human and we do not expect this to be different in mice. We will carefully monitor their behaviour and any mouse in distress will be culled. Surgical procedures may induce infections and this may make the mice ill, or delay their recovery. Animals will be carefully monitored for any signs of infection and sterile equipment is used to prevent them. When the experiment is finished we will take brain or eye tissue, blood, and organs and analysis them for signs of nerve damage and inflammation. We will also look at the gut and look in poo samples, to get information about bacteria that can cause inflammation as a result of lifestyle and diet. We typically use a range of techniques, and state-of-the-art microscope to zoom into the brain and stain cells for disease markers, or measure genes following treatment of the animals. Similar techniques are used to analyse human tissues or cells grown in dishes, so we can compare the results.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We intend to use cells grown in dishes, where possible, for example nerves or microglia. We also intent to use brain and eye tissue from people that have died with late stage dementia or vision loss. However, this work is aiming to understand the role of inflammation before clinical symptom occur and it is unethical to conduct experimental on humans where removal of parts of the brain or eye is required for investigating the role of inflammation at the early stages of disease. Due to the complex interaction between the immune system and the nervous system there is no alternative that would entirely replace the use of living animals.</p>
2. Reduction	<p>The design of the individual experiments will be optimized to ensure that the maximum amount of</p>

<p>Explain how you will assure the use of minimum numbers of animals</p>	<p>data is obtained from the minimum amount of resource. We have already optimized many of our methods which means we can use small amounts of tissue for analysis. We will use the ARRIVE guideline to inform our experimental design.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse models which are proposed in our programme have many features in common with human neurodegenerative diseases, including loss of nerves, the activation of the immune system and typical behaviours such as memory loss and mood changes. We already showed that real bacterial infections in mice induce symptoms seen in humans and that a high fat diet results in subtle inflammation in the blood, similar as seen in humans. Previous studies using mice have provided a wealth of information on the role of inflammation in disease and these have resulted in novel treatments. A good example is a novel anti-inflammatory drug for people with arthritis that blocks inflammation and delays the progression of disease. This anti-inflammatory drug is now one of the most successful drug to date. The knowledge obtained from these mouse studies can be used in our models of dementia and vision loss. The immune system of the mouse has been studied for decades and the parallels with the immune system of humans are well known. Importantly, mice are also the only species where genetic manipulation has been carried out to permit further comparison and this allows us to investigate how genetics risk factors of dementia and vision loss are related to inflammation, life style choice or infection.</p> <p>To reduce lasting harm we will restrict our methods to induce mild systemic inflammation and regularly monitor for pain, distress and sickness behaviours using a well-being score and a pain score. If this score is exceeded, we will terminate the experiment.</p>

Project	The Role of Pattern Recognition Receptors in Immunity and Homeostasis	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our objective is to understand how molecules found on our immune cells, called pathogen recognition receptors (PRRs), enable our immune system to combat disease causing organisms and how these PRRs are involved in the development of autoimmune diseases of the joints (arthritis), lung (asthma) and eye (uveitis). Experimentally, to understand how particular PRRs are involved, we compare immune responses of normal mice to those of mice lacking that PRR and/or other components of the immune system. We may also need to use drugs to alter the immune system or types of	

	<p>immune response so as to understand the underlying mechanisms of resistance or susceptibility to disease. Using these approaches we can determine which PRRs are important, and the mechanisms that they use to control the development of disease.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The direct benefit of our research is the furthering of scientific knowledge of the underlying mechanisms of our immune system. These advances allow us to understand the disease process, and lay the foundation for new and better treatments for humans in the future. Our work has already led to substantial advances, as evidenced by our publication in scientific journals and the impact these have had on other scientists, measured by the number of times our work is cited by them. Our discoveries have already led directly to a greater understanding of what can cause disease in people, such as genetic alterations that cause predisposition to infections, and also a novel therapy for a fungal skin infection.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>29700 mice and 20 rats will be used in experimental procedures during the 5 year PPL. These numbers represent the theoretical maximum, and in practice will likely be less. Estimates are based on the required group sizes for experiments, experience on how many experiments are required to complete the studies, and on the number of researchers that will be working under this PPL</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Mice are given substances that can induce diseases which we need to study, including inflammation, eye disease, arthritis, and asthma. We also retain a small number of mice and allow them to age, to see what diseases develop, like in older humans. While the majority (approximately 70%) of mice used in our experiments will have no significant adverse effects, in some studies the mice can become unwell for a few days. These animals may show weight loss, become less active, have ruffled fur and appear hunched. During models of arthritis, their limb joints may become slightly red and swollen, and the mice may limp when walking on solid surfaces. We also use models of</p>

	<p>infectious disease, where mice are infected with microbes that cause illness. In most studies the mice can become very unwell and therefore this protocol is listed as severe. Animals will show up to 30% weight loss, become less active and isolated, have ruffled fur and appear hunched. In all our experiments, mice which become very ill are closely monitored and killed as soon as possible, once we have the scientific data we need. We anticipate that no more than 20% of the animals will show severe severity and for less than 24hrs. The time when we kill the animals has to have strong scientific justification and be agreed with the senior animal technicians and the veterinary surgeon. We have already established a very thorough system for monitoring these animals, which we use to minimise pain and suffering as much as possible. All animals are killed at the end of the study and their cells, organs or tissues are used for scientific analyses.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Our mouse models are for used for studying diseases found in people for which there are no other laboratory or computer based alternatives. However, we make use of non-animal models (such as human cells or laboratory grown cells) whenever possible and constantly look for new methods that would enable us to replace animals in research.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We reduce the numbers of animal we use for experiments, by maximising the number of scientific measurements we make for each animal and identifying and using the correct numbers of animals to give us the most robust scientific results. We also use highly inbred strains of mice of the same sex and age to increase the robustness of our results and cease breeding of mouse strains that we do not use. We also carefully monitor the breeding of all our mouse lines, to minimise the production of excess animals. In our experience, these approaches help to reduce our animal usage by at least 25%.</p>

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are a worldwide used model to study human disease and the working of the immune system. Most of the tools we need to use for our investigations have been developed for working in mice, including genetically altered animals. We also use rats to generate new tools that we can use in our mouse models, such as monoclonal antibodies. The use of other animal species will not provide the key insights we need to understand human disease. We make sure that our experimental approaches are the most appropriate and performed as best as they can be. We are constantly scanning the scientific literature and talking to other scientists to make sure we are using the best possible approaches. Where we start a new line of experimentations, we first make use of a small number of animals (a pilot study) to learn about the impact of these new approaches on the animals and how best to minimise any suffering. Where there is potential suffering for the animals, particularly during our infection protocols, this is minimised by ensuring that all the people performing the experiments are appropriately trained, and through the use of techniques for the alleviation of this suffering (e.g. such as pain relief medication). In all our experiments, we are constantly looking for new ways to improve the well-being of our animals.</p>
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Project	The role of respiratory sinus arrhythmia in biventricular pacing to improve cardiac function	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The heart is a muscular organ that pumps blood around the body to sustain life. A weakening in the pumping action of the heart, due to injury or disease, is called heart failure. Typical signs of heart failure include breathlessness, swelling of the legs and heart rhythm problems. Heart failure is a progressive condition and many patients die due to worsening pumping action of the heart. Some patients are suitable for treatment with a small electronic device, called a pacemaker, which provide electrical impulses to coordinate the pumping action of the heart	

	<p>chambers. These specialised pacemakers have revolutionized the treatment of heart failure have been shown to both improve the function of the heart and reduce the symptoms of heart failure. However, in up to a third of patients they fail to impart any discernable health benefits.</p> <p>In healthy people, the heart rate changes as we breathe - heart rate increases slightly when breathing in and decreases slightly when breathing out. This is known as respiratory sinus arrhythmia (RSA). However in heart failure patient RSA is often lost and is associated with a poorer outcomes for patients.</p> <p>Current pacemakers regulate the heart rate and can respond to levels of activity but do not adjust heart rate as the patient breathes. The purpose of this study is to assess if a novel pacemaker which is able to adjust heart rate in response to breathing, in the way that a healthy heart functions, will improve the outcome for patients suffering from heart failure.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>If successful, the study will pave the way to first in man trials of this new pacemaker that regulates heart beat in a manner that replicates normal heart function. We propose that this new device will improve the heart function of patients suffering from heart failure, above and beyond that achieved using currently available devices. In particular, the new device is expected to benefit the one third of patients who currently receive no discernable benefit from the standard available pacemaker.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The study will be conducted in pigs and sheep as their cardiac anatomy, size and heart rate closely replicates that of humans. Data generated during the initial phase will be used to determine the group size needed to achieve statistical significance for each of the various data sources needed to determine the effectiveness of the intervention and the advice of biostatisticians will be obtained to determine the group size needed before commencing the main device study. We estimate approximately 90 animals will be used in total during the</p>

	project. The study duration will be 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The severity level for this work is moderate as the model system requires surgical procedures and imaging to be performed. These procedures will be performed by experienced surgeons and to NHS standards. The procedures undertaken are not expected to compromise the well being of the animals once they have recovered from the surgical procedure. Throughout the study period, animals will be monitored closely and given painkillers as necessary. At the end of the study they will be killed to enable tissues to be collected to determine the effectiveness of the treatment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The purpose of the study is to assess the efficacy of a novel cardiac pacemaker for the treatment of heart failure. It is not possible to undertake this study without the use of animals as there are no suitable tissue/organ culture or computer models. Furthermore, successful studies in animals are a requirement before progressing the new treatments into human clinical trials. Consequently, large animal studies that replicate the clinical scenario are the only viable means of progressing this work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The study design aims to minimise animal numbers. The initial phase of the study involves the development of an animal model that replicates the pathological cardiac remodelling that occurs in patients suffering from heart failure. Data generated during this initial phase will be used to determine the group size needed to achieve statistical significance for each of the various data sources needed to assess efficacy. The main experiment will involve three experimental groups to compare the effectiveness of the novel pacemaker with a conventional pacemaker. Following the initial modelling phase we will seek the advice of biostatisticians with regards to the study design and group size needed before commencing the study.

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The pig and sheep are the most suitable animal models for these studies because their physical size and thoracic anatomy enables use of sampling, imaging and interventions used clinically in human patients. All surgical procedures will be undertaken by cardiac NHS specialists working in a state of the art facility that matches or exceeds that found within the very best NHS units. Post-operative care and pain control will match that offered to human patients undergoing similar procedures. The procedures undertaken are not expected to compromise the well being of the animals following recovery. All animals will be group housed with companions, or singly housed close to other animals. Animals will be kept on deep straw beds and provided with toys, novel objects and food treats throughout the study.</p>
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Project	The role of the microbiota in nutrition, metabolic diseases and colorectal cancer
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research <input checked="" type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The gut bacteria and other organisms in the gut (together known as 'microbiota') are known to be involved in many diseases such as obesity, diabetes and cancer. However, many questions regarding the role of the microbiota on our health and in disease remain unanswered. For example, do they in some way control our risk of disease? Weight loss surgery has become increasingly popular in treating morbid obesity and diabetes and it causes gut microbiota changes. Although there are benefits of weight loss surgery (reduced body weight and reduction of type 2 diabetes needing treatment), opinions differ on whether

	<p>it is helpful or harmful as it also increases bowel cancer risk. This research aims to make animal 'models' with different microbiota in order to investigate their role in weight loss surgery, diseases such as diabetes, and bowel cancer.</p> <p>The objectives include</p> <ol style="list-style-type: none"> 1. Can the microbiota alone produce the same benefits to humans as weight loss surgery such as lowering the risk of type 2 diabetes needing treatment? 2. Do microbiota changes resulting from weight loss surgery cause bowel cancer? 3. Can different parts of our diet influence the risk of bowel cancer? 4. Does the microbiota in mothers affect that of their children which goes on to affect the risk of these children getting diseases like diabetes in later in life?
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This project will increase knowledge of how the microbiota affects health and diseases like diabetes and cancer, which in turn will benefit all scientists and clinicians working in this field and we hope ultimately to lead to better ways of treating and preventing such diseases. We aim to make a significant contribution to research into the potential benefits of transplanting microbiota into disease sufferers. This may ultimately lead to us being able to manage these diseases in a more patient-friendly way, identifying associated diet or lifestyle changes which will reduce the need for surgery. This will minimise the cost to society of managing such diseases, at the same time benefiting patients.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Rats (n=250) Mice (n=5060) Over 5 years</p>
<p>In the context of what you propose to</p>	<p>Animals are likely to lose weight after</p>

<p>do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>microbiota are transplanted to their gut and also if they are put on a restricted calorie diet. Any animal whose body weight falls below the agreed expected normal limits, and any showing signs of distress (this is extremely unlikely) will be humanely killed. Animals will be anaesthetised when they undergo imaging which will expose them to potentially harmful radiation, but radiation doses will be kept as low as possible. Where relatively higher doses need to be used to obtain useful scientific information, the animal will be humanely killed whilst still under anaesthesia. Animals will be continually monitored during experiments for signs of distress and humanely killed if this is seen. Any animals which are expected to develop disease symptoms such as tumours will be continually monitored daily throughout the experiments and researchers will contact Named Veterinary Surgeon for advice immediately when unexpected signs occur. At the end of the experiment all animals will be killed using a humane method.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The main emphasis of the project is on the interactions between diet, gut bacteria and the host (animal), which cannot be achieved without using animals. We have been doing studies in obese patients who underwent weight loss surgery and have some ideas about how their weight loss after surgery affects, or is affected by, the type of bacteria in their gut. However, it is not ethical (morally right) to conduct further experiments on humans in order to find out if our theories are correct. Although microbiota transplant is used for treating patients infected with <i>Clostridioides difficile</i> (bacteria causing diarrhoea, often after antibiotic use), this method is yet to be used for other disease treatment. If we are to make further advances with this type of treatment and apply it further, the animal experiments are crucial at this stage. Therefore, there is no feasible alternative that would replace the animal use.</p>

<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We have consulted the departmental statisticians on experimental design and group size to minimize the number of animals used and maximise the information gained.</p> <p>Standard methods will be agreed for all experiments and procedures and researchers will be trained to fully understand the aims of the experiment and how to carry out procedures correctly so that these do not have to be repeated.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rats and mice will be used in the proposed project. These animals are widely used by scientists to 'model' cancers of the bowel and will allow us to investigate the role of the microbiota in cancer and to study if changing diet or the microbiota helps to reduce the risk of cancer.</p> <p>The microbiota will be transplanted through a tube directly into the animal's stomach, which will cause minimal discomfort. Anaesthetics will be used if animals are to undergo a procedure which might be uncomfortable or painful and pain relief administered as it would be in humans. Liquid food is easier to eat and digest and will be fed when necessary rather than dry food. Animals will be introduced to a different type of caging if needed for the procedure, by gradually increasing the time spent there until they are familiar with it. All animals will be housed in groups where possible, with nesting material and play tunnels, and fed according to current institutional 'best practice'.</p>

Project	The role of TRP ion channels in pain
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	Regulatory use and routine production
	Protection of the natural environment in the interests of the health or welfare of humans or animals
	Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Pain is a common medical condition associated with many diseases such as arthritis, and poses significant challenge for everyday life of the patients. Cold is widely used as an analgesic for alleviating pain. Paradoxically, cold also triggers pain. The opposite effects of cold may be the reason why the cold therapy has only a modest effect. However, it is poorly understood how cold exerts such contrasting effects on pain. The objectives of this project are: (1) to determine sensory nerve cells responsible for transmitting pain and analgesia, respectively, evoked by cold; (2) to determine the role of these nerve cells in chronic pain; (3) to determine the role of TRP channel proteins in pain. This research could reveal novel pain and analgesic pathways leading to more</p>

	effective pain therapies.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Scientifically, the project could reveal novel proteins and nerve circuits responsible for carrying pain and analgesia, respectively. It would thus advance our understanding of pain transduction and the opposing effects of cold on pain. Practically, the project could act as a fundamental basis for guiding and improving the current practice of cold therapy. Finally, in the long term, the project could lead to the development of novel analgesics that target on pain transducing molecules and pathways revealed in this project. The project could thus be beneficial to many of the patients suffering from pain.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used. It is estimated that roughly <u>3000</u> mice will be used over five years of time.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will induce inflammatory pain, arthritis pain and <u>neuropathic pain</u> in mice followed by assessing pain behaviors of mice. Therefore, mice would inevitably experience moderate severity of pain <u>in the affected limbs and paws</u> and have difficulty in free-walking. All the animals will be culled at the end of experiments using a Schedule 1 method. <u>Furthermore, animals showing any signs of autotomy such as constant biting their paws and toes will also be culled using a Schedule 1 method.</u>
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The main objective of the project is to test the role of channel proteins and their regulators in pain behaviours in animals. The research thus necessitates the use of whole animals for assessing systemic pain behavioural responses. Cell and tissue models cannot be used to predict or synthesize systemic pain behaviours, and thus cannot be used as a replacement, though we examine functions of isolated cells to predict animal behaviours
2. Reduction Explain how you will assure the use of minimum numbers	Power calculations will be used to estimate the minimal number of animals required for experiments based on literature and previous experience. Furthermore, unbiased experimental design will be

of animals	carried out to minimize the number of animals to be used, while obtaining more information.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Species of animals:</p> <p>Mice were chosen for the following reasons: first, pain behaviours of mice are well characterized. It will make our results comparable to the results obtained from other labs. Secondly, the transgenic mice lines to be used in the project are already available, thus obviating the need to generate transgenic lines from a different species from scratch.</p> <p>Animal models:</p> <p>To examine the specific effect of cold on arthritis pain, acute arthritis pain and chronic rheumatoid arthritis and osteoarthritis pain models were chosen. These pain models are comparable to arthritis pain in humans and are thus representative, and have been widely used to study pain mechanisms. Moreover, arthritis pain in these models can be conveniently induced in one knee of mice, while another knee can act as a control, thereby capable of reducing the overall suffering of animals. We will aim to reduce the duration of arthritis pain to less than 4 weeks in order to minimize pain suffering. However, analgesics cannot be given to animals during arthritis pain, because it is our primary objective to monitor the effect of cold on pain behaviours.</p> <p><u>We will also generate neuropathic pain models in which sciatic nerve controlling the hind limbs is partially injured. These models produce less injury and severity compared to other severe neuropathic pain models involving complete transection of sciatic nerve. However, these models mimic human pain and recapitulate the typical mechanisms and symptoms of neuropathic pain, and have been widely used.</u></p> <p>Measures to minimise welfare costs:</p> <p>All the pain assessment will be carried out aiming to reduce the exposure time of mice to painful stimuli and to avoid tissue injury. Drug administration will be carried out in accordance with the LASA guidelines. Animals with signs of ill-health and <u>regular abnormal behaviours</u> will be humanely culled using a Schedule 1 method.</p>

Project	The sensory mechanisms of the animal magnetic sense in birds and fish	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Many animals appear to detect changes in the Earth's magnetic field but remarkably, we still do not know exactly where in the body the magnetic sense is located. A recent discovery has demonstrated that electromagnetic noise similar to that produced by every day electrical equipment used by humans stops birds tested in laboratory conditions from detecting the magnetic field. Crucially, this electromagnetic noise is at a level below that considered safe for humans. It is not clear however whether this would extend to birds navigating in the wild, where in principle they would have access to	

	<p>other cues such as the sun, odours, visual cues or sound. We therefore need to test this phenomenon on free flying birds to assess its impact. On the other hand, in order to be able to fully understand and trace the location of the magnetic sense within the body, laboratory studies are required that can take advantage of the latest techniques and trace magnetic responses of the nervous system. This will help us to understand the structure and function of the magnetic sense.</p> <p>On this basis, we aim to investigate the magnetic sense from two standpoints, using the most appropriate animal for each task. First, to understand how the magnetic sense is used in the navigation system we will use the homing pigeon. Homing pigeons are able to return to their home loft from places they have never been to before, and have been demonstrated to use a number of different cues to do this, including the Earth's magnetic field. Understanding the role of the magnetic sense and whether electromagnetic noise disrupts it will inform us on whether pigeons can overcome the disturbance effect of electromagnetic noise. Second, to understand where the animal magnetic sense is located in the body and how it works, we will use Zebrafish. Genetic and molecular techniques exist in this species to observe the nervous system in action. We therefore aim to establish a behavioural approach to clearly demonstrate that zebrafish can respond to a changing magnetic field which will open the possibility of investigating the magnetic sense using techniques not available in birds.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Establishing a reliable behavioural approach for studying the magnetic sense using the zebrafish will provide the potential to trace the location of the magnetic sense using powerful research tools including the ability to assess their nervous system in detail. Understanding when electromagnetic noise disrupts the magnetic compass sense will help us to inform the safety standards that apply to the rapidly expanding wireless phone and mobile broadband industry. This project will also provide excellent research</p>

	and teaching opportunities REDACTED .
What species and approximate numbers of animals do you expect to use over what period of time?	Purpose-bred (AB strain) zebrafish, maximum of 300 individuals over 5 years. Homing pigeons bred by UK professional breeders, up to 100 individuals over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	To train zebrafish to respond to a magnetic field, they will receive a short (less than 1 sec) electric impulse (most likely less than 3V comparable with a gentle pinch) which should not exceed the mild level of severity. Homing pigeons will receive one of seven treatments: a clock-shift which shifts their perception of daytime, a magnet attached to head, a magnetic pulse, electromagnetic noise, a local anaesthetic (such as Xylocain, used by dentists to numb areas of tissue) to the inner beak, eye caps or a perforated eardrum. All of these treatments are reversible, and with the exception of the perforated eardrum, non-invasive. None of the treatments have been shown to have a permanent effect on a pigeons' sensory system or their ability to home, and so we do not expect this to exceed mild severity. After the project, zebrafish will be humanely euthanized according to the ASPA regulation, and homing pigeons will be kept alive REDACTED .
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Because the location of a magnetic sense remains unknown, studies require using live animals such as zebrafish. To assess the effects of electromagnetic noise in the wild we need to use homing pigeons to see the effect of the treatment on an actively navigating animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Overall, the design of experiments will be based on a thorough research of the relevant literature. Only established approaches will be used to decrease variability and increase the chance of finding a significant effect with the minimum numbers of animals required. Where possible, the same animals will be used throughout the project. All data collected from free-ranging birds will be also shared between several researchers so that the same data will be used

	for separate and independent analyses. This will allow us to avoid duplicating experiments.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>To establish a laboratory method for investigating the magnetic sense, the zebrafish is the most suitable animal. The tools available to investigate its nervous system non-invasively and in detail will, after the establishment of a behavioural response to magnetic fields, allow us to make significant advances in tracing the location of the magnetic sense. Animals will be checked daily during experiments for signs of stress such as behavioural changes or loss of appetite and will be removed from the experiment if assessed to be showing such signs.</p> <p>The homing pigeon is the ideal animal to assess the risks of the disturbing effect of electromagnetic noise for free-ranging birds as they home to their loft with high accuracy and motivation. They are large enough that they can fly with miniature devices affixed as backpacks with harnesses during short-term flights that will not disrupt their behaviour. We will use the lightest devices available to further minimise effects. During the experiments, the behaviour of animals will be closely monitored for signs of pain, distress and ill health by the experimenters, experienced technicians and the named veterinarian. Any animal showing such signs will be removed from the experiment. Releases will be carried out only under good weather conditions (no rain or strong wind). Sites that have evidence for high bird of prey activity will be avoided. For the disturbance of navigational cues we will use only reversible, painless and temporary treatments.</p>

Project	The social behaviour circuit in zebrafish	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Humans are fundamentally social animals. We spend the majority of our time with other people. Our ability to communicate with them, is unparalleled. However, even the most complex social skill requires a basic behaviour, “social preference”, which is the ability to recognise, find it rewarding and approach other members of our species. This essential “social preference” is hard-wired into our brain. For example, new-borns immediately prefer to look at faces. If this social preference is lost, then our entire social development is affected, as testified by several neurological disorders (for instance autism).</p> <p>This research projects aims to understand how</p>	

	<p>social preference is built into the brain, such that we can better understand how it might be impaired. In addition, we want to understand how/if social preference responses can be modulated in the presence of other stimuli salient stimuli, such as somatosensory stimulations. This is difficult to study in humans because the brain circuits involved are established before we are born. Zebrafish, however, are small transparent fish that develop ex utero allowing to follow the development from a single cell into a social organism within just a few weeks. Therefore, they provide a unique opportunity to watch the development of the neural underlying social preference behaviour, and to identify what goes wrong in developmental diseases like autism.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The primary aim of the project is to answer basic biological questions such as what are the zebrafish circuits that process social visual information, how they develop, how they process social information together, how they can be impaired during development and how they can be modulated. By establishing the zebrafish as animal model to study social behaviour in larvae and juvenile fish for the first time, it will be possible to 1) identify environmental and genetic factors that can cause impairment of the anatomical and functional circuit; 2) screen for drugs that could reduce or rescue alterations of social behaviour, 3) test how drugs that are already in use in humans can cause social impairments or modify somatosensory responses during development.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The projects will look at the development of zebrafish from larvae to juvenile. We expect to use about 70,000 zebrafish for experiments and maintaining the line over the course of 5 years. The vast majority will be used for breeding and maintaining our genetic lines.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity?</p>	<p>The majority of the experimental procedures will only cause mild or undetectable adverse effects on zebrafish. During the experiment we will routinely monitor the state of health of the</p>

<p>What will happen to the animals at the end?</p>	<p>fish by imaging specific brain areas. In some experiments we will remove some of the elements of the social or somatosensory neuronal circuit in order to verify their causal key role in processing visual social or somatosensory responses. These experiments will be necessary to prove the direct involvement of a specific brain area to a social function or behavioural output. At the end of these experiments larvae or juvenile zebrafish will be euthanized using an overdose of anaesthetic. This is a procedure approved by the Home Office.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The social brain network comprises multiple structures that are scattered throughout the brain. In order to fully understand how these areas are established, and how they process social information during the whole development we will need to monitor brain activity in a living animal that is presented with social information</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will reduce the animal number to the minimum by:</p> <ol style="list-style-type: none"> 1) always maintaining only the minimum number of fish per line to maintain the colony. 2) making statistical power calculations that will give us an idea of the minimum number of fish per experiment that can provide significant results 3) Using powerful imaging methods that allow acquiring better quality and larger amount of data with fewer fish.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Zebrafish are the best animal model for this project for the following reasons:</p> <ol style="list-style-type: none"> 1) They are vertebrate and therefore their brain structure is similar to humans 2) They are transparent and therefore allow non-invasive imaging of brain activity throughout the whole brain and with single cell

	<p>resolution. This method is harmless and allows us to monitor many more cells simultaneously and reduce number of experiments.</p> <p>3) Their development occurs <i>ex utero</i>, so we can follow the development of the whole brain from fertilization.</p>
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Project	The study of epithelial cancer genes	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>We aim to identify the genes responsible for the development of different types of breast cancer and lung cancer.</p> <p>In particular, the research will focus on an aggressive form of breast cancer which has a high possibility of spreading to other tissues, and patients with this cancer have low rates of survival. Identifying genes that are responsible for the development of breast cancer is essential for the development of treatments in the future.</p> <p>We will also be studying lung cancer. Nearly 45,000 new cases of lung cancer are diagnosed annually and nearly 35,000 people</p>	

	<p>die from the disease in the UK every year. Broadly speaking there are two major types of lung cancer - small cell lung cancer (12% of cases) and non-small cell lung cancer (NSCLC) (88% of cases). There is an urgent need for the development of more effective treatments for NSCLC as currently only 16% of patients survive for 5 years or more after their initial diagnoses. To improve that, a better understanding of how the various types of lung cancer develop is required.</p> <p>We will carry out genetic analysis to identify and characterise key factors that drive the development of breast and lung cancers in the hope that, in the future, this knowledge may help in the development of new treatments.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Results from this project could have an impact on the 100,000 breast and lung cancer patients diagnosed every year in the UK. Our study aims to: 1) Increase the understanding of how types of breast and lung cancers develop; 2) Identify new biological 'markers' to enable better cancer diagnosis.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mouse – 43,180 over 5 years. Out of this number, 20,000 mice will be used for breeding and maintenance of genetically altered animals. 22,000 will be used for breast and lung cancer studies.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>REDACTED Under this licence we propose to study the role of candidate genes in driving breast and lung cancers. To achieve this we will use different methods to induce tumours in mice: 1) we will breed and use genetically modified mice carrying mutations found in humans that are known to increase the susceptibility to cancer; 2) we will use chemicals (delivered orally or via injection) to induce tumour development and; 3) in some cases, we will grow human cancer cells in mice, and; 4) in some cases, we will surgically transplant modified breast cells into the cleared breast tissue (Fatpad) of mice to test the effect of the certain genetic mutations on cancer development. We will also use these different mouse models of cancer to</p>

	<p>assess the effect of certain drugs on tumour growth. We expect the majority of the animals will have mild adverse effects from our work, indeed about half will only be used for breeding and maintenance of genetically altered animals and not subject to any intervention. For some animals, a moderate severity limit is expected, particularly if the animals develop tumours. Animals that will undergo surgery are expected to recover from the procedure with minimal complications. All surgical procedures will be performed under aseptic conditions therefore, reducing chances of contamination and complications.] However, we have protocols and humane end-points in place to minimise suffering in these animals, and will not allow any animal to suffer more than a moderate level of pain, suffering, distress or lasting harm. Animals expected to develop tumours will be constantly and carefully monitored for any signs of ill health or distress, and no tumour will be allowed to grow beyond 1.2cm². We expect all tumour bearing mice to exhibit no more than moderate severity levels of distress.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Cancer is a complex disease that develops in intact tissues. It is necessary to have a realistic model, which is amenable to genetic, and biochemical studies whilst maintaining the tissue architecture. The mouse allows us to perform detailed genetic and biochemical studies whilst maintaining the 3D organisation and normal physiological environment of tumours in the body. We will aim to use human cell lines in culture dishes in the laboratory, whenever possible, to perform some of our studies.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will restrict our analysis of genes using mice to those showing potential clinical relevance in large patient datasets.</p> <p>1) We will use the latest gene editing technology (called CRISPR/Cas9) to perform our experiments which will allow us to reduce</p>

	<p>the number of animals needed to generate a genetically modified mouse.</p> <p>2) When possible we will perform pilot studies on human cell lines in the laboratory before moving on to animal experiments.</p> <p>3) Statisticians have been consulted on experimental design to minimise the number of animals used whilst still obtaining meaningful results.</p> <p>REDACTED When possible and experimentally appropriate to do so, mice from the core colonies which have not been genetically modified will be used as controls for genetically altered animals. This will mean that we do not need to breed extra mice to use as controls.]</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse provides a good model for various human diseases. The genetic and physiological similarities between human and mouse are significant thus the mouse provides a good model to study cancer biology.</p> <p>We constantly review our surgical procedures to minimize the impact on animal welfare. We have recently changed how we carry out the surgical procedures in this study which will reduce the chances of post-surgical complications and also reduce the amount of time an animal is under general anaesthesia.</p> <p>We use pain-killers to minimise any discomfort the animals might feel after surgery.</p> <p>For all tumour experiments no animals will be allowed to suffer unnecessarily. No animal will be allowed to suffer from ulcerated tumours or any effects on movement, vision, eating, excreting or breathing. All animals will be monitored closely for signs of deteriorating health or suffering.</p> <p>At present, for our surgical procedures, we make a cut in the skin in the shape of a letter 'T', but we are currently trying out the use of a single straight cut to see if that minimises scar tissue formation.</p>

	<p>We use tissue from ear-clips which are taken for routine animal identification purposes to obtain the DNA we need to carry out genetic analysis. This means that we do not need to take any additional samples, such as from the tail, in order to get samples to carry out genetic analysis.</p> <p>In addition, the animals will be housed in a facility, which is equipped with world-class equipment, and highly trained staff that regard animal welfare as a priority. The life of every mouse, including its health status, is captured in a bespoke database.</p>
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Project	The testing of bone scaffolds in sheep	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	In this study we will test the ability of a biodegradable polymer based scaffold to encourage bone growth. This will be tested in a bony defect in the knee of sheep. The material to be tested is a 3-dimensional (3D) scaffold with a high number of linked pores, which should allow cells to migrate through the defect whilst providing support for the bone to grow. In addition the scaffold will be coated in substances which encourage bone to grow and have stem cells added which should increase the rate of bone growth.	
What are the potential benefits likely to derive from this project (how	Worldwide there are a large number of surgeries carried out that require bone	

<p>science could be advanced or humans or animals could benefit from the project)?</p>	<p>grafting, which is classed as the gold standard and therefore serves as a point of reference against which other bone graft materials may be compared. This can be due to fractures, osteoarthritis and osteoporosis, which are more prevalent with an aging population. Bone grafting has significant risks associated with it, for example rejection and infection, multiple surgeries, increased hospital stays and greater demand and costs put on the NHS. There is also usually a lack of sufficient bone graft, therefore donor bone may be needed. The material we are working with will hopefully be able to support bone growth, cell migration and the formation of blood vessels within a defect site, and could benefit humans. This in turn should reduce the number of surgeries and infections associated with bone grafting and provide a material which encourages better bone fill to a defect site.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>76 sheep over 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We expect that the animals will be slightly lame for a couple of days and then will have no adverse effects of the surgery. In addition the animals are generally standing and eating in about 1 hour post-op. Animals will be humanely killed to allow us to harvest the knees and investigate the amount of new bone that may have grown into the defect site. We will use micro-computed tomography and histology to look at the quality and the amounts of new bone within the defect.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Cell based studies are useful to look at how cells will behave in contact with the scaffold, however cell assays alone cannot adequately model the complete array of effects important in bone modelling or repair. Using stem cells we have shown that cells were viable, proliferated, and were evenly distributed throughout the scaffold, suggesting that the material would not be toxic and encourage</p>

	<p>bone growth. Predictions of degradation rates and pore numbers have been obtained from these studies for varying formulations; however none of these assays can adequately model the <i>in vivo</i> environment of a whole animal. Therefore we need to test the material in an animal model before it can be translated into use in a human.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use both hind legs in an animal to reduce the overall number of animals used. Statistical analysis has been employed to calculate the minimum number of animals we could use and still have a scientifically relevant study. This value is a minimum of 6 defects per experimental group. Data will be analysed using a suitable statistical package and statistical tests, for example one way analysis of variance and post-hoc testing. All experiments will be conducted in a manner that will allow high quality publication.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The use of sheep in orthopaedic research is increasing. Sheep are useful models as they have a similar bone and joint structure, body weight and have a comparable rate of bone remodelling as humans. The model involves initially aspirating cells from the sternum of a skeletally mature female sheep and culturing them, before creating a bony defect to return them within the scaffold material. All surgery is carried out aseptically in dedicated facilities with experienced staff. Control defects to compare the scaffold's success to will be created using either bone graft from the sheep or leaving the defect empty. Control sheep will only undergo a single anaesthetic event.</p> <p>All animals will receive pain relief during and after surgery. Antibiotics will also be given to prevent infection. After complete recovery from anaesthesia the animals will be returned to group housing. From previous studies we do not expect the animals to be lame for a significant period of time, but if they are unable to stand or not showing signs of improvement they will be killed to prevent any suffering. A scoring system will be used to monitor the animal's well-being after surgery.</p>

Project	Therapeutic Antibodies in Haematological Disorders	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The main objective of this project licence is to understand how novel monoclonal antibodies, an antibody produced by a single clone of cells or cell line and consisting of identical antibody molecules, effect animal models of blood disorders. This understanding will underpin the development of these new treatments for these blood disorders. Present therapies for blood disorders suffer from side effects and patients developing resistance to the treatments or not responding initially. This leads to many patients unfortunately still suffering from the clinical symptoms of these debilitating conditions which reduce both the quality of life and can reduce	

	<p>lifespan.</p> <p>With the data provided by this project licence we intend to develop new therapeutics for blood disorders (including anaemia and haemophilia), bringing the opportunity of long term relief to a larger number of patients than can currently benefit from present therapies. We aim to generate new treatments, based on a class of drug called monoclonal antibodies. During the course of discovering new monoclonal antibodies, we will also be addressing the questions of which patients will respond and whether our treatments can help those who do not respond to the currently available therapies. This licence will also provide essential supporting data for the antibody therapies being evaluated, enabling us to determine the way our molecules are working.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The potential benefits of this project will be the development of new knowledge of blood disorders, the provision of data which will underpin the development of novel therapeutic antibodies leading to the progression of these new therapies into clinical development and ultimately onto the market bringing benefit to patients.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use approximately 5,500 adult rats and 5,000 adult mice over 5 years for this project</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The animals used in this licence will be used to develop me antibody therapies to treat haematological conditions. This will include establishing the pharmacokinetic (how the organism effects the drugs and how long the drug is effective) and pharmacodynamic (how the body reacts to treatment with an agent) of therapeutic antibodies in both normal and diseased animals. Animal used under this licence will also be used to establish if therapeutic antibody therapies are effective at treating animal models (of anaemia and haemophilia) of haematological diseases and to establish if they are better than present therapies at treating these models. The majority of the adverse expected under this licence (e.g. weight loss, pallor and hunched posture) will be associated with the anaemia and inflammation</p>

	<p>associated with these models. This anaemia and inflammation may have effect the whole animal, and can affect the general welfare of the animal. We have put in place measures to closely monitor the effects of this anaemia and inflammation on animal health enabling us to monitor the impact on the animal. Animal condition will be checked daily and a closer inspection and weighing of the animal will be carried out three times a week any changes in condition, weight and / or behaviour will be noted and animals deviating from normal condition and/or behaviour will be assessed. If necessary, following consultation animal care technician and /or the veterinary surgeon, mice maybe further closely monitored or an intervention such as the supply of dietary supplements or animals may be killed. Adverse events can occur, following the administration of compounds depending on the intended mechanism of action of the molecules being tested. Where adverse events are noted, if necessary, following consultation with the veterinarian and appointed animal technician, mice maybe further closely monitored or an intervention such as the supply of dietary supplements may be given, or animals may be killed. When mice are anaesthetised there will be close and continuous monitoring to ensure that there is no possibility of mice recovering consciousness until procedures have finished. At the end of the experiments, animals will be killed, and tissues taken for further analysis. This analysis is an important and integral part of the project as this data will help us to decide which types of inflammatory response are likely to respond to our therapies and therefore which patients are most likely to benefit.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Haematological diseases involve complex process involving many cells types which interact with each other. This interaction is not just between involves many types of cells in different organs. It is not currently possible to model these aspects of complex interactions without the use of animal models, as we cannot reproduce the overall complexity of the types of cells involved and how they interact with each other in an</p>

	<p>vitro system. We will do this as we test our lead monoclonal antibodies in vitro first, only picking those that have the right characteristics to progress to in vivo models</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will regularly consult the most current papers on the subject to make sure we have the most up to date scientific knowledge in the area of research we are working in. We are also working in collaboration with a laboratory REDACTED who are working on improving the reproducibility and translatability of these models. We will initially run studies with as few animals as possible to make sure that our experimental technique is correct and the models we have chosen are the most appropriate. In this way, for each project, only a few antibodies will need to be tested in vivo. We will also use statistical methods to ensure that we are using the fewest animals per experiment to obtaining meaningful data.</p> <p>In addition, we will be taking many samples that will tell us about the changes to the immune system at the end of each study to ensure that we gain the maximum amount of information from each animal and minimise the number of experiments that need to be carried out</p> <p>We will also develop imaging techniques to enable us to monitor inflammation longitudinally which will enable us to use fewer animals and time points.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mouse models have been successfully used for the development of therapeutics for a wide range of haematological conditions. It is well established that antibodies can be used to treat patients with haematological diseases. It is also well established that rodent models can be used to develop treatments for haematological diseases and that these rodent models are translatable to the clinic.</p> <p>Rodents will be housed in state –of –the art conditions with care and welfare provided by an excellent and highly trained team of technicians.</p> <p>We will ensure that only agents that have passed stringent analysis for quality will be used in rodent</p>

	<p>studies. When conducting studies, we will select the protocol where we are using the lowest concentration of challenge possible during the study to meet our experimental requirements. We will actively monitor the anaemia and the impact that has on condition of the animal to ensure that no animal suffers unduly.</p> <p>We will continue to meet with local and international groups that work in the field of haematological conditions to refine experimental techniques and bring the best advice to bear on our projects so that we can always obtain the best information from the studies we conduct</p>
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Project	Therapeutic control of inflammatory lung diseases	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Lung inflammation, that occurs in diseases like asthma and chronic obstructive pulmonary disease (COPD), which cannot be adequately controlled by moderate doses of inhaled corticosteroids, is a significant medical problem that results in early death and serious detriment to patient's quality of life. Although corticosteroids are the best available treatment to limit inflammation, there are large populations of patients (e.g. COPD) where they are not effective. The use of high inhaled doses or oral dosage forms puts patients at risk of serious adverse drug reactions (e.g. osteoporosis, hypertension, glaucoma and weight gain). Patients need to take corticosteroids for life, so	

	<p>these serious adverse effects can be a major problem. Our primary aim is to understand why some forms of lung inflammation are resistant to corticosteroid treatment.</p> <p>A subsidiary aim is to examine the potential link between lung inflammation and cardiovascular disease. Clinical studies have identified that patients with diseases involving lung inflammation are at greater risk of developing cardiovascular disease. There is no known mechanism for how lung inflammation can influence the structure and function of the cardiovascular disease. It is not possible to study this link in patients as cardiovascular changes will have already occurred by the time lung disease is diagnosed. Our inflammatory models provide the opportunity for a mechanistic study of how lung inflammation can alter cardiovascular structure and function at an early stage.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Through a better understanding of why some lung inflammation is less sensitive to corticosteroid treatment, we will be able to find ways to reverse this insensitivity or identify alternative treatments to provide more effective control. This will allow the effective control of inflammatory lung diseases, such as steroid-resistant asthma and COPD, reducing the decline in quality of life and early death of these patients. Our study of the link between lung inflammation and changes in cardiovascular structure and function will allow the identification of which inflammatory pathways are important. This will inform clinical practice in determining which patients are at risk of getting hypertension as well as identifying potential ways of breaking the link between lung inflammation and cardiovascular disease.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We propose to use mice and guinea-pigs in these studies. Over a 5 year period we may use up to 3000 mice and 500 guinea-pigs.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity?</p>	<p>We will be inducing lung inflammation which can make it difficult for animals to breath, like an asthma attack (moderate severity), that is transient in nature, resolving back to normal in</p>

<p>What will happen to the animals at the end?</p>	<p>an hour or so. It is not normally possible to visually distinguish between animals on a procedure from control animals where no inflammation has been induced. There are very rare events (less than 1%) where an animal may experience an unexpected and severe inflammatory response (anaphylactic shock, severe severity). The onset and outcome of anaphylactic shock is very fast, making it very difficult to identify and stop. Some animals will be restrained for limited periods of time (up to 5 minutes). The stress of this is reduced by training animals to get used to the restraint. The level of discomfort during training is moderate and of very short duration (increasing duration from 30 secs). Once trained, the restraint is well tolerated and of mild severity.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Inflammation is a complex process that evolves over time and involves the constant interaction of tissue and blood-borne components. Due to this, it is not currently possible to study inflammation in anything other than intact animals.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We have carefully designed our experiments to get the maximum information possible from any individual animal. An example of this is our experimental design that allows us to address two separate aims thereby reducing the total number of animals required for the project overall. We will only use the numbers of animals required to make our studies statistically valid.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Guinea-pigs are the best small rodent to use to study the pulmonary inflammation due to similarities with the structure of human lungs and inflammatory process. Unfortunately, the scientific tools (e.g. research antibodies and transgenic animals) we need to study the mechanism underlying resistance to corticosteroids are not available for guinea-pigs. Therefore, we will need to use mice for these studies, as the required tools are available. Mechanistic findings about inflammation in mice have been shown to be relevant to humans</p>

	<p>giving us confidence that mice are an appropriate species to use in our studies.</p> <p>We will use sedation to minimize animal stress, and anaesthetic to minimize discomfort wherever possible. Checks and interventions are planned to intervene if any animal suffers any unnecessary discomfort.</p>
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Project	Therapeutic intervention in a sheep model of REDACTED	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	4 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We have previously generated a sheep model of REDACTED-identifies researcher and establishment.. Previous mouse models of this disease were not ideal for testing intervention strategies as the REDACTED of a mouse differs significantly from that of a human. The REDACTED of a sheep is much more similar to human in both size and structure. The aim of the current project is to test a REDACTED treatment for REDACTED in our sheep.	
What are the potential benefits likely to derive from this project (how science could be advanced or	Our model of REDACTED aims to better evaluate a gene therapy. Our sheep model of this human disease has the potential to	

<p>humans or animals could benefit from the project)?</p>	<p>overcome limitations of existing animal models and provide a tool to both evaluate therapies and further improve our understanding of the disease. In the longer term we anticipate that such large animal models of human disease will become increasingly common as we refine therapeutic strategies.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>39 sheep REDACTED over a period of 4 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>REDACTED For MRI imaging REDACTED sheep will be anaesthetised by single injection of a suitable drug into a vein in the neck and remain unconscious throughout the scanning process. A sample of blood and/or REDACTED may also be taken at this time. For delivery of REDACTED therapy to the REDACTED, the animals will remain unconscious following the first MRI scan. REDACTED. Following any surgical intervention, animals may be singly housed (with eye to eye contact with for company). Sheep cope well with anaesthesia and recover to standing within 10-15 minutes after cessation of anaesthesia. One risk associated with the above procedures is infection. However, our good sterile practice means that this is very rare. At the end of these procedures all animals will be killed to provide tissues for analysis.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Where possible we do use alternatives to animals. REDACTED.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>Every new experiment is carefully evaluated by experts in statistics, ethics and animal care and requires official approval before it can proceed. We must set out clearly the goals and the experimental design we will apply to answer our questions. This process ensures the minimum number of animals is used to meet our</p>

	objectives.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>REDACTED. Additionally, the dosage of any intervention required will be more similar to that for a human.</p> <p>Initial observations were of a small number of animals (3), enabling us to refine the REDACTED therapy study proposed here. Such a progressive approach allows us to better understand the outcomes of our REDACTED and identify experimental and humane end points that minimise any suffering experienced by the animals.</p>

Project	Therapeutic intervention in a sheep model of REDACTED	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	4 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We have previously generated a sheep model of REDACTED-identifies researcher and establishment.. Previous mouse models of this disease were not ideal for testing intervention strategies as the REDACTED of a mouse differs significantly from that of a human. The REDACTED of a sheep is much more similar to human in both size and structure. The aim of the current project is to test a REDACTED treatment for REDACTED in our sheep.	
What are the potential benefits likely to derive from this project (how science could be advanced or	Our model of REDACTED aims to better evaluate a gene therapy. Our sheep model of this human disease has the potential to	

humans or animals could benefit from the project)?	overcome limitations of existing animal models and provide a tool to both evaluate therapies and further improve our understanding of the disease. In the longer term we anticipate that such large animal models of human disease will become increasingly common as we refine therapeutic strategies.
What species and approximate numbers of animals do you expect to use over what period of time?	39 sheep REDACTED over a period of 4 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	REDACTED For MRI imaging REDACTED sheep will be anaesthetised by single injection of a suitable drug into a vein in the neck and remain unconscious throughout the scanning process. A sample of blood and/or REDACTED may also be taken at this time. For delivery of REDACTED therapy to the REDACTED, the animals will remain unconscious following the first MRI scan. REDACTED. Following any surgical intervention, animals may be singly housed (with eye to eye contact with for company). Sheep cope well with anaesthesia and recover to standing within 10-15 minutes after cessation of anaesthesia. One risk associated with the above procedures is infection. However, our good sterile practice means that this is very rare. At the end of these procedures all animals will be killed to provide tissues for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible we do use alternatives to animals. REDACTED.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Every new experiment is carefully evaluated by experts in statistics, ethics and animal care and requires official approval before it can proceed. We must set out clearly the goals and the experimental design we will apply to answer our questions. This process ensures the minimum number of animals is used to meet our

	objectives.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>REDACTED. Additionally, the dosage of any intervention required will be more similar to that for a human.</p> <p>Initial observations were of a small number of animals (3), enabling us to refine the REDACTED therapy study proposed here. Such a progressive approach allows us to better understand the outcomes of our REDACTED and identify experimental and humane end points that minimise any suffering experienced by the animals.</p>

Project	THERAPEUTIC TARGETS IN INFLAMMATORY BOWEL DISEASE
Key Words (max. 5 words)	
Expected duration of the project (yrs)	3 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	Regulatory use and routine production
	Protection of the natural environment in the interests of the health or welfare of humans or animals
	Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The proposed programme aims to the investigation of much needed new therapeutic applications for chronic inflammatory bowel diseases (IBD). IBD include Crohn's Disease (CD) and Ulcerative Colitis (UC), and affect more than 200,000 individuals in the United Kingdom, and these numbers steadily increase. This increase is most noticeable in developed and developing countries which are adapting a 'westernised' lifestyle and diet. Genetics cannot explain this phenomenon; environmental factors, including diet and toxins, affect the way genes are turned on and off through chemical changes called epigenetic modifications.

	<p>In addition, current medical treatments for IBD target the patient's defence (immune) system and may be ineffective or often limited by unwanted effects. Thus, the identification of novel therapeutics for IBD patients is of great importance. This project aims to exploit specific epigenetic mechanisms affected in IBD patients for the development of new drug treatments.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The identification of novel therapeutic targets in IBD. We will test the effects of new compounds on disease progression. Molecular analyses will reveal new mechanisms regulating disease development. We will address the value and benefits of treatments that do not target exclusively or directly the patient's immune system, an approach that comes with several limitations. This study encompasses analyses of both the efficiency and safety of new drugs that can be rapidly brought to the clinic.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>It is estimated that up to 430 wild type and genetically altered mice will be used for this project over a 3-year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Two models of colitis will be developed. One will involve administering in the drinking water a chemical (Dextran Sulphate Sodium Salt, DSS), and the other will involve the transfer of immune cells (T cells) by intraperitoneal injection. The signs of colon inflammation in these models are similar to the ones observed in patients with IBD and include weight loss, blood in stools or diarrhoea. Intracolonic administration of specific medicines will be applied to assess their ability to inhibit disease. This approach resembles a route of drug administration commonly used in patients (enema). All protocols have been designed to achieve the desired objectives without compromising the animal's welfare. Adverse effects such as transient discomfort from injections, and weight loss, slightly loose stool and slight presence of blood in the colon are expected. For some of the animals (control groups), severity will be mild. Cumulative severity will be moderate. Candidate medicines will be administered intracolonicly at low volumes to anaesthetised mice, at the lowest possible therapeutic concentration, in order minimise</p>

	potential side effects. The overall severity of the project is expected to be 'Moderate', and all animals will be culled at the end of each study.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The requirement to use animal models stems from the fact that IBD is a multifactorial disease, and impossible to replace with other approaches. The pathogenesis involves host-microbiome interactions, cell-cell interactions, activation of inflammatory cells, loss of epithelial integrity and mucosal homeostasis. The extensive research data already available in mouse models, as well as the availability of inbred strains, offers an excellent model for studying human diseases. Compared to other mammals, IBD develops in a short timeframe in mice and it is based on well-established protocols. A phylogenetically lower species cannot replace the use of mice. Mouse models are important in this study, as <i>in vitro</i> or <i>in silico</i> assays, to recapitulate the human IBD are completely missing.</p> <p>For the identification of the substances to be tested in mice, we have employed cell-based assays. By employing <i>in vitro</i> assays, we replace a large number of mice that would be required to test the whole range of compounds.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will analyse the effects of the tested substances on functional endpoints relevant to IBD, the inflammation and mucosal healing <i>in vitro</i>. Molecular analyses will address the most efficient concentration of the candidate medicines with the minimum toxic effects. Thus, the use of mice for titration of compound doses will be avoided or significantly reduced.</p> <p>Upon completion of the experiments, mouse tissues and fluids will be collected to verify the lack of toxic effects. Combination of the evaluation of drug efficiency with the analysis of toxicity in the same animals, further reduces the number of mice used.</p> <p>The variation between individual mice and the variable development of the disease has been taken into consideration in order to ensure the</p>

	<p>delivery of valid findings. Experiments have been designed to include the minimum possible number of mice needed in order to reach statistical significance in the anticipated results.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>This study will employ two different mouse models of IBD, DSS and T cell transfer. We have selected these mouse models for our studies following the screening of multiple models of IBD because they reproduce the mechanisms observed in patients with colitis. Chemically-induced colitis (with DSS) recapitulates the loss of epithelial cell barrier integrity, whereas the immune cell transfer-induced model (transfer of T cells) recapitulates the persistent aggressive inflammatory response, both of these elements are the major host-related pathogenetic mechanisms which appear to drive human disease.</p> <p>Both models are considered essential. First, because they reproduce the mechanisms identified in patients, and second, because this study aims to formulate the design of a clinical trial and should encompass the concept of variability observed in human disease. Notably, it is now widely accepted that to assess the clinical efficacy of a drug, its ability to reverse disease in at least two different animal models of chronic intestinal inflammation is required.</p> <p>A pilot study will be used to assess the exact time frame of disease development. This would allow the application of treatments within specified time limits to exclude the possibility of increased severity. The protocols employed are well established. Every effort will be made to reduce and relieve pain in the mice. We have developed a protocol for the intracolonic delivery of therapeutics in order to increase the efficiency and minimise potential systemic side effects. The protocols employed are well established. Male mice are selected for the DSS model due to reproducibility and susceptibility, and female animals will be used for the T cell model because they are less aggressive than males. This will avoid the negative impact of stress and wounds.</p> <p>In the DSS model, upon each cycle of DSS administration animals experience gradual weight loss and reduced stool consistency. Progressively,</p>

after the third cycle animals may present diarrhoea and bloody stools. The clinical symptoms should not exceed moderate discomfort and between cycles animals are expected to recover. In the T cell transfer model, the signs of colitis develop after three weeks as slow progressive weight loss which is later accompanied by loose stools and diarrhoea. Because disease is gradually worsening we aim to perform experiments upon detection of early signs of inflammation, and if increased weight loss, rectal bleeding and diarrhoea are observed they will be terminated.

Every effort will be made to reduce and relieve the pain of the mice. Animals will be kept for the minimum possible time outside the cages for weighing, observation and administration of therapeutics. We have developed a protocol for the intracolonic delivery of candidate therapeutics in a small volume in order to increase the efficiency in directly targeting the diseased tissue and minimise potential systemic side-effects. Candidate medicines will be used at their lowest therapeutic concentration and dose volume to minimise discomfort. When required, injections will be performed according to established methods and needle sizes. We expect that the candidate medicines will have no, or only minor, adverse effects. Mice will be monitored daily and at least twice per day (more often if indicated) when disease develops for pain/distress and if they reach the humane endpoint indicated by weight loss of 15% (T cell transfer model) or 18% (DSS model), they will be culled before the study's scientific endpoint. If any welfare concerns arise, then the named animal care and welfare officer and/or the named veterinary surgeon will be consulted.

Project	Therapy of rheumatoid arthritis	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Tumour necrosis factor (TNF) blocking drugs are a major advance in the treatment of rheumatoid arthritis and are now widely used in the clinic. However, TNF inhibitors does not cure the disease and therefore years of therapy may be required, resulting in high costs and the potential for serious infections, cancer and other adverse outcomes. In addition, about a third of patients fail to respond adequately to anti-TNF therapy and in a significant proportion of patients who initially respond well, there is a progressive loss of efficacy. In this project we aim to devise novel strategies to treat rheumatoid arthritis with the ultimate aim of achieving long-term disease remission. In healthy individuals regulatory T cells prevent</p>	

	<p>and control autoimmunity and inflammation. However, in rheumatoid arthritis these cells are defective and unable to control disease. Under our previous license we showed that this defect is caused by epigenetic DNA methylation, a process which controls gene expression and also contributes to the development of cancer. Hence, an important aspect of this project will be to evaluate the potential of inhibitors of DNA methylation to induce long-lasting disease remission.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Rheumatoid arthritis affects 1% of the world's population and causes chronic pain, disability and premature death. Current drugs do not cure the disease and require continuous treatment to prevent relapse. Furthermore, most drugs weaken the immune system and have harmful side-effects. This research aims to develop new treatments that provide long-term reductions in disease activity without the need for continuous drug administration. We also predict that new drugs will be identified that are effective in reducing pain and helping patients to lead more active and productive lives and our aim is take at least one of the drugs developed from this license through to clinical trials in patients.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>16000 over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Mice will be bred and maintained. A proportion of the mice will be immunised with an antigen in order to provoke an autoimmune response. Other mice will be injected with a protein into one knee, which results in transient arthritis in the injected knee. Overall, up to 50% will develop arthritis of moderate severity and these will be treated with promising anti-arthritis drugs. Adverse effects include pain, which will be controlled by the administration of analgesia, skin ulceration at the injection site and reduced movement. Mice may also experience diarrhoea during irradiation and reconstitution of the bone marrow or when kept in a germ-free environment. Some of the drugs may have unexpected adverse effects and</p>

	these will be monitored carefully. The mice will be humanely killed at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal models are necessary because arthritis is not a static process confined to a single tissue and culture techniques do not offer a realistic alternative. Rather, the inflammatory process is dynamic and highly complex, involving trafficking of cells from distant sites to the joint via the circulation. Hence, modelling the effects of treatment on arthritis must at some stage involve whole animals, rather than isolated tissue extracts.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The use of animals will be minimised through careful planning of experiments and by the use of cell based culture systems whenever possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use a mouse model known as collagen-induced arthritis which is less severe than other models and bears most similarities to human disease. Collagen-induced arthritis has previously been shown to be predictive of human rheumatoid arthritis. To reduce pain during arthritis, analgesics will be provided and subcutaneous injection on the flank reduces the risk of ulceration. The duration of active arthritis will be kept to a minimum (10 days) and severity limits will be in place to ensure no mouse suffers unduly. Mice with arthritis will be provided with easier access to food and supplemental bedding will be provided. Irradiation will be sub-lethal and split into two smaller doses to reduce the likelihood of adverse effects and mice will always be reconstituted within 24 hours. The use of germ-free mice avoids the use of antibiotics. Where possible we will use a monoarthritic mouse model which is of short duration and affects only one joint.

Project	Thrombus formation and its resolution	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Blood clots (thrombosis) are a major cause of death and may cause long term conditions including leg ulceration and limb loss. Treatments include surgery and drugs that break up the clot or stop it developing further, allowing its natural removal by the body. Surgery can lead to further clotting, while the drugs used can cause fatal bleeding (e.g. stroke). Thrombosis in the veins can also lead to a greater risk of heart attacks.</p> <p>The aims of this project are to better understand the mechanisms that regulate formation and natural removal of a clot and to use these to design experiments that aim to reveal: (i) new</p>	

	<p>targets for treatment that prevent clots forming and promote their removal and the restoration of blood flow through a vessel, without posing a serious risk of bleeding; (ii) new more specific and sensitive markers of the presence of a clot and its composition; (iii) novel imaging targets that can be used to non-invasively assess clots; and (iv) how thrombosis in the veins can promote disease progression in the arterial system, leading to heart attacks and stroke.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Current treatment of clots is mainly by the use of anti-clotting agents that can lead to unwanted bleeding (e.g. a stroke). Markers of the presence of a clot, especially in smaller blood vessels (e.g. calf veins) are not very specific and can lead to unnecessary treatment or lack of treatment. A better understanding of how clots form may reveal novel treatment targets that do not have the potentially fatal side effect of excessive bleeding and may also reveal more specific markers of the presence of a clot. Treatment of more extensive clot involves surgery and the use of clot-dissolving enzymes (lysis), which can also lead to a fatal bleed. Young clots can more readily be dissolved than older 'fibrous' clots, but aging of a clot is currently dependent on a subjective examination of the patient. More objective methods of determining the composition of a clot are therefore needed. Understanding the cellular and molecular changes that take place in the clot and circulation during clot formation and resolution could help us to develop novel imaging (e.g. based on MRI) and biochemical measurements that are informative of clot structure and susceptibility to lysis, and to develop treatment regimens that prevent the progression of disease in the arteries. These would better inform treatment options for patients, reduce the risk of potential serious bleeding and reduce mortality from conditions such as heart attacks.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The complex and dynamic cell environment in blood and surrounding blood vessel cannot be replicated in the laboratory. Mice and rats will be used in this project as models of thrombosis and the formation of fatty plaques that give rise to heart disease and stroke in man, are well</p>

	<p>established in these animals. All the tools necessary to study clots in these animals are readily available. Genetically modified mice, in particular, allow us to study the effect of enhancing or inhibiting particular factors in our models. We will carry out parallel studies in patients with blood clots and fatty arteries, as well as more detailed work in the laboratory to investigate the importance of specific cells and molecules revealed by the animal studies. We expect to use up to 9000 animals over the 5yr period of this study.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Our long experience with these models is that rats and mice tolerate blockage and manipulation of blood flow in their blood vessels very well. If pain is anticipated during a procedure (e.g. surgery) then the procedure will be carried out with appropriate anaesthesia. Adverse effects are most commonly associated with post-procedure discomfort. This will be minimised by administration of pain relief until the animal has fully recovered. We need to use genetically modified mice fed a high fat diet to initiate the development of fatty plaque in their arteries in experiments in which we wish to study the effect of a clot in the veins on the growth of fatty plaques that lead to a heart attack and stroke in man. The genetically modified mice that are given a high fat diet normally fair well with this diet. The strain of mouse that we use for these genetic modifications can however develop skin ulceration as they age, which can be exacerbated by the high fat diet. We have put in place treatment options to alleviate these symptoms in the mice. Animals will be humanely killed at the end of an experiment. Some animals will be used to breed animals for use in study protocols. We will only breed genetically manipulated animals that have at most a mild effect on normal behaviour and health. If we create an animal with a more severe effect, the animal will be immediately, humanely killed.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p>	<p>Animal models are used because the complex molecular and cellular environment that</p>

<p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>regulates clot development and its removal cannot be replicated in the laboratory. These processes involve a dynamic interaction between cells and molecules in the blood and local vessel wall.</p> <p>We will use mouse and rat models of thrombosis in the veins and arteries, that we and others have developed, to investigate the cells and molecules responsible for clot formation and its natural removal by the body. Genetically modified animals will help us to highlight the importance of specific cells and factors in these processes. These studies will provide the platform from where we can design interventional studies aimed at stimulating specific beneficial processes, as well new imaging methods and analytical methods that are informative of the presence, size and composition of a clot. These studies will be supported by laboratory-based analysis of the function cells and factors revealed in the animal models to be involved in the processes that give rise to clots and dissolve clots.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Animal numbers will be minimised by in the following ways:</p> <p>(i) Carrying out appropriate calculations (based on our experience of the variability in various end points such as clot size) of the requisite numbers of animals needed to provide a robust statistical analysis of these end points.</p> <p>(ii) Where possible, longitudinal analysis (changes over time) in, e.g. clot size in the same animal using non invasive scanning techniques such as microCT.</p> <p>(iii) Following humane killing, single animals can be used as donors to provide cells, from tissues such as bone marrow, that can be grown in the lab, to provide sufficient numbers of cells for experiments in a number of other animals. In some circumstances we will use the much larger reservoir of cells obtainable from human blood for experiments in mice that will accept human cells without rejecting them, thereby removing the need for donor mice.in these instances.</p> <p>(iv) Laboratory-based techniques to screen the</p>

	<p>activity or function of cells or factors revealed by the observational studies to be involved in clot formation and removal. e.g. the activity of factors that affect the growth of small blood vessels (that form inside clots to restore blood flow through the blood vessel) can be assessed using cells that form blood vessels, grown in the laboratory.</p> <p>(v) Using animal models in which multiple factors or cells can be screened prior to administration into the more complicated and severe clot model;</p> <p>(vi) Carrying out parallel studies of the measurement of cells and blood borne factors in patients with blood clots.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rats and mice will be used, as they are the least sentient and smallest animals in which we are able to reproducibly induce clot formation and assess its prevention/removal by the body or by our interventions. All the tools necessary to study clots in these animals are readily available. Genetically modified mice, in particular, allow us to study the effect of removing or inhibiting particular factors in our model. We will carry out parallel studies in patients with blood clots, as well as more detailed work in the laboratory to investigate the importance of specific cells and molecules revealed by the animal studies.</p> <p>Adverse events from procedures carried out in these studies will be minimal and mostly relate to post-surgical discomfort, which is controlled by the use of appropriate pain relief until the animal has recovered. Genetically modified mice that are given a high fat diet normally fair well with this diet. The strain of mouse that we use for these genetic modifications can however develop skin ulceration as they age, which can be exacerbated by the high fat diet. We have put in place treatment options to alleviate these symptoms in these mice.</p>

Project	Time-dependent mechanisms in learning and memory	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Links can form between the memories of events that occur in close temporal proximity. These links or associations influence adaptive behaviour. The strength of these links is affected by the temporal properties of events. The aim of the project is to investigate the psychological processes that result in time-sensitive learning and the neural substrates that are involved.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit)	The project will provide information that will advance our knowledge of how learning is achieved in the brain. This is of fundamental importance for a wide range of academic	

from the project)?	disciplines such as Psychology, Neuroscience, Psychiatry, Artificial Intelligence, Ethology. In addition, identifying the psychological processes and neural substrates responsible for normal cognition will aid our understanding of abnormal cognitive processes that occur in neuropsychiatric diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	It is anticipated that approximately 2500 mice and 250 rats will be used over a period of five years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of the work will involve behavioural studies in which the level of severity is mild. For some studies the severity level may be moderate due to the cumulative combination of behaviour procedures. The effects of these moderate procedures will be specific to cognition and behavioural performance on learning and memory tasks. Animals will be killed humanely at the end of the study. In some circumstances it will be necessary to collect brain tissue for analysis under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to establish the neural substrates that are necessary for learning and memory it is necessary to manipulate neural function in a manner that is not ethical nor practical in humans. Computational models, whilst useful for generating novel predictions, rely on empirical data from experiments. Therefore, although I hope that the work will lead to the development of computational models that will determine future research directions, they will not, ultimately, replace the need for the animal research proposed.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used will be kept to the minimum necessary to achieve the scientific goals by several means. First, where appropriate, with behavioural studies, manipulations of different factors will be conducted within the same animal. This will reduce the total number of animals necessary. Second, counterbalancing of non-crucial factors

	<p>will rule out potential non-specific explanations of the results. This will reduce the total number of experiments necessary to reach conclusions. Third, statistical analyses have been conducted to calculate the numbers of animals necessary to avoid false negatives. Fourth, procedures will be constantly evaluated with the aim of increasing sensitivity of manipulations and measures. This will ultimately lead to decreasing the numbers of animals necessary for answering specific questions.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rodents will be used because (i) there are clear structural and functional equivalents between rodent brains and human brains. (ii) Cognitive states can be studied easily in rodents and they are the lowest vertebrate group in which the behavioural tasks have been developed. (iii) Genetically altered rodents provide a means of examining the functions of specific genes, physiological processes, and anatomical systems in cognition.</p> <p>The health of animals throughout all procedures will be monitored daily.</p>

Project	Tissue and tumour adaptations to suboptimal environments
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X Basic research
	Translational and applied research
	Regulatory use and routine production
	Protection of the natural environment in the interests of the health or welfare of humans or animals
	Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Growing tissues are able to adapt to stresses in suboptimal environments, such as limited amounts of nutrients. This type of stress adaptation is critical for tissues to grow and function healthily from embryo to adult. Unfortunately, similar adaptations are also used by tumours to promote their uncontrolled growth during cancer. It is not yet clear which genetic and metabolic changes are most important for tissues to adapt to stress, nor how similar the adaptations are between normal and cancer tissues. The overall aim of this project is to discover the genetic and metabolic adaptations</p>

	<p>that are important for the growth of normal and cancerous brain tissue in the presence of only limited nutrients or a poor blood supply. We will compare adaptations between brains, brain tumours and several other organs in order to pinpoint their different vulnerabilities to stress. In the context of brain tumours, drugs targeting stress adaptations will be tested as potential anti-cancer therapies.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>There are three potential benefits likely to derive from this project. First, advances in fundamental knowledge via the identification of genes that regulate the growth of normal tissues and tumours. This will help to pinpoint the vulnerabilities of different growing organs to metabolic disease and cancer. Second, the development of improved experimental methods for studying metabolic disease and cancer, which can then be shared with other researchers. And third, the identification of drugs or combinations of drugs that, in the longer term, may be developed for treating human cancer and metabolic disease.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice. 2000 per year.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The expected adverse effects on the wildtype and genetically altered mice in this project are a moderate loss of body weight and the formation of small tumours under the skin. Genetically altered mice may also develop other adverse outcomes, which will depend upon which gene is being altered. Many of these adverse effects will present at embryonic and fetal stages, and are not usually compatible with continued life. The maximum expected level of severity for any procedure conducted within this project is moderate and follows strict guidelines in accordance with the Home Office. At the end of procedures, animals will be killed by an approved method.</p>
<p>Application of the 3Rs</p>	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>It is necessary to use animals to study the growth of organs and tumours because this process is subject to regulation inside the body from numerous other tissues and circulating hormones. It is not yet possible to recreate this level of biological complexity in a test tube or petri dish. Therefore, studies in the context of the whole intact animal are needed to identify metabolic and anti-cancer therapies that will ultimately be meaningful for human clinical studies. The proposed project also makes extensive use of non-protected animal alternatives such as the insect <i>Drosophila</i> and mammalian cells grown in a Petri dish.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Wherever possible, we will always use non-protected animal alternatives. Alternatives that we use extensively include mammalian cells grown in a Petri dish and also the insect <i>Drosophila</i>. The numbers of mice needed to be bred for this project will be reduced by sharing mice with other researchers. Experimental designs will use the minimal number of mice required to obtain statistically significant data. We will maximise the amount of data obtained from each mouse by studying multiple tissues, by analysing them using several different methods. We review our breeding strategies regularly and cryopreserve any strains which are not under current investigation.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice share similar genetics, metabolism and physiology with humans and so are an appropriate mammal for providing insights into human diseases. They are not a primate or an endangered species. Mice also have well-established laboratory procedures and advanced genetics, which both help to expedite research progress. In all cases, animal suffering will be minimised by following strict guidelines in accordance with the Home Office and by regularly monitoring animals in consultation with an animal care and welfare officer and a veterinary surgeon.</p>

Project	Tolerability and PK profiling of Compounds	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This licence will enable us to provide support services to the Pharmaceutical and Biotechnology industries to assist in the development of novel antimicrobial compounds.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	There is an acute shortage of compounds to treat microbial organisms that do not respond to available antimicrobials and newly-emerging diseases. The WHO recognises that antimicrobial resistance and infections are some of the greatest threats to humans. This licence will allow development of novel antimicrobials which will primarily benefit humans and animals.	

<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The number of animals used will be dependent on the service requirements of clients and the number of drugs in the development pipeline but will be approximately 50,000 Mice, 25,000 Rats, 5,000 hamsters, 5,000 cotton rats, 500 Guinea pigs and 500 rabbits over the 5 year period of the licence.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>A safe dose required for preclinical studies will be identified by an iterative process starting with a low dose, identified from in vitro studies, will be given to two mice and if it is tolerated the next two mice will be given a higher dose or lower dose if it was not tolerated. This will be repeated until a safe clinically relevant dose is found. In our experience this requires 2-4 cycles. Following treatment the mice will be closely monitored for any ill effects such as prolonged change in breathing, hunched and ungroomed appearance or display fits. Any animal showing adverse effects will be humanely euthanized. All mice at the end of the study (typically 24h post-treatment), will be humanely euthanized and post mortem carried out to look for any damage/change to internal organs. Based on our experience over the past 5 years ~80% mice will be in the mild severity band and ~20% will be in the moderate severity band.</p> <p>Pharmacokinetic studies are pivotal to drug development as it is critical to have sufficient drug present at the target site to inhibit the microbe causing an infection. Pharmacokinetic studies will use treatment doses that are well tolerated and so no adverse effects of the treatment are expected. Blood and at times tissue samples, are collected post dose to allow measurement of drug. When only blood samples are required, whenever possible animals will have micro samples of blood taken at different times during the study. When larger samples or tissues are required terminal samples are taken at the time of euthanasia. At the time of euthanasia ~90% of animals in PK studies will be in the mild severity banding and ~10% will be in the moderate severity banding.</p>
<p>Application of the 3Rs</p>	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We run an extensive range of <i>in vitro</i> assays (termed ADME and DMPK) to identify the most suitable compounds to progress into animal studies. The sets of assays performed are designed to prevent compounds with unsuitable physical properties, pharmacokinetic properties or toxicity progressing into animal studies. We use large panels of assays that include:</p> <ul style="list-style-type: none"> ● Drug uptake studies through human intestinal and lung cells ● Drug degradation studies following exposure to liver cell or cell metabolic enzymes ● 3D Hepatotoxicity assay to screen for liver toxicity ● 3D Combined hypertrophy and structural cardiotoxicity assay (uses beating cardiac 3D spheroids derived from human stem cells). <p>However, currently there are no <i>in vitro</i> models or mini-host systems that can fully replace animals that more closely mimic the clinical spectrum seen in humans. Comparative gene expression studies and immunological responses show substantial differences with vertebrates so can limit translation when using non-animal alternatives.</p> <p>We offer clients <i>in vitro</i> biofilm and hollow fibre models for screening of compounds. In addition where possible antimicrobial of interest are screened in <i>Galleria mellonella</i> (wax moth) larva infection models for efficacy confirmation before going into animal studies. Compounds that show no or limited efficacy are rejected at this stage. However, pathogenicity, response to therapy and importantly pharmacology differs greatly between <i>Galleria</i> compared to human and animals and therefore animal use is unavoidable.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The experimental design and analytical methods are fully supported by a statistician.</p> <p>Prior to all experiments literature is reviewed to ensure best practice in terms of experimental</p>

	<p>design. We aim to publish all models both successful and failed ones which should help reduce animal use.</p> <p>Where possible we collect multiple samples from a single animal to reduce the number of animals required. In addition where possible we combine multiple drugs into a cassette to reduce animal usage.</p> <p>For all experiments we include:</p> <ul style="list-style-type: none"> ● Statement of the objectives. ● Description of the experiment. ● Deliverable statement. ● No protocol will be executed until approved by senior staff
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Whilst mice will be our first choice for development of new antimicrobials there are likely to be instances where this species is not relevant. Specific examples where mice might not be appropriate would include host specificity (where it is not possible to establish an infection in mice), or where blood sampling is required to assess biomarkers for assessment of disease progression. Where blood sampling is necessary the rat is a better species to use, both because serial sampling is possible and multiple samples of sufficient volume to analyse can be collected. Further, the metabolism of the target test drug may require the use of a species where it is more likely to reflect that in the human. In these cases the species that more closely mimics the human infection or can provide the volume of samples required for bioanalysis will be used. For example, cotton rats are most suitable for chronic nasal colonisation with <i>Staphylococcus aureus</i> due to natural carriage and are one of the few non-human hosts of RSV.</p> <p>We will use the following to minimise harm to animals:</p> <ul style="list-style-type: none"> ● Ensure that where possible animals are kept in their social/cage mate groups. ● Only trained competent personnel carry

	<p>out procedures.</p> <ul style="list-style-type: none">● Ensure that administration and sampling limits are adhered to.● Where pain is likely then prophylactic analgesic agreed with the named vet is used.● Use rigorous monitoring of clinical conditions to ensure animals are euthanized within agreed severity bandings.● Continually assess published literature to ensure latest refinements are used and avoid duplicating work.
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Project	Tolerability and PK profiling of Substances	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>There are many common and rare human diseases where treatment is sub-optimal or there is no effective treatment available. To develop effective treatments new drug need to have no side effects after dosing and be effective in controlling the disease for which it was developed.</p> <p>This licence will enable us to provide information on whether a proposed drug has any side effects following dosing and whether it is taken up into the body in correct amount and at the place in the body required to control the disease.</p>	

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This project will provide first in-animal data that will be used to rank compounds for tolerability and drug exposure that is a critical step in drug development. These studies will allow substances to be ranked for safety and exposure so the best examples can progress into further drug development and eventually to clinical trials and into the clinic for patient and animal use.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>This project licence will only use rats and mice and of these approximately 80% will be mice and ~20% rats. The number of animals used will be dependent on the service requirements but is likely to be about 100,000 over the duration of the licence.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>A safe dose will be identified starting with a low dose that will be given two animals and if it is tolerated the next two animals will be given a higher dose or lower dose if the first dose was not tolerated. This will be repeated until a safe clinically relevant dose is found. In majority of studies the drug will be given to the animals by a route that does not require any surgery (oral dose, injection into skin, vein etc) which will cause brief discomfort or pain. However, in some cases (<10%) animal will have lines implanted to access the circulation or under the skin. These will be carried out surgically and animals will be given pain relief to mitigate any pain from the procedure. All animals taking part in a study will be regularly monitored for signs of ill-health (loss of weight, change in coat condition, reduced response to stimulation or lack of interaction with cage mates) and will be humanely killed if their condition does not change. At the end of the study, typically 24h in duration, it is necessary for the animals to be humanely killed and post-mortem carried out to look for any damage or change to internal organs. These studies will be followed by drug distribution in the blood and tissues but using the safe dose identified above. These studies are typically only 24h in duration with several blood sampling time points during this time, which will cause brief discomfort or pain. At the end of the study tissue samples are taken for</p>

	bioanalysis after the animal has been humanely killed. The majority of animals (~80%) in these studies will undergo experimental procedures classified as mild, and ~20% classified as moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Use an extensive panel of non-animal alternatives and computer modelling to identify and select the most suitable compounds before testing in animals. Whilst non-animal and computer models are highly predictive and stop the development of many compounds before they are tested on animals, currently there are no non-animal models that can fully replace animals due to the complexity of a complete animals with multiple interacting systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experimental designs and analytical methods used in this project will be scrutinised by a statistician to ensure good quality data is obtained with the minimum number of animals and appropriate statistical tests are applied. Extensive literature searches will be carried out prior to any study to ensure best study design and method are being used as well as check the work has not already been carried out. Where historical or published data is not available, a small pilot study before a full study will be carried out and data used to statistically calculate the minimum number of animals required in each group to ensure 80% chance of finding a meaningful result. Where possible we collect multiple samples from a single animal to reduce the number of animals required. In addition where possible we combine multiple drugs into a cassette to reduce animal usage.
3. Refinement Explain the choice of species and why the animal model(s) you will	Mice will be the first choice for studies but there are likely to be instances where this species is not relevant such as due to drug interactions or clearance is not the same as in humans or

<p>use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>where larger volume of blood might be necessary. In these case rats will be used.</p> <p>We will use the following to minimise harm to animals:</p> <ul style="list-style-type: none"> ● The most appropriate species will be used ● Animals are kept in their social/cage mate groups. ● Only trained competent personnel carry out procedures. ● Ensure that administration and sampling limits are adhered to. ● Collect the minimum volume of blood on the fewest occasions ● Where pain is likely then prophylactic analgesic agreed with the named vet is used. ● Use rigorous monitoring of clinical condition to ensure animals are euthanized within agreed severity bandings. ● Continually assess published literature to ensure latest refinements are used and avoid duplicating work.
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Project	Tolerance and addiction to drugs of abuse	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>1: To investigate how tolerance develops to opioid drugs.</p> <p>Drugs such as morphine are effective pain relievers (analgesics). They work by activating certain receptors in the brain (opioid receptors). Although they are powerful and effective analgesics, chronic use progressive loss of dru effect (tolerance).</p> <p>A novel finding in the field is that of 'biased agonists'. These activate the receptor in a different way to standard agonists such as morphine. It is hypothesized that 'biased agonists' at opioid receptors will be better analgesics by reducing tolerance. Another</p>	

	<p>problem with current opioid analgesics is that they can be abused, for their euphoric effects, which can lead to addiction; it has been hypothesized that 'biased agonists' can overcome this problem. We are conducting experiments that will test those hypotheses.</p> <p>2: To investigate the role of learning and memory in drug addiction.</p> <p>Drug addiction is poorly treated with approximately 70% of quit attempts failing within a year. One key trigger that enhances the risk of relapse is being exposed to a cue linked with drug-taking. Environmental conditions (eg. sights, smells, sounds) experienced during drug-taking can become linked with the feeling of the drug itself. This forms 'Pavlovian Conditioning' memories so that re-exposure to the environmental 'cue' can result in craving for the drug.</p> <p>We know, therefore, that learning and memory processes form a component of addictive behaviour, but precisely where in the brain these processes occur and by what mechanisms is unclear. This project aims to determine the learning and memory processes that contribute to addictive behaviour, and provide evidence for novel anti-addiction therapies to be designed.</p> <p>By gaining greater understanding of these memory processes and devising strategies to inhibit these memories novel anti-addiction therapies can be designed.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>'Biased agonists' at opioid receptors have the potential for being effective analgesics with fewer side-effects to current analgesics used in the clinic. This project will provide evidence as to whether 'biased agonists' are superior analgesics, and what their effects are following long-term treatment. These findings will underpin future work developing these drugs for clinical use. This project will demonstrate the brain regions and neuronal processes underlying learning and memory processes that contribute to addictive behaviour, and how these can be inhibited. These findings will suggest potential new medicines that can treat</p>

	addiction.
What species and approximate numbers of animals do you expect to use over what period of time?	1110 standard laboratory rats, 1480 standard laboratory mice, 1000 Genetically-altered mice.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	<p>Most animals will be standard laboratory rats or mice. However, some will be genetically-altered but only where the generic alteration itself causes no harm. Around 47% of animals will be humanely killed to provide brain tissue. This results in no more harm than when a Vet puts down an animal (the animal is killed while under general anaesthesia). Around 15% of animals will be given rewarding substances (drugs that can lead to addiction) in a particular new environment. The new environment itself is non-harmful and non-stressful. The only harm experienced by these animals is due to drug injection. Around 3% of animals will undergo the same procedure as above but will experience one single acute stressful event (restraint stress, where the animal is placed in a confined space for around 30 minutes). Around 10% of animals will be given repeated injections of an analgesic drug and the amount of pain-relief caused by those drugs will be measured using warm water on the tail tip. The animal will experience mild and transient pain that it can fully escape from. The harm is similar to a human dipping their toe into a bath that is too hot, then removing the toe. Around 7% of animals will have minor surgery to implant a device under the skin that can release a medicine slowly over a period of time. They are expected to recover quickly and will be given painkillers and post-operative care just like people recovering in hospital. Around 5% of animals will have minor surgery where a non-harmful indicator is injected into the brain. The animals are expected to recover quickly and will be given painkillers and post-operative care just like people recovering in hospital. Approximately 13% of animals will be used for breeding genetically-altered mice; procedures which will cause no harm. At the end of all procedures animals will be killed by a humane method and tissues taken for analysis after</p>

	death.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Non-animal alternatives like experiments with cultured cells, and computer modelling, are intrinsic to this research and are part of the overall research plan. However, it is only possible to understand the long-term effects of new drugs on the human brain by using mammalian brain tissue.</p> <p>Addiction and memory are complex behaviours involving a network of different brain regions that can only be studied in whole animals. Non-mammalian species do not have the same brain networks as mammals and so cannot be used.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The minimum number of animals is determined using statistical methods. We have extensive experience in these techniques to ensure the robustness of statistical analysis.</p> <p>Wherever possible we will generate multiple types of data from each animal undergoing a procedure. For example, we will generate whole-animal behavioural data, then take brain tissue from that animal to investigate changes at a neuronal/synaptic level.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rodents are the lowest mammalian species that are appropriate for this work. The anatomical distribution of relevant receptors and neuronal networks in rodents is similar to in humans. This is not generally the case in invertebrate species.</p> <p>In around half of the animals used, the only licenced procedure will be humane killing (killing the animal under general anaesthesia). This is a more refined way than 'Schedule 1' methods for preparing viable brain tissue, but results in no more harm to the animal than a 'Schedule 1' method.</p> <p>When animals are given drugs that can cause addiction, this can be used to model aspects of human drug addiction but exploits normal</p>

	<p>rodent behaviour, involves no surgery and causes no lasting harm; the drugs are not given at sufficiently high doses to cause harm such as drug withdrawal or addiction itself. One of the key triggers for relapse back to drug taking in human addicts is an acute stressful event. This can be modelled in rodents. The acute stressful event (restraint stress) is the most refined stressor that still results in a measurable response in the behavioural model.</p> <p>When the analgesic effects of drugs are tested using warm water on the tail tip, pain is mild, transient and escapable. Temperature-limits and time-limits are in place in the protocol to ensure no lasting harm occurs. This model yields robust data regarding opioid receptor activation.</p> <p>Only where necessary will minor surgery occur. Surgery will be carried out aseptically and painkillers will be administered to the animal. All animals are expected to undergo a rapid recovery. We are experienced in all of the surgical procedures to be used and all animals are expected to undergo a rapid and full recovery, using standard peri- and post-operative care.</p>
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Project	Towards the development of biomaterials for regulating tissue formation in health and disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Healing is an essential process that occurs after injury. As we age, we become less able to heal skin wounds, liver damage and bone fractures effectively, which represents a major burden for the National Health Service. We aim to understand which molecular signals are particularly important during skin, liver and bone healing. Healing involves numerous cell types that work together, but must receive specific, specialised instructions from different molecules. We aim to try to revolutionise the way these molecules, or related substances, can be	

	<p>delivered to wounds or diseased sites (skin, liver and bone). Currently, most substances are administered to the whole body by injection or topically, through a cream. There are several disadvantages to this, including the fact that numerous different cell types may be exposed to the same signal, which may give the correct 'instruction' for some cells, but not for others. Ultimately, this may have negative implications for healing. We aim to attach molecules to nano-particles or biocompatible surfaces. By doing this, the molecules remain biologically active and can be delivered specifically to the site of interest, to restrict which cells receive these signals. We also aim to generate 3D tissues (e.g. bone and liver tissues) outside the body and transplant them in the injured site (e.g. fractured bone) to accelerate healing. On the other hand, we also aim to use similar engineered scaffolds with molecules that slow down or even stop growth, and deliver them to tumours to limit their progression.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>We hope that our research will be the beginning of developing new ways to deliver therapeutic substances and tissues generated outside the body for the promotion of healing damage or limiting cancer growth. Not only this, but we hope to uncover some of the mechanisms by which healing can be 'regenerative', meaning that the site of injury appears no different from prior to injury, and can fulfil most of the same functions. For example, scarring following skin injury not only affects the appearance of the skin but also its ability to regulate temperature (by using hairs and sweat glands) and its capacity for movement (its elasticity). Additionally, we aim to use our engineered scaffolds in the opposite way, to target tumours locally and slow down or limit the progression of the cancer.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We approximate that we will use 7720 mice over 5 years. This number includes all animals used for breeding, those used for looking at their tissues after they have been humanely killed, and those used in experiments while they are alive.</p>
<p>In the context of what you propose</p>	<p>This project uses animals to test new ways of</p>

<p>to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>administering therapies for healing of skin, liver and bone, and also to limit or reduce the growth of bone cancer. All used procedures are either mild or moderate, meaning they have only a short effect on the wellbeing of the mice, or a small effect over a longer period of time. All injury or damage to the three tissues (skin, liver and bone) will be generated under anaesthetic and with the addition of pain relief so that they are under as little distress as possible. Mice will be monitored closely following any procedures to observe for adverse effects such as weight loss or discomfort. Steps will be taken to minimise the possibility of this, and if in the rare instances when it does occur, animals will be euthanized following consultation with the vet. Measurements will be taken while the mice are healing from the surgery, and at the end of each experiment mice will be humanely killed so that their tissues will be analysed in more detail.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Although various aspects of wound healing can be examined by using cells in vitro (grown on glass plates), the healing process involves many different cell types, and knowing the interactions they have with each other and their environment is key to understanding how they work in the body. Therefore, in order to gain a full understanding of how new therapies may influence the process (both positively or negatively), it is necessary to use animals that work in a similar way to humans, such as mice. Lower organisms, such as worms and flies, which do not feel pain, can't be used as they are too different from humans – an example being they don't have bones. Where possible, we will substitute the use of living animals (in vivo) with in vitro experiments. Similarly, while we can study some aspects of cancer cells in vitro, the in vivo studies are required to investigate cancer development and its interaction with the rest of the body, including blood supply, the immune system and the extracellular matrix that surrounds cells. Alongside our animal experiments, we will also perform in vitro experiments that will give us important information regarding dosing and types of</p>

	molecules that we will then use in our animals.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We have consulted a statistician to establish the minimal number of animals required to observe a clear outcome not due to chance (statistically significant). To prevent unnecessary breeding, we will keep stocks of frozen mouse sperm and embryos. We predict that advances in non-invasive imaging technology will potentially reduce the number of animals used in this project, as we can take repeated measurements of tissues inside the body without causing harm to the animal.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Our studies will use mice, as these animals have been extensively used in the types of studies that we will be pursuing, and it is known that they show promising results in trials of new therapies. Many of the procedures we will carry out are well established and we will continue to optimise them. Also, we have read the literature extensively, firstly so that we don't repeat experiments that have been done before, and secondly so that the techniques we use are as up to date as possible.</p> <p>When a new procedure is involved, training is first carried out on dead animals. Whenever possible, we will carry out procedures for the shortest time periods to minimise discomfort or harm to the mice. We will also refine our experiments by eliminating known influences on the rate healing. For example, hair follicles undergo periodic cycling between an active growth phase and a resting phase. The stage of hair follicle growth influences the rate of healing, and so we will only perform surgery on mice that are in the resting phase.</p>

Project	Toxicology of Pharmaceuticals
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input checked="" type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To undertake safety assessment of potential new human pharmaceutical materials, by the conduct of tests required by regulatory authorities, to enable approval of suitable materials to be used in human volunteers and patients.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The successful conduct of tests will establish scientific information on the safety of potential new human medicines. This information is designed to be used to help bring to market those materials which are safe, and ultimately shown to be effective in the treatment, prevention or diagnosis of human diseases. Without these studies, progression of new medicines to early human studies and to patients could not occur safely. The conduct of animal tests before human subjects can

	<p>be exposed to new medicines is a legal and ethical requirement. The ultimate benefit of the project is therefore the development of safer and more effective medicines. This is by identifying safe new medicines for healthy volunteers and patients and rejecting unsuitable or unsafe candidate drugs before humans would receive them. Validation and refinement of test methods may be completed for specific techniques, and may be published to the wider scientific community.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Within the five year life of the project it is estimated that the following may be used: • 50000 rats, mice or hamsters • 2000 rabbits • 3500 dogs • 500 pigs • 2800 macaques • 100 marmosets</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The aim of toxicity studies is to find out the relationship between the amount of the new medicine given to the animals and any effects seen. Medicines are given by the same route that humans would receive them, samples are taken for analysis; animals may be confined or restrained during the conduct of the studies. A small number of animals undergo surgical procedures to enable the dosing or sampling. The kind of effects seen in animals might include decreased activity or food consumption. Occasionally more severe effects may be seen in some animals. In this case action will be taken to alleviate suffering such as stopping the treatment, or humanely killing the animal. Rarely, sudden severe effects may be noted. In this case the affected animals are humanely killed without delay. Animals will typically be humanely killed at the conclusion of a test to allow examination of body tissues to be undertaken. On occasion, animals which have not been significantly or permanently harmed by conduct of a study may be re-used in a subsequent study, under veterinary guidance. The study designs and potential effects of test items result in consideration of most of the work authorised by the licence as moderate.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p>	<p>The purpose of the project is to find out how a whole living animal responds to a new medicine.</p>

<p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Cell cultures, tissue slices and many other non-living systems exist and can provide information on the way drugs act and how they produce particular effects. Non-animal methods are used routinely to identify only those most promising potential new medicines for further testing in animals and humans. But there is no non-animal alternative that can replace the whole living animal, and so animal tests and then human tests remain necessary in the development of safe and effective medicines. Alternatives have been introduced for skin tests and eye tests and are used. That is why there is no skin and eye testing for irritancy included in this project.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Requests for animal studies are reviewed by scientific staff to ensure that such work is required before it is agreed.</p> <p>Relevant government guidelines containing information on study designs will be used where available. Where there is no definitive guidance on numbers of animals, we will use experience of related programmes, taking account of the need to use sufficient animals for studies to provide robust results, without excess.</p> <p>Initial short screening tests using small group sizes, are often used to select the most promising lead compounds and appropriate dose levels for formal testing.</p> <p>Studies are performed in accordance with the principles of Good Laboratory Practice (GLP), This requires the highest standards of staff training, study planning and data recording and storage. This minimises any risk that studies are unsuccessful and have to be repeated.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rats and mice will be used for the majority of the work of the project. However, government authorities require new drugs to be tested in non-rodents as well as rodents in order to ensure as far as possible that the new medicine is safe. These non-rodent studies form a very low percentage of the overall testing. Species with special protection are used where they are the only suitable species, or in case of dogs, where there are no other practicable species available. This is because, after man, more is known about the anatomy,</p>

	<p>medicine and disease of the dog than any other animal. Pigs are sometimes used especially for skin treatments. Monkeys are used only when no other species is suitable and when the medicine is being developed for the treatment, diagnosis or prevention of debilitating or life-threatening conditions in humans, such as cancer or Parkinson's.</p> <p>In every case the utmost care has been taken to ensure that the animals are maintained in enriched, comfortable safe surroundings during the tests. They are trained wherever possible to avoid stress and are cared for by dedicated staff who are familiar with their husbandry needs. Any confinement or restraint is restricted to the minimum required, under guidance issued by the site's Animal Welfare and Ethical Review Body (AWERB). Dose volumes and blood sampling volumes are similarly controlled by the AWERB. The institute has won awards for its husbandry and care of animals.</p>
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Project	Toxicology testing of chemicals
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input type="checkbox"/> Basic research
	<input type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input checked="" type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To produce good quality information on the toxicity of chemicals, largely those that will be used in the oil and gas industry. This information will then be used by the regulator to decide if the chemical should be used, and what controls will have to be placed on the chemical's use.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This will protect the environment by letting the regulator know how harmful a chemical will be, and preventing or restricting the chemical's use.
What species and	6000 sheepshead minnows 100 zebrafish 100 turbot

approximate numbers of animals do you expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The fish could suffer toxic effects from exposure to the chemicals, up to and including death. The procedures are Severe. The fish will be euthanized after testing.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The Offshore Chemical Regulation Guidelines (2005/1) state that it is mandatory to carry out a fish toxicity test on all oilfield chemicals or their components.</p> <p>While we also conduct toxicity studies on algae and crustaceans, these are in addition to the fish testing and cannot replace it.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Our standard test is done at a concentration determined by the algae and crustacean tests. By using these preliminary tests we significantly reduce the number of fish that we use.</p> <p>We only run a set of tests when we have 3 or more chemicals to test. This reduces the number of fish that we use as controls.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We strictly follow the guidelines to ensure that our results will be accepted by the regulator.</p> <p>Fish that are not going to survive the test are euthanised immediately to relieve suffering.</p> <p>If a chemical is having a significant effect on a group of test fish, the test is immediately terminated and the fish euthanised to prevent any further suffering.</p>

Project	Training in complex surgical procedures	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
	<input checked="" type="checkbox"/>	Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project will train surgeons in advanced, therapeutic, minimally invasive, surgical procedures.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	In many cases minimally invasive (keyhole) surgical procedures are significantly better for patients than open procedures as they are associated with less post-operative adhesions, less time in hospital, faster recovery, less pain, easier post-operative care and much faster return to active life. Consequently many new minimally invasive procedures are being developed to replace larger, open procedures - particularly in response to the Governments	

	<p>new screening programmes for bowel cancer and aortic aneurysm among others. These screening programmes are identifying 30-40% more patients requiring surgical intervention for their conditions and the number of surgeons qualified in the new procedures is very limited. Un- or insufficiently trained use of these new procedures results in unacceptable death rates and long term side effects. We aim to teach surgeons these new, complex procedures, in terminally anaesthetised animals, to ensure rapid competency and safety. These courses will ensure an adequate supply of appropriately trained surgeons who will be able to fulfil the needs of our increasing numbers of patients using new minimally invasive procedures safely and effectively.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>300 pigs and 100 sheep over the course of the licence</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>As all protocols are non-recovery, at the end of the procedures, animals are given an anaesthetic overdose and then all possible organs are harvested for use in other studies being conducted by a range of scientists at the institution as well as for use in other training courses.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>As yet, there are no simulators that truly represent the full physiological state necessary to teach these procedures. Current simulators are unable to replicate the blood and lymph flow of tissues and are also not able to replicate tissue responses to stimuli, muscular activity in bowel, effects of surgery affected by temperature, or tissue changes relative to procedures. We will endeavour to develop better simulators as these courses progress.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>By carrying out a number of procedures in one animal we can reduce the number needed and, as all animals will be deeply and terminally anaesthetised, there will be no suffering or adverse effects. Using 2 animals per 3 or 6</p>

	surgeons depending on the course also reduces the number of animals needed.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The pig and sheep have been chosen for these courses as we need to represent the same size and physiology as humans, in particular with regard to blood system, lymph system, tissue response and general anatomy. Principally, animals are terminally anaesthetised and therefore insentient throughout. They are carefully monitored using staff trained, skilled and experienced in ensuring effective prolonged anaesthesia in these species.</p>

Project	Training in Complex Surgical Procedures	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	This project aims to continue delivering training to surgeons on advanced, therapeutic, minimally invasive, surgical procedures including endoscopy, laparoscopy, robotic and vascular surgery.	
Why is it important to undertake this work?	Surgical procedures continue to improve and ongoing training is required to ensure patient safety. Surgical techniques and technology are also evolving and minimally invasive surgical (MIS) procedures are gaining popularity worldwide nowadays. These procedures include keyhole or natural orifice surgery that can be carried out using endoscopy (camera test through natural orifice), laparoscopy (keyhole through the abdominal wall) and thoracoscopic (keyhole through the chest wall). More	

	<p>recently, all these techniques could be facilitated with the robotic techniques. It is proven that MIS procedures are significantly better for patients compared to open procedures as they are associated with less trauma resulting in faster recovery, less pain, less post-operative adhesions, easier post-operative care and much faster return to active life. Consequently, many new minimally invasive procedures are being developed to replace larger, open procedures across all specialities such as colorectal, urology, gynaecology and other digestive disease and vascular surgery. Optimal training on these advanced skills is required as insufficiently trained use of these new procedures results in unacceptable complication rate including higher death rates.</p> <p>As a non-animal alternative has yet to be devised that satisfies the requirements of the training of surgeons in these advanced techniques, training on live animals is required to ensure competency before practising on patients. There is also a need to improve the efficiency of the current training pathways due to the increasing demand to train more surgeons to cope with the increasing demand of the National Health Service (NHS). With the adoption of certain national screening programmes in a number of specialities such as colorectal cancer and vascular surgery, these are contributing to identifying 30-40% more patients who require surgical intervention for their conditions and the number of surgeons qualified in the new procedures is very limited, resulting in long waiting lists across the NHS. Through this licence, we aim to continue delivering teaching to surgeons on these complex procedures, in terminally anaesthetised animals, to ensure an adequate supply of appropriately trained and competent surgeons.</p>
<p>What outputs do you think you will see at the end of this project?</p>	<p>How will the course attendees use their knowledge or skills in the future career?</p> <p>Course attendees are carefully selected to ensure that they will practice the attained skills immediately or shortly after the course. Some of the courses are part of a wider training curriculum that encompasses clinical proctorship (mentoring) process immediately after the course. This paradigm shift from a single course (for one-two days) into a whole curriculum of training ensures that the learned skills are applied in clinical practice and under a safe educational environment and directly impacted on patient care and the delegates' career progression.</p> <p>What are the principle outcomes from these courses?</p> <p>The main outcomes of these courses are to enable the delegates to acquire the skills on:</p>

	<ul style="list-style-type: none"> ● Performing safe dissection and optimal anasomosis of very small blood vessels ● Safe application of endovascular stenting into blood vessels ● Safe removal of benign or malignant diseases pathology using endoscopy, laparoscopy or robotic technique <p>How are these outcomes important to the people in the course?</p> <p>these are considered essential skills in the relevant fields and important for surgeons to progress to the next phase of training (clinical mentorship)</p>
<p>Who or what will benefit from these outputs, and how?</p>	<p>Surgical procedures continue to improve and ongoing training is required to ensure patient safety. Surgical techniques and technology are also evolving and minimally invasive surgical (MIS) procedures are gaining popularity worldwide nowadays. These procedures include keyhole or natural orifice surgery that can be carried out using endoscopy (camera test through natural orifice), laparoscopy (keyhole through the stomach wall) and thoracoscopic (keyhole through the chest wall). More recently, all these techniques could be facilitates with the robotic techniques. It is proven that MIS procedures are significantly better for patients compared to open procedures as they are associated with less trauma resulting in faster recovery, less pain, less post-operative adhesions, easier post-operative care and much faster return to active life. Consequently, many new minimally invasive procedures are being developed to replace larger, open procedures across all specialities such colorectal, urology, gynaecology and other digestive disease and vascular surgery. Optimal training on these advanced skills is required as insufficiently trained use of these new procedures results in unacceptable complication rate including higher death rates.</p> <p>As non-animal alternative has yet to be devised that satisfies the requirements of the training of surgeons in these advanced techniques, training on live animals is required to ensure competency before practicing on patients. There is also a need to improve the efficiency of the current training pathways due to the increasing demand to train more surgeons to cope with the increasing demand of the National Health Service (NHS). With the adoption of certain national screening programme in a number of specialities such as colorectal cancer and vascular surgery, these are contributing to identifying 30-40% more patients who requiring surgical intervention for their conditions and the number of surgeons qualified in the new procedures is very limited, resulting in long waiting lists across the NHS. Through this licence, we aim to continue delivering teaching to</p>

	surgeons on these complex procedures, in terminally anaesthetised animals, to ensure an adequate supply of appropriately trained and competent surgeons.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	Planning to implement a quality assurance of training to measure the impact of teaching on live animals and the outcome will be published in peer review journals.
Explain why you are using these types of animals and your choice of life stages.	<p>Pigs are the animals of choice for central vascular access and navigation as the central vascular system is very similar to that of humans. However, pig's legs are too short for peripheral long wire access and sheep would be the animal of choice for these manoeuvres. We will investigate modification of the equipment used for EVAR training to possibly allow simultaneous or sequential training of both central EVAR procedures and peripheral endovascular procedures in the same animal thus introducing reduction and refinement. For all laparoscopic and endoscopic training, with the exception of head and neck, the animal of choice will be the pig due to its close comparison to man with respect to upper and lower GI tract. However for training involving head and neck, the sheep presents closer resemblance to human anatomy and would therefore, for these courses, be the animal of choice. We will investigate the potential for simultaneous or sequential training in endoscopic head and neck procedures and lower peripheral endovascular procedures in the same animal and, if possible, will adopt that process to reduce numbers of animals used. .</p> <p>For microvascular surgery, that there is currently no acceptable alternative to the use, ultimately, of live rats for gaining microsurgical skills. Simulated tissues can be used to gain basic technical skills but it is only in a live model complicated by the problems of thrombosis, haemorrhage and biochemical reactions to tissue manipulation that the true viability of an anastomosis can be tested.</p> <p>Alternatives (simulated tissue – silicone membranes and 'mock vessels made from soft rubber) are used on the first morning to familiarise surgeons with the use of the microscope, correct handling of micro instruments and sutures, correct anastomotic technique and the placement and tying of micro sutures. Only</p>

	<p>when competence has been achieved in this exercise can the surgeons go on to complete anastomosis in anaesthetised animals.</p> <p>Videos are used to demonstrate all exercises of the course and appropriate videos are shown continually during the week for student reference. This avoids the use of separate animals for demonstration purposes.</p>
Typically, what will be done to an animal used in your project?	<p>Animals will be anaesthetised and the steps vary according to each protocol.</p> <p>In Endovascular course, an access to the blood vessel is obtained and stent is inserted and placed in a blood vessels under X-ray. For microsurgery, blood vessels will be cut and rejoined and for robotic and key hole surgery; an access to the abdomen will be carried out prior to inflating the stomach and set up the robot to start removing certain organs.</p> <p>All animals will be terminated at the end of each procedure.</p>
What are the expected impacts and/or adverse effects for the animals during your project?	<p>The impact on the animals will be minimal since they will be under non-recovery anaesthesia for the entirety of the regulated procedures being applied</p>
What are the expected severities and the proportion of animals in each category (per animal type)?	<p>Non-recovery in all courses</p>
What will happen to animals at the end of this project?	<p>killed</p>
Why do you need to use animals to achieve the aim of your project?	<p>As yet, there are no simulators that truly represent the full physiological state necessary to teach these complex procedures. Current simulators are unable to replicate the blood and lymph flow of tissues and are also not able to replicate tissue responses to stimuli, muscular activity in bowel, effects of surgery affected by temperature, or tissue changes relative to procedures. We will endeavour however, to develop better</p>

	<p>simulators as these courses progress.</p> <p>It is the nature of animal laboratories that progress in the knowledge required to perform their various tasks is constantly sought in the scientific press, meetings and conferences as well as personal communications between laboratories. A non-animal alternative has yet to be devised that satisfies the requirements of the training of surgeons in microsurgery.</p> <p>It is important, however, to be able to develop a feel for live perfused tissue and learn how to deal with the problems of haemorrhage, blood clots and the way that real tissue and blood vessels behave during handling. A living animal is therefore essential and the rat is chosen for microvascular course as it is the smallest animal in which this work can reasonably be performed. For minimally invasive surgery, the pig and sheep have been chosen as we need to represent the same size and physiology as humans, in particular with regard to blood system, lymph system, tissue response and general anatomy.</p>
<p>Which non-animal alternatives did you consider for use in this project?</p>	<p>We currently use simulated tissues and tubing can be used for very basic exercises and these are used at the start of our workshops to establish each participants' competency is achieved on the basic tasks as well as in the use of various equipment such as the microscopes, the small surgical instruments and robot along with the correct surgical technique before they progress to animal work. we also use cadaver animal (small and large bowel) for teaching basic skills.</p>
<p>Why were they not suitable?</p>	<p>A non-animal alternative has yet to be devised that satisfies the requirements of the training of surgeons in microsurgery. Physiological realism with vascular and minimally invasive surgery is also required for these advanced skills teaching.</p>
<p>Enter the estimated number of animals of each type used in this project.</p>	<p>pigs: 1300 rats: 300 sheep: 50</p>
<p>How have you estimated the numbers of animals you will use?</p>	<p>This is based on the current and planned number of courses during the next 5 years across all the three protocols</p>

<p>What steps did you take during the experimental design phase to reduce the number of animals being used in this project?</p>	<p>By carrying out a number of procedures in one animal we can reduce the number needed and, as all animals will be deeply and terminally anaesthetised, there will be no suffering or adverse effects. Using 2 animals per 3 or 6 surgeons depending on the course also reduces the number of animals needed.</p> <p>We also use animal work for advanced skills and after achieving competency on basic manual dexterity skills which are learned on simulated tissues, tubing and ex-vivo to reduce the number of animals required. We have also recorded our own instructional videos which have removed the need for live demonstrations thus further reducing the number of animals used.</p> <p>In microvascular training, small animal anaesthetic technology has improved considerably over the last 20 years resulting in less animals dying prematurely (rats can now be safely anaesthetised for up to 7 hours) which reduces the number of animals needed.</p>
<p>What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?</p>	<p>Maximising the number exercises carried out on each animal have also minimised the number utilised.</p>
<p>Which animal models and methods will you use during this project?</p>	<p>Rats for microvascular surgery</p> <p>Pigs/sheep for endovascular, laparoscopic, endoscopic and robotic training</p>
<p>Why can't you use animals that are less sentient?</p>	<p>Adult animals are required to survive long surgery including anaesthesia and therefore less sentient animals such as fish or amphibia, or invertebrates are not suitable.</p> <p>Also adults required due to the size of the tissues and organs used providing a more faithful model for surgeons to practise on..</p>
<p>How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the</p>	<p>Involved in research and publication of novel models that can replace or complement live animals</p>

project?	
<p>How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?</p>	<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p> <p>Principally, animals are terminally anaesthetised and therefore insentient throughout. They are carefully monitored using staff trained, skilled and experienced in ensuring effective prolonged anaesthesia in these species. Through running several courses, a number of refinement strategies have been in place.</p> <p>Rats are easily anaesthetised and provide easily accessible vessels of a similar diameter to human finger's vessels. When repaired, these vessels can be assessed for viability of the anastomosis up to a few hours post-operatively.</p> <p>We have also taken note of the way other microsurgical courses are run, and their types of refinement, both in this country and abroad and where applicable have introduced new ideas. Through our experience we have been able to structure the courses such that the maximum number of exercises is carried out in each animal used. This has come in part from refinement of instructional technique and in part from refinement of exercises.</p> <p>Anaesthesia is induced and maintained by skilled assistants. The depth of anaesthesia is assessed frequently and body temperature is maintained by heat from bench lamps and by covering the rats with a small surgical drape. Local anaesthetic is applied topically throughout the exercises (on advice from the veterinarian). Fluids are applied topically via the wound site, to ensure hydration of the animal and to ensure care of the operative field. The named Veterinary Officer and the Named Animal Care and Welfare Officer may also periodically monitor animals throughout the course to ensure animal welfare compliance.</p>
<p>What published best practice guidance will you follow to ensure experiments are conducted in the most</p>	<p>LASA Guidelines on Aseptic technique</p> <p>Quality Improvement Guidelines for Endovascular Treatment of Iliac Artery Occlusive Disease</p> <p>Dimitrios Tsetis Æ Raman Uberoi. Cardiovasc Intervent Radiol</p>

refined way?	<p>(2008) 31:238–2</p> <p>Guidelines for the optimization of microsurgery in atherosclerotic patients. Chen HC1, Coskunfirat OK, Ozkan O, Mardini S, Cigna E, Salgado CJ, Spanio S. Microsurgery. 2006;26(5):356-62.</p> <p>A CONSENSUS DOCUMENT ON ROBOTIC SURGERY This document was reviewed and approved by the Board of Governors of the Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) in Nov 2007. Prepared by the SAGES-MIRA Robotic Surgery Consensus Group</p>
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Project	Transcriptional regulation of angiogenesis
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research <input checked="" type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The main objective of this work is to understand how genes are switched on and off during vessel development, with a particular interest in how this occurs in disease states such as tumour growth. Insights into the molecular processes controlling vessel growth, and a clear understanding of the underlying genetic programs, are essential to the development strategies to modulate vessel growth in humans, and would be applicable to diseases as diverse as macular degeneration in the eye, inflammatory disorders, cancer and heart disease
What are the potential benefits	The benefits stemming from this work include

<p>likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>increased understanding about how blood vessel growth is controlled. Excess blood vessel growth occurs during many human diseases, and in cancer, vessel growth is necessary for solid tumours to survive. Additionally, in some human diseases it is desirable to deliberately stimulate particular blood vessels to grow, for example after vessel damage in the arm or leg, or in the heart after a heart attack. Not all blood vessels are the same (for example, the cells that make up arterial and venous vessels express different sets of genes, as do growing blood vessels when compared to stable, quiescent vessels), so it is crucial to understand both what makes vessels grow and stop growing, and also to understand what type of vessels are downstream of any particular pathway or intervention. The knowledge derived from the work described here will help development of therapies to prevent, modulate or encourage vascular growth in multiple different disease states.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use mice and zebrafish for our work. Over five years, we expect to use 26,000 mice and 6,000 zebrafish.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The vast majority of animal use (over 80% of mice, 100% of zebrafish) will consist solely of the maintenance and breeding of genetically modified animals followed by death and post-mortem analysis of blood vessel development. For these animals, few adverse effects are anticipated, and where adverse effects are detected, in most cases the animals will be killed immediately. For maintenance and breeding, most will only experience mild severity, with the possibility of moderate severity in genetically modified animals. To study blood vessel development, a minority of animals will be administered substances, including via mini-pumps, to alter gene expression and/or induce vessel growth. Vessel growth may also be encouraged through either the implantation of matrix or tumour cells, into which the growth of vessels, and of the tumour itself (tumour growth is dependent on effective development of new vessels) will be measured. Additionally, a small number of mice, including pregnant females, will be subject to hypoxic environments (lower than usual amounts of</p>

	<p>oxygen) for up to 6 days: hypoxia is known to cause increased blood vessel growth and we are interested in understanding how it does this, and how it is different in healthy or damaged tissues. Adverse effects may include discomfort at site of administration (e.g. inflammation, oedema, scratching) and a possibility of infection with a limit of only moderate severity. Tumours grown in mice will not be permitted to grow beyond 12.5mm wide, but may result in adverse effects with a limit of only moderate severity, including reduced body weight, reduced food and water consumption and partial piloerection. Animals in hypoxic environment may appear transiently distressed, but are expected to adapt quickly to the reduced oxygen. Pilot experiments will ensure this is the case. Animal numbers will be kept to the minimal required to give statistically sound results. At the end the mice will be killed.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Our work uses the study of gene enhancers. These are the regions that control the switching on and off of genes. Putative enhancers require validation in animal models, as cell-based assays are unable to reliably determine the activity, nor specific expression pattern, of putative enhancer regions. However, we have replaced transgenic mice with transgenic pre-free feeding zebrafish embryos for our initial analysis of enhancers.</p> <p>Once identified as a potential regulatory protein, we will investigate the role of this protein in animal models where it has been mutated or deleted. Although we can and will also perturb gene expression in cells in culture, it is challenging to clearly detect the effects this has on vessel development. Endothelial cells grown in culture adopt a proliferative mode, unlike that found in mammals, and cell culture cannot accurately mimic events <i>in vivo</i>. Endothelial cells behave differently in their natural environment, where they are in tubular structures, surrounded by accessory cells and receiving intrinsic and extrinsic signals.</p>
<p>2. Reduction</p>	<p>Putative enhancer will be initially screened in transient transgenic zebrafish (before day 5 post</p>

<p>Explain how you will assure the use of minimum numbers of animals</p>	<p>fertilisation) in place of transgenic mice, using a three-colour reporter-gene system which will allow us to analyse three enhancers in each transgenic zebrafish.</p> <p>Where suitable lines exist, animals will be obtained from the relevant supplier. As the technology develops, we will also consider using genome editing technologies (e.g. Cas9/CRISPR) to generate transient gene deletions reducing the need for stable animal lines and consequently much greater number of breeding mice. To make a quantitative analysis of angiogenesis we will use the retinal angiogenesis model and Ad-VEGF tumour surrogate models, both which develop blood vessels in a known, sequential manner that reduces necessary time-points.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Analysis of enhancer/promoters in transgenic zebrafish allows most analysis to be done before zebrafish are free feeding. Live imaging allows study of the formation of the vessel system in the same fish.</p> <p>Aseptic precautions will be taken to reduce the risk of infection and care will be taken to ensure the animals are properly restrained during injections. Analgesia will be used when necessary to minimise welfare costs to the animal. A matrix angiogenesis assay will permit modelling of vascular growth in a healthy mouse after a minimally invasive procedure, whereas the Ad-VEGF tumour surrogate model simulates the tumour environment for blood vessel growth without subjecting the mouse to any tumour burden.</p>

Project	Translational pharmacology for drug discovery	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The burden on patients, carers and society of disorders such as Alzheimer’s disease, chronic pain and inflammatory conditions is immense and growing with an increasingly ageing population. Unfortunately, current treatments in all of these areas have substantial limitations in terms of the level of effectiveness provided and/or the undesirable side effects caused. The development of new safe and effective medicines are an important facet of how society approaches such unmet medical need.</p> <p>The aims of this project are to continue our efforts to help facilitate and optimise the advancement of potential new medicines being developed by other</p>	

	<p>drug discovery scientists (e.g. pharmaceutical & biotechnology companies, academic institutions) for chronic disorders of the brain and inflammation. This will be in the form of providing robust evidence from pre-clinical rodent models of likely therapeutic benefit in the clinic, an indication of the levels of drug in blood required to produce such benefit, and a recommendation for the types of patient and clinical outcome measures most suited to the new treatment. The models and technologies that will be used have been refined over many years and have strong translational relevance to the human condition.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The pre-clinical evidence produced by this project will help identify the best new drugs for progression into human clinical trials and is expected to reduce the currently high number of failures observed in the clinic. Importantly, the scientific translational approach being taken has previously been successful in advancing several new drugs that have proven to have some clinical benefit in Alzheimer's disease and various chronic pain conditions.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Over the 5 years of the proposed licence we estimate that we will use: Rat: 15000 Mouse: 5000 Gerbil: 500</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>A proportion of the techniques used under this licence are minimally invasive and therefore classified as mild. Some animals may have undergone procedure that will cause some pain and discomfort, but will be kept to the minimum possible and these will be classified as moderate. We anticipate only a small number of animals may show adverse effects and where do so they will be culled.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>A range of chronic disorders are covered by this licence, including cognitive deficits associated with Alzheimer's Disease and a range of painful (e.g. osteoarthritis) and inflammatory conditions (e.g. rheumatoid arthritis) and neuropathic conditions (diabetic neuropathy). These are all conditions where the whole organism (i.e. intact</p>

	<p>nervous system) is required in order to measure a cognitive or painful response.</p> <p>No in vitro systems are in existence that can replicate the whole functioning organism.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of animals will be kept to the minimum required through good experimental design.</p> <p>For most experiments, sample sizes have been set using power analysis, generally using a significance level of 5%, a power of 80-90%, to detect a difference between groups of 25%. For most procedures numbers of animals per group will be in the 8-12 range depending on the protocol in use. We will continue to monitor group sizes and modify as appropriate based on their analysis.</p> <p>Most experiments will involve parallel groups, though in some instances a cross-over design may be used if deemed appropriate.</p> <p>Substances can be administered using a cross-over design, whereby each animal receives all treatments and acts as its own control e.g. where animals have been surgically prepared for EEG or trained to perform a task such as touch screen. Within animal comparisons, are less variable than between animal comparisons, so this will allow the use of smaller groups of animals, to consistently achieve statistical significance.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Choice of species</p> <p>The majority of experiments carried out under this project licence will be in rats and mice. Rats are widely used to provide data in drug discovery because there is a large body of data describing some of the similarities and differences between rat and human physiology. Mice are sometimes used for example in situations where antibodies optimised for use in humans retain their affinity for the equivalent mechanistic target in mouse but not in rat. We may use genetically modified mice, usually this is because no specific inhibitors for the mechanism of interest have yet to become available for the target in question.</p>

Choice of models

The models described in this licence have been extensively used to identify candidate drug molecules, and are of the lowest severity that will allow decision making data to be obtained and where possible a human correlate exists.

Minimisation of suffering

Telemetry devices maybe implanted to measures physiological parameters such as body temperatures, blood pressure & heat rate, this will minimise the stress that maybe experienced with repeated measures such as rectal probe & tail cuff, eliminates the requirement for restraint/tethering and allows the continuous collection of data without the need for any manipulation. Therefore the benefit of the surgical implantation will improve the overall lifetime experience of the animal compared to repeated procedures.

STZ injection produces neuropathy by evoking typical symptoms of diabetes and therefore, animals will drink more than usual and this will be taken into consideration during the husbandry care. 2 % sucrose is added to the drinking water to help avoid the initial hyperglycaemia, and animals will stay group housed to help maintain body temperatures.

All animals undergoing nerve injury for the induction of neuropathic pain, are placed onto an environmental enrichment protocol, where from arrival day enrichment is changed on a Monday (castle) Wednesday (house) and Friday (tubes), to help reduce the incidents of autotomy. Choice of types enrichment may vary depending on availability.

For all pain protocols, we will continue to encourage the use of spontaneous behaviours/ non-invasive endpoints to reduce pain and suffering experienced by the animal, such as weight bearing, burrowing, paw volume and any other more naturalistic behaviours.

Project	Treatment and Prevention of Auto-immune and Immune-mediated Diseases																
Key Words (max. 5 words)																	
Expected duration of the project (yrs)	5 Years 0 Months																
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<table border="1"> <tr> <td data-bbox="719 638 746 728"></td> <td data-bbox="746 638 1402 728">Basic research</td> </tr> <tr> <td data-bbox="719 728 746 817">X</td> <td data-bbox="746 728 1402 817">Translational and applied research</td> </tr> <tr> <td data-bbox="719 817 746 907">X</td> <td data-bbox="746 817 1402 907">Regulatory use and routine production</td> </tr> <tr> <td data-bbox="719 907 746 1077"></td> <td data-bbox="746 907 1402 1077">Protection of the natural environment in the interests of the health or welfare of humans or animals</td> </tr> <tr> <td data-bbox="719 1077 746 1167"></td> <td data-bbox="746 1077 1402 1167">Preservation of species</td> </tr> <tr> <td data-bbox="719 1167 746 1256"></td> <td data-bbox="746 1167 1402 1256">Higher education or training</td> </tr> <tr> <td data-bbox="719 1256 746 1346"></td> <td data-bbox="746 1256 1402 1346">Forensic enquiries</td> </tr> <tr> <td data-bbox="719 1346 746 1480"></td> <td data-bbox="746 1346 1402 1480">Maintenance of colonies of genetically altered animals</td> </tr> </table>		Basic research	X	Translational and applied research	X	Regulatory use and routine production		Protection of the natural environment in the interests of the health or welfare of humans or animals		Preservation of species		Higher education or training		Forensic enquiries		Maintenance of colonies of genetically altered animals
	Basic research																
X	Translational and applied research																
X	Regulatory use and routine production																
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	Preservation of species																
	Higher education or training																
	Forensic enquiries																
	Maintenance of colonies of genetically altered animals																
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to develop novel therapies to treat auto-immune diseases.																
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Auto-immune diseases affect millions of people worldwide causing pain, impaired function and diminished quality of life. We will be facilitating the development of new therapies for diseases which cause significant ill health and for which current treatments are inadequate. By contributing to the development of new immune-modulatory drugs, our project will benefit the patients, improving their quality of																

	<p>life and reducing suffering. By providing high quality services and scientific expertise, we can make the testing of such drugs more informative.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The estimated number of animals to be used over the duration of the project (five years) is 11500. Mice will be used in more than 90 % of studies with rats being used in the remainder. No other species are to be used.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We expect animals to develop some clinical signs relating to the diseases induced under this project licence. Inducing auto-immune encephalomyelitis under our treatment and prevention of experimental autoimmune encephalomyelitis project is likely to cause adverse effects such as bodyweight loss, paresis and paralysis of the tail and hind limbs. The expected level of severity for this model is severe. Specific measures are taken to limit harms such as frequent monitoring and scoring of disease, specific clinical signs and non-specific clinical signs. Moderate signs are not tolerated for more than 24 hours and severe signs will not be tolerated. At the end of an experiment, all animals will be humanely killed to enable further in vitro testing of samples. For our treatment and prevention of systemic lupus erythematosus (SLE) projects the adverse effects we expect to see are impaired kidney function, increased protein in urine, swelling to the paws, changes to breathing patterns and abdominal distention. On our treatment and prevention of psoriasis we expect to see changes to the skin such as skin thickening, flaking, crusting, redness and itching. Treatment and prevention of dry eye syndrome (Sjogren's) can cause reduced tear and saliva production and onset of diabetes. For mice and rats used on our treatment and prevention of experimental autoimmune uveitis (EAU) we can expect the animals to develop inflammation to the front and back of the eye. Under our antigenic challenge protocol expected adverse effects are changes to body and coat condition, decreased activity, changes to breathing patterns and irritation or break in the skin around adjuvant admin-</p>

	<p>istration site. The adverse effects expected under the Treatment and Prevention of Amyloidosis include bodyweight loss, changes to coat condition and posture, changes to breathing patterns and decreased activity levels. The expected level of severity for all the above models is moderate. Measures are taken to limit harms such as frequent monitoring of disease-specific clinical signs and non-specific clinical signs for early identification of adverse events. Moderate signs are not tolerated for more than 24 hours and severe signs will not be tolerated. At the end of an experiment, all animals will be humanely killed to enable further in vitro testing of samples. Expected adverse events: Animals showing signs of several major human diseases will be given new treatments by injection or slow release implants and the effects on the diseases measured. Diseases modelled include multiple sclerosis, in which animals may show weakness, difficulty moving and bodyweight loss. In other models, animals may develop increased protein in urine, crusting, skin flaking, skin thickening, reduced saliva and tear function; or eye inflammation; changes to body condition or weight loss. Most protocols show moderate signs of disease, other than EAE which displays severe disease. Most animals will develop only mild disease, a small number of animals may develop more severe disease. Measures taken to limit harms: frequent monitoring of animals for early identification of adverse events, Animals showing moderate signs for more than 24 hours will normally be humanely killed. Models of multiple sclerosis can be more severe, and animals will be monitored very closely and given extra care. At the end of an experiment, all animals will be humanely killed to enable further in vitro testing of samples.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Immunology involves multiple systems, multiple organs and multiple cell types. The complexity of the immune response cannot be reproduced in laboratory tests.</p>

	<p>In addition, the symptoms of auto-immune diseases – paralysis, eye inflammation, reduced salivary/lachrymal function, skin flaking and thickening - cannot be modelled in a laboratory. Experiments on cell lines and on cell cultures will be performed. However, the limitations of these methods do not allow them to replace the use of experimental animals: there is no alternative to the use of a living animal that would allow the objectives to be met.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Statistical tests will be performed at the planning stage to determine the necessary number of animals to be used to obtain scientifically sound data. Where suitable, previous experimental data from our establishment will be used to allow for comparison. In addition, if possible, we encourage the use of a shared control or untreated group among different studies using the same model.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Most of our studies will be done in mice, which are the lowest species that develop these diseases in the same way as humans. Rats are occasionally used when the disease cannot be modelled in mice, if the test compound does not work in mice, or if a larger animal is needed.</p> <p>Animal suffering will be limited by ensuring that the models used cause the least amount of harm to the animals. The mildest disease inducing agent or dose will be used, and studies will be kept as short as possible. Animals are monitored frequently for signs of discomfort, and appropriate action taken promptly. We will monitor animals closely throughout the studies, and they will be treated or humanely killed if they develop signs of excessive suffering.</p> <p>Animals are housed in groups and kept in an appropriate environment with plentiful bedding and nesting material and suitable object that allow them to express normal behaviour. All staff are trained in good animal handling procedures. Animals are always handled gently and humanely, especially animals which may</p>

	<p>be in pain. Animals may be acclimatised to being handled prior to the experiment starting so that they are less stressed once the study begins.</p> <p>Animals are provided with a bowl of mashed food on the cage floor if moving may be uncomfortable. When substances need to be administered, we will give the smallest volume possible and administer it in the way that causes the least distress.</p>
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Project	Treatment and Prevention of Diabetes
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Basic research
	X Translational and applied research
	X Regulatory use and routine production
	Protection of the natural environment in the interests of the health or welfare of humans or animals
	Preservation of species
	Higher education or training
	Forensic enquiries
	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to develop novel therapies to treat type I and type II diabetes including pain caused by nerve damage, and kidney damage.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Diabetes affects patients causing pain, impaired function and diminished quality of life. By contributing to the development of new drugs, our project will benefit the patients improving their quality of life and reducing symptoms. By providing high quality services and scientific expertise, we can make the testing of such drugs quick and reliable, ensuring that effective treatments are identified at the earliest opportunity. This means benefits

	to patients are realised in a timely manner and potentially harmful or ineffective therapies are identified long before they get to the stage of being given to people.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use rats and mice. The estimated number of animals to be used over the duration of the project is 3000. Mice will be used in more than 90 % of studies with rats being used in the remainder. No other species are to be used.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals to be used include types of rats and mice that develop diabetes spontaneously. Other rats and mice may be treated with compounds to induce diabetes or may be given a high fat diet. Animals are expected to develop high blood sugar, and other signs associated with diabetes in people such as kidney problems, nerve damage and altered sensation. Animals will be given treatments by injection, in food or water, or by devices implanted under the skin. They may also have small blood samples taken. Animals may experience moderate distress as a result of the procedures. Animals will be closely monitored and any animals experiencing more than moderate effects will be humanely killed. Measures taken to limit harms: frequent monitoring of disease-specific clinical signs and non-specific clinical signs for early identification of adverse events, moderate signs tolerated for no more than 24 hours, severe signs not tolerated. At the end of an experiment, all animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complexity of the immune response cannot be wholly reproduced <i>in vitro</i> . <i>In vitro</i> experiments on cell lines and <i>ex vivo</i> experiments on cell cultures will be performed. However, the limitations of these methods do not allow them to replace the use of experimental animals: there is no alternative to the use of a living animal that would allow the objectives to be met.

<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of animals used will be the minimum required to ensure meaningful data is acquired.</p> <p>Statistical tests will be performed at the planning stage to determine the necessary number of animals to be used to obtain scientifically sound data. Where suitable, previous experimental data from our establishment will be used to allow for comparison. In addition, if possible, we encourage the use of a shared control or untreated group among different studies using the same model.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Most of our studies will be done in mice, which are the lowest species that develop these diseases in the same way as humans. Rats are used when the disease cannot be modelled in mice, if the test compound does not work in mice, or if a larger animal is needed.</p> <p>Animal suffering will be limited by ensuring that the models used cause the least amount of harm to the animals. The mildest disease inducing agent or dose will be used, and studies will be kept as short as possible. Animals are monitored frequently for signs of discomfort, and appropriate action taken promptly. We will monitor animals closely throughout the studies, and they will be treated or humanely killed if they develop signs of excessive suffering.</p> <p>Animals are housed in groups and kept in an appropriate environment with plentiful bedding and nesting material and suitable object that allow them to express normal behaviour. All staff are trained in good animal handling procedures. Animals are always handled gently and humanely, especially animals which may be in pain. Animals may be acclimatised to being handled prior to the experiment starting so that they are less stressed once the study begins.</p> <p>Animals are provided with a bowl of mashed food on the cage floor if moving may be uncomfortable. When substances need to be administered, we will give the smallest volume</p>

	possible and administer it in the way that causes the least distress.
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Project	Treatment and Prevention of Inflammatory Diseases	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to develop novel therapies to treat inflammatory diseases.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Inflammatory diseases affect millions of people worldwide causing pain, impaired function and diminished quality of life. By contributing to the development of new anti-inflammatory drugs, our project will benefit the patients, improving their quality of life and reducing suffering. By providing high quality services and scientific expertise, we can make the testing of such drugs more cost effective, more informative and	

	reduce the need for companies to set up the models in house.
What species and approximate numbers of animals do you expect to use over what period of time?	The estimated number of animals to be used over the duration of the project (five years) is 13000. Mice will be used in more than 90 % of studies with rats being used in the remainder. No other species are to be used.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	When inducing inflammatory diseases, we expect to see some clinical signs relating to the disease. When inducing arthritis in mice and rats under our treatment and prevention of joint inflammation protocol, this is likely to cause adverse effects such as joint swelling and reduced mobility. For our treatment and prevention of gastro-intestinal inflammation projects the adverse effects we expect to see are bodyweight loss, diarrhoea, intestinal bleeding and abdominal discomfort. On our treatment and prevention of skin inflammation we expect to see changes to the skin such as skin thickening, flaking, crusting and red-ness. Treatment and prevention of lung inflammation and fibrosis (including Chronic obstructive pulmonary disease COPD and Asthma) can cause changes to breathing patterns which is expected as part of the disease. For mice and rats used on our treatment and prevention of liver inflammation (Hepatitis) we can expect to observe some body weight loss and changes to appearance such as coat condition, posture and lethargy. Adverse effects expected on the treatment and prevention of peritoneal inflammation project include body weight loss and changes to appearance such as coat condition, posture and lethargy. Adverse effects expected during treatment and prevention of kidney inflammation (Nephritis) can cause increased protein in the urine, body weight loss and abdominal swelling; changes in appearance or behaviour may also be observed. For mice and rats used on peripheral inflammation projects some discomfort, swelling and abnormal behaviour may be observed during the project. Our air pouch model is not expected to cause adverse effects. The expected level of severity for all the above models is moderate. Measures are taken to limit harms such as

	frequent monitoring of disease-specific clinical signs and non-specific clinical signs for early identification of adverse events. Moderate signs are not tolerated for more than 24 hours and severe signs will not be tolerated. At the end of an experiment, all animals will be humanely killed to enable further in vitro testing of samples.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Inflammation, the immune system's response to the presence of antigens, involves multiple systems, multiple organs and multiple cell types. The complexity of the inflammatory response cannot be reproduced in laboratory tests. In addition, the symptoms of inflammation - heat, redness, swelling and pain- cannot be modelled in a laboratory. Experiments on cell lines and on cell cultures will be performed. However, the limitations of these methods do not allow them to replace the use of experimental animals: there is no alternative to the use of a living animal that would allow the objectives to be met.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical tests will be performed at the planning stage to determine the necessary number of animals to be used to obtain scientifically sound data. Where suitable, previous experimental data from our establishment will be used to allow for comparison. In addition, if possible, we encourage the use of a shared control or untreated group among different studies using the same model.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Most of our studies will be done in mice, which are the lowest species that develop these diseases in the same way as humans. Rats are occasionally used when the disease cannot be modelled in mice, if the test compound does not work in mice, or if a larger animal is needed. Animal suffering will be limited by ensuring that the models used cause the least amount of harm to the animals. The mildest disease inducing agent or dose will be used, and studies will be kept as short as possible. Animals are monitored frequently for signs of discomfort, and

	<p>appropriate action taken promptly. We will monitor animals closely throughout the studies, and they will be treated or humanely killed if they develop signs of excessive suffering.</p> <p>Animals are housed in groups and kept in an appropriate environment with plentiful bedding and nesting material and suitable object that allow them to express normal behaviour. All staff are trained in good animal handling procedures. Animals are always handled gently and humanely, especially animals which may be in pain. Animals may be acclimatised to being handled prior to the experiment starting so that they are less stressed once the study begins.</p> <p>Animals are provided with a bowl of mashed food on the cage floor if moving may be uncomfortable. When substances need to be administered, we will give the smallest volume possible and administer it in the way that causes the least distress.</p>
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Project	Treatment of abnormal retinal development	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Albinism is a condition characterised by pigment deficiency and abnormal retinal development, resulting in visual impairment. Unfortunately, there are no treatments available for infants and young children affected by albinism. Normal retinal development is known to continue at least until 5 years of age and there is evidence to suggest that retinal development in children with albinism is also ongoing, although occurring more slowly and in an abnormal pattern in comparison to normal controls. L-DOPA, a signalling molecule which is essential for normal retinal development is deficient in albinism. Exactly how L-DOPA deficiency results in abnormal retinal development is unclear. It is	

	<p>also unclear if L-DOPA supplementation in early infancy/childhood can help to correct abnormal retinal development in albinism and optimise visual function.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>We aim to: (a) establish proof of concept for the earlier treatment of albinism with L-DOPA; (b) define the therapeutic time window within which L-DOPA treatment will be effective at rescuing abnormal retinal development in albinism and (c) explore the biochemical mechanisms underlying abnormal retinal development in albinism and potentially identify novel therapeutic targets.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We would estimate a total of approximately 300 animals for the project. Exact numbers will depend on the results of breeding and the experimental data. Mice have been chosen as the knockout mouse is the only animal model of albinism in which preliminary data exists for the use of oral L-DOPA (including drug dosages and formulation) in rescuing retinal function. The minimum number of animals will be calculated for each part of the project based on power calculations and statistical estimates.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The administration of L-DOPA to mice may result in side effects similar to those experienced by humans including nausea, loss of appetite, movement disorders, sleep disturbances and agitation. Close attention will be made to the health and behaviour of the animals throughout all experiments and when at rest. Appropriate action will be taken where an animal is thought to be distressed at any time. Eye-drops will be used in very similar techniques to those employed in humans with the same use of local anaesthetic drops and general anaesthetic where appropriate. Some examination techniques may include restraint for short periods and pre-injection of visualising substances such as fluorescein as is used in humans. Minimal discomfort is expected from these procedures which will be limited in number and frequency. In order to record eye movements in some mice it will be necessary to attach a small fixing plate to the head by an adhesive using a surgical technique. This will be</p>

	<p>done under general anaesthetic with appropriate subsequent pain-killers and a long recovery period. Subsequent eye movement tests will be done by minimal animal contact and are limited in time and frequency. Some animals experience discomfort but in most cases only in the period immediately after surgery. Additional pain-killers will be used as necessary.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>In order to establish L-DOPA as a treatment in infants and young children with albinism, it is necessary to define exactly when treatment will be effective. In humans, albinism is a very large and variable group of conditions, making it extremely difficult to diagnose the specific subtype at an early age. This limits the precision with which the potential therapeutic window can be determined, necessitating the use of a genetically well-characterised animal model of albinism, where it is possible to test the efficacy of L-DOPA treatment at specific and earlier ages.</p> <p>In addition, the safety of L-DOPA treatment has not been established in infants under 3 years of age and it would be unethical to commence L-DOPA treatment trials in human infants and young children prior to demonstration of proof of concept and safety in animals first.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The numbers of animals needed will be determined by accurate statistical calculations, allowing us to use the minimum number of animals possible per protocol and by adopting a longitudinal study design.</p> <p>In order to minimise the numbers of mice needed & any possible distress, the functional & anatomical effects of administering L-DOPA to mice at different ages on retinal development will be determined by performing longitudinal, non-invasive eye examinations.</p> <p>By including up to 3 eye movement recording sessions per study mouse, we are reducing the number of mice needed to provide high quality eye movement data required for data analysis.</p>

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>For this study, I have chosen the only genetically well characterised animal model of albinism in which preliminary data exists for the use of oral L-DOPA (including drug dosages and formulation) in rescuing retinal function. In addition, electroretinography (ERG) have been established as a measure of visual function in both control and albino mice. Optical coherence tomography (OCT) retinal imaging in mice has also been shown to be reliable. This makes them an ideal model for longitudinal monitoring of retinal development response to L-DOPA treatment.</p> <p>We will reduce suffering by minimising the number and frequency of procedures, minimising the stress and suffering of mice during procedures and reducing the number of animals used. For example, for eye examination, animals will need to be restrained. We have established protocols for causing minimal stress and minimising the time of restraint. Strict rules will be applied to the number of procedures or examinations per week and per animal and for the duration of the technique. All procedures will be completed with close attention to animal stress signs and general anaesthetics, local anaesthetics and systemic pain killers will be used as they are when these procedures are carried out in human children.</p>
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Project	Treatment of Neurodegenerative Disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project will focus on developing treatments for neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), Alzheimer’s disease (AD), Huntington’s Disease (HD) and Parkinson’s disease (PD). These diseases have a significant impact on individuals as well as socio-economic costs for society. There are currently no cures and treatments only address the symptoms but do nothing to stop the progression. In addition, many of the treatments are either poorly tolerated or associated with side effects or safety issues. There is a clear need for better treatments with improved side effects and safety profiles. Therefore, the purpose of this project is to investigate new medicines to treat neurodegenerative diseases and improve our	

	<p>understanding of the disease mechanisms.</p> <p>Additionally, in order to bring new medicines to patients, regulatory authorities require information on how drugs and other substances affect mammalian physiology. This must be studied in animals before progression of medicines into the clinic.</p> <p>Data will be generated by dosing animals (either naive or following induction of neuroinflammation) with test compounds and assessing the effects on animal behaviour with subsequent analysis of tissues to assess neurochemical changes.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>In this project, we will use animal models of neurodegenerative disease to assess the effect of novel compounds on cognitive enhancement in AD and PD - and reversal of motor deficits in ALS and PD and reduction of dyskinesia (a movement disorder which is a side effect of dopamine replacement therapy) in PD. We also aim to investigate therapies with the potential to slow down or halt the progression of neurodegenerative disease – currently a major unmet clinical need. This will help us further understand neurodegenerative disease mechanisms, which in turn will accelerate the goals of finding new, improved and more effective medicines to treat neurodegenerative disease. Data generated will also help to inform Artificial intelligence and Machine Learning so that better compounds are designed, meaning fewer animals are needed for research. The long-term benefit of this project is compounds moving forward into pre-clinical development and ultimately clinical testing in humans</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will only use rats and mice in this project. We expect to use around 6000 rats and 3000 wild type mice.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the</p>	<p>The experiments are designed and conducted by highly trained personnel to ensure animals suffer the minimum amount of distress. Naive animals or those dosed with compounds known to induce neuroinflammation, a key driver of neurodegeneration (usually once but at times repeated dosed may be</p>

end?	required) will be used under this licence. Animals will be dosed with novel compounds aimed at improving disease relevant deficits which will be assessed using behavioural testing (such as locomotor activity or novel object discrimination tests) when required for biomarker analysis blood samples may be taken and tissue will be taken post mortem (or under terminal anaesthesia). This is minimally invasive, and animals will be monitored for signs of discomfort or distress. Low doses of compound will be dosed initially and animals will be closely monitored for adverse drug reactions.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The complex circuitry of the brain and the resultant loss of brain cells controlling movement and other functions cannot be modelled by computer simulation or cell culture; therefore animals must be used for these studies. Rats and mice are the lowest vertebrate species in which established and well characterised protocols exist for modelling the effects of neurodegenerative dysfunction of the CNS</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Before novel medicines are tested in animals, they are extensively screened using in vitro systems including using enzyme assays, receptor screens and cell culture where relevant to assess the mechanism of action and potential benefits. Only those treatments that show a positive potential to treat neurodegenerative disease will be assessed in appropriate animal models.</p> <p>The minimum number of animals will be used for each study in order to obtain information and data required.</p> <p>Pilot studies may also be performed prior to the large scale assessment of drug activity to test conditions and procedures. These strategies will minimise the adverse effects and reduce animal numbers to a minimum.</p> <p>Behavioural assessment can be used to determine the symptomatic effect of new treatment strategies, and to predict the long term effects and side effects of these agents. In addition they can be used to determine their ability to slow the progression of the disease and hence give an indication of the</p>

	<p>effectiveness in neurodegenerative disease. These tests can be performed throughout the duration of the study, thus reducing animal usage. Data generated will also help to inform Artificial intelligence and Machine Learning so that better targets are identified and better compounds are designed, meaning fewer animals are needed for research.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use established protocols that limit the suffering of the animals and reduce the numbers to a minimum. New study protocols will be extensively reviewed prior to implementation and steps will be taken to minimize potential adverse effects.</p> <p>Good husbandry and care practices based on veterinary advice will be used throughout and all animals will be sufficiently monitored to allow adverse effects to be identified at an early stage and steps taken to minimise them.</p>

Project	Tumour Models for Therapy of Advanced Cancer	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to utilise clinically-relevant animal models to understand the role of specific genetic abnormalities causing cancer development and progression and to evaluate novel therapeutic approaches for advanced malignant disease.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Once cancer spreads (metastasises), cure rates significantly diminish and over 90% of cancer deaths are due to secondary cancers at distant sites in the body. We are developing therapies targeting genetic alterations associated with tumour growth, tissue invasion, cancer spread and also the cancer's blood supply	

	<p>(angiogenesis) on which sustained growth and the opportunity to disseminate via the blood stream depends. We need to model both common cancers and rarer cancers which are hard to cure and also their major sites of metastasis to ensure that our new drugs are capable of tackling these unmet clinical needs. While targeted therapies have shown some promise, the development of drug resistance and the need for rationally-designed combinations of agents is becoming a major issue which will be addressed in this licence. We measure the efficacy of an agent (on primary tumours and/or metastases) in relation to its levels in the blood and/or tumour to inform the optimum starting dose and schedule in man. In parallel, we develop quantitative biomarkers of response which help us to understand determinants of sensitivity or resistance and to confirm that efficacy is tightly linked to the desired mechanism of action. This knowledge and the technology we develop in our models is directly transferrable to the clinic. We also check normal tissues at autopsy, and aim to define the 'biologically effective dose': the minimum dose of the drug that gives therapeutic benefit without significant adverse effects. The most promising compounds proceed to clinical development and trials in cancer patients.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We use immunocompromised mice -the simplest species suitable for such complex pathophysiological studies in which human tumour cells can be grown. Over a 5-year period, we expect to use no more than 7000 mice.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>All procedures are designated as moderate. Adverse effects are related to the implantation and growth of tumours (superficial and within internal organs) surgery to remove primary tumours, ovaries or testes, anaesthetics for surgery or imaging and the effects of therapeutic agents. All animals will be humanely culled by a Schedule 1 method or by collecting blood at the end of the studies.</p>
<p>Application of the 3Rs</p>	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Human cancers develop in 3-dimensional (3-D) space within specific tissues in the body, each providing a unique growth environment which cannot be adequately modelled in 2-D cell cultures grown on plastic dishes in the lab. Cultured cells are provided with constant, optimal levels of oxygen and nutrients, and are all growing at the same rate. This is rarely the case in the body, and variations in these parameters can significantly influence responses to therapy. Metastasis in particular (the major cause of treatment failure) is exclusively an <i>in vivo</i> phenomenon, since during this process tumour cells from a primary cancer must access the blood circulation, spread around the body and colonise new organ sites. Similarly, the effects of drugs must be tested <i>in vivo</i> to determine that adequate levels are achieved in tumour tissues, that adverse effects on normal tissues are minimised and that efficacy tracks with effects on appropriate biomarkers.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>All compounds are first tested in tissue culture for potency, specificity and stability using both simple tumour cell monolayers and more complex 3-D functional assays (e.g invasion). Failure at any of these stages, limits the number of compounds going forward for testing in animals. As far as possible we use cells in which we express luminescent or fluorescent markers that emit light, enabling detection of tumours inside mice using optical imaging, which is quick and requires only light anaesthetic. Otherwise we use methods such as magnetic resonance imaging or ultrasound to locate and follow the development of internal tumours and their response to therapy. Thus fewer mice are required and studies can be terminated before the animals experience significant symptoms. We ensure that we obtain the maximum possible information from each tumour, assessing not only tumour growth rates but also correlating efficacy with drug levels and biomarker responses to give statistically robust data in proof of concept trials</p>
<p>3. Refinement</p> <p>Explain the choice of species and</p>	<p>Mice are the lowest species that are appropriate for <i>in vivo</i> drug development studies and are</p>

<p>why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>widely used for this purpose. Most of our work is carried out using well-characterised human tumour cells, grown in the appropriate anatomical site in naturally immunodeficient adult mice to avoid tissue rejection. This enables us to study human cancers in the correct tissue microenvironment. The animals are maintained in individually ventilated cages using sterile food and bedding and all procedures are carried out in special cabinets using strict aseptic techniques to avoid infections.</p> <p>Suffering will be minimised by keeping tumour burdens within tolerable and acceptable limits and according to NCRI guidelines. Compounds to be evaluated will have been selected for potency, stability and tolerability in other projects. They are delivered using previously determined well-tolerated doses and schedules, and are generally of low toxicity (e.g. agents targeted to molecules selectively overexpressed or mutated in human cancers).</p> <p>By using cutting-edge preclinical imaging technologies, such as ultrasound and magnetic resonance imaging, we will ensure that injection of tumour cells into the animals is performed with highest possible precision. This will increase success rates and reduce the impact of procedures (e.g., less surgery) on animal wellbeing. Imaging also allows for monitoring of tumour growth and better definition of clinical endpoints critical for timely termination of experimental procedures thus significantly refining our <i>in vivo</i> methodology.</p>
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Project	Type 2 inflammation in health and disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>There is a global need to cope with parasitic worm (helminth) infection and allergies. Helminth infections affect a third of the world's human population and most mammals, while allergy is at epidemic levels in the developed world, and an increasing concern around the globe. This project aims to help our understanding of these conditions, as well as their relationship to one another.</p> <p>The overarching aim of our research is to determine which cells are involved, and which mechanisms and pathways are used, to initiate, maintain and regulate a form of inflammation, called 'Type 2', found in parasitic worm</p>	

	<p>(helminth) infection and allergic responses.</p> <p>Specific objectives:</p> <ol style="list-style-type: none"> 1. To define the function and importance of specialised immune cells called dendritic cells in initiation, maintenance and regulation of immunity and inflammation when the body is challenged with substances that alter immune responses 2. To define the function and importance of dendritic cells in initiation, maintenance and regulation of the immune response and inflammation against allergens and during infection with a helminth called <i>Schistosoma mansoni</i> <p>Our ultimate goal is identification of cellular and molecular targets for rational development of therapeutics.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The primary benefit of our work will be to discover new knowledge about the initiation, maintenance and regulation of Type 2 inflammation by identifying fundamental mechanisms that control inflammation. This will ultimately provide novel candidates for the development of therapeutics targeting cells or their products (in any inflammatory disease). It also has the potential to direct drug development.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>The increased availability of genetically altered mice relevant to study of the immune system has allowed us to elevate and refine the questions we can address. Approximately 39,700 mice over 5 years will enable us to maintain genetically altered colonies (approximately 22,000) as well as investigate immune challenge with substances (8,000), and interrogate models of helminth infection (4,500), airway inflammation (4,200) and intestinal inflammation (1,000).</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at</p>	<p>Most of the animals undergoing experimental procedures, even with immunological manipulation, will experience mild, and no more than moderate, severity limits. Breeding and maintenance of genetically altered mice with</p>

the end?

specific deletions in immune function genes or transgenic expression of immune receptors are not expected to exhibit any harmful phenotype. However, as genetic alteration of key molecules or cell types can sometimes result in harmful phenotypes, it is our intention to only carry out experiments using such animals when absolutely necessary (1,000 over 5 years). Our assessment of the importance of dendritic cells during Type 2 inflammation generated by the body in response to challenge with substances can result in local or systemic inflammation and pathology. This can manifest as weight loss, the involuntary bristling of fur, reduced spontaneous activity and reduced response to external stimuli. However, in most cases, only a small proportion of experimental animals will develop beyond mild symptoms to moderate severity limits. Studies on immune and inflammatory aspects of infection with schistosomes will generate life cycle stages (eggs) of the parasite. From approximately 6 weeks post infection, schistosome infected mice may show swollen and distended abdomens, normally associated with weight gain. Some animals (up to 25% across experiments, depending on strain of mouse, infectious dose and time post-infection and, from our previous experience, approximately 10% over the lifespan of the licence) can suddenly succumb to infection from week 5 onwards, with no overt warning signs or evidence of suffering. However, the majority of our experiments will not involve strains of mice, doses or times post-infection that will lead to this level of disease severity. In most cases, only a small proportion of experimental animals will develop beyond mild symptoms. In investigating lung inflammation by airway manipulation using established models, some animals (5-10%) may experience temporary (less than 24 hours) respiratory symptoms resulting in moderate severity limits. However, most experimental animals will not develop beyond mild symptoms. The investigation of established models of inflammation of the small or large intestines generally result in moderate symptoms including weight loss, inactivity and loss of appetite, which can sometimes lead to bleeding and prolapse, resulting in moderate severity limits. Some protocols will involve general procedures such

	<p>as restraint, injection or use of anaesthesia. All of these provide the possibility of adverse effects, but none beyond moderate severity. All animals will be humanely killed at the end of each Protocol.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The mammalian immune system is highly complex, with many different cells and molecules working in combination to produce a co-ordinated response. Thus, the use of lower organisms such as <i>Drosophila</i> is not feasible, as they do not possess a complex immune system seen in mammals. Similarly, in vitro cell culture models cannot give an accurate reflection of the cellular and molecular complexity of a mammalian immune system. Thus, use of mammals is essential, with mice proving an invaluable tool in studying immunity and inflammation in the past 25 years.</p> <p>Our research also depends on generating the egg stage of <i>Schistosoma mansoni</i> for use in subsequent studies. There is no alternative means of generating this life cycle stage other than in a mammalian host.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We reduce numbers of these animals in our experiments by using littermates where possible as controls. Increasingly, we are generating frozen embryos or sperm for later use. In many of our preliminary experiments, we generate primary cells from bone marrow isolated from only a few animals to test our hypotheses, before we embark on more extensive <i>in vivo</i> experiments that require use of larger numbers of animals. This approach also reduces the number of genetically altered animals that have to be bred to facilitate the research.</p> <p>Our animal work is designed in consultation with statisticians and/or using the NC3Rs Experimental Design Assistant, in order to use the minimal possible animals in experimental groups that will still achieve significant results.</p>

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use inbred laboratory mice and genetically altered mice for the vast majority of our research as they provide a range of refined approaches not available in any other species for investigation of immune cells and their products.</p> <p>Mice are the most established animal model for study of the parasitic worm that we work with (schistosomes). Further, <i>Schistosoma mansoni</i> in mice is the most established model for human schistosomiasis. Parasite migration, maturation, egg deposition, and pathological consequences of infection in the mouse are similar to the processes in humans. A proportion of <i>Schistosoma mansoni</i> infected mice may show signs associated with hepatosplenic disease from approximately 6 weeks post infection. Doses are carefully adjusted to minimise adverse effects.</p> <p>Sensitisation and challenge of mice with allergens or allergen-loaded cells generates airway inflammation that is an accepted model for studying mechanisms underlying human asthma. Similarly, dextran sodium sulphate (DSS), methotrexate or cell transfer provide accepted models of intestinal inflammation that are the foundation of innovative research into colitis and inflammatory bowel disease in humans.</p> <p>We are constantly assessing and refining our methods to give the best possible scientific approach coupled with the minimal severity and numbers of animals used. In all studies, animals will be closely monitored and should any unreasonable or unexpected loss in condition be observed, the animals will be humanely killed.</p>
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Project	Understanding and influencing neural responses in the rodent visual system	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of this study is to better understand the nature of information processing in the brain, using the mouse visual system as a model.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our work aims to obtain a greater understanding of how the nervous system processes information. Once we have performed this work, others will be able to build on it with an aim to develop therapies of neurological and psychiatric disorders including blindness, and schizophrenia.	

<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>3,000 animals/year, 15,000 for 5 years total across all protocols.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The main experiments in this project involve training mice to perform visual discrimination tasks to indicate their visual perception, while monitoring or influencing their brain activity. To allow access to the brain via optical or electronic means, the mice undergo surgical procedures. During these procedures they are fully anaesthetised and they do not experience pain. Effective analgesia is provided after each surgery. Use of sterile techniques, strict analgesic regimes and careful monitoring of the animals during and after the surgery minimises any potential pain or distress experienced by the animals. After recovery, they are trained to perform a task and water is given to them as a reward in each trial of the task. To ensure motivation, water access is controlled so the mice obtain most of their water by performing the task. However, care is taken to ensure adequate hydration, so that each day the mice receive the correct amount of fluids. Animals are also supplied with supplementary gels or mild sweeteners to keep them motivated and to ensure their weight is at a healthy level. After the experiments, the mice will be euthanized.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Measurements of visual performance are currently only possible in live animals. Moreover, the part of the brain that we study, the cerebral cortex, is present only in mammals, and since we need to measure its responses during visual performance, we can do this only in live mammals.</p> <p>Computer simulations cannot give us the information we seek: Although technology to simulate neuronal circuits function is becoming increasingly powerful, they are currently too different from the actual brain. The data collected in this project will help make those simulations more realistic so that one day we may be able to forgo animal recordings, but this is a distant goal.</p>

	<p>Achieving it would mean that we have understood the cerebral cortex, which is the very goal of our research.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Our approach exploits new technology that allows recording from large numbers of neurons simultaneously. Indeed, through these new forms of imaging and electrical recording, we can monitor the activity of tens to hundreds times more neurons than an experiment using conventional methods. This makes it possible to use far fewer mice than in previous studies. For instance, we have refined the methods of two-photon imaging so that they provide data from thousands of neurons at a time, reducing the number of animals needed for a study. Similarly, we adopted next-generation Neuropixels probes that dramatically increase the number of neurons that can be recorded at a time. Again, this reduces the number of animals needed in our studies.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Research in mechanisms of brain function such as decision making has been traditionally performed in non-human primates. However, mice are a species whose needs are easier to meet in a laboratory environment, so we have been early advocates of moving this research to mice. Indeed, mice are becoming the prevalent mammalian species in biomedical research, as only in mice is it possible to use extremely powerful techniques of genetic manipulation and targeting. This allows us to study the activity of specific cell types that are relevant to our research questions, and provides us with data of extremely high quality, while also reducing the number of mice required for the study. The techniques of simultaneous recordings described above rely heavily on these techniques. We take multiple and stringent measures to prevent welfare issues and minimise harm:</p> <ul style="list-style-type: none"> ● We continuously refine the anaesthetic and analgesic regimes for pain relief during surgery and recovery. ● Our techniques in subsequent experiments are often minimally invasive, as they involve imaging.

	<ul style="list-style-type: none">● We spearheaded the recent design and manufacture of extremely thin recording probes, which has greatly reduced any potential mechanical damage to the brain tissue.● We continually refine the head attachment system to minimise discomfort, by choosing light-weight head attachments, and streamlining their shape so as not to restrict normal mouse behaviour in their home cage.● By progressively acclimatising the mice to the behavioural environments, we make them comfortable with head fixing.● We made several refinements to the design of our training rigs, in order to provide the best possible environment for the animals to train in. This includes introducing sound proof boxes around training rigs to maintain a quiet environment, and thermoregulator fans to maintain the appropriate temperature.● We developed a robust database that has greatly enhanced the visualisation, calculations and monitoring of daily weights and water amounts for animals on water restriction. The database computes daily water amounts, defines the required action to be taken when the weight is closer to 80%, and generates auto reminders to users when action needs to be taken. The database allows monitoring by personnel involved in husbandry, in experiments, and in management, thus minimizing potential issues or errors.● By carefully controlling the minimum amount of water received every day and by recently introducing supplements such as a sweet water, we avoid dehydration and reduce weight loss, while maintaining appropriate levels of motivation.
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Project	Understanding and tackling the metabolism of brain tumours	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Brain tumours continue to kill more children and adults under the age of 40 years than any other cancer. Brain tumour deaths are rising, and represent ~3% of all deaths from cancer. Glioma is a type of brain tumour with microscopic similarities with non-neuronal cells of the central nervous system (i.e. glial cells), and high-grade gliomas have an unfavourable prognosis. In the majority of cases, glioma patients undergo surgery and treatments, which extend their life expectancy for months, at the expense of their quality of life. There are many reasons for this devastating impact of brain tumours in the UK,	

	<p>but they can be grouped into two basic factors: the anatomical localization of this cancer, and the lack of understanding of its biology.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>A better understanding of the biology that enables brain tumours to grow and invade normal tissue is needed for developing better clinical options to treat this cancer. This project aims to improve the understanding of brain tumours biology, and to translate this knowledge into interventions that can improve the prognosis of brain tumour patients. In particular, by studying tumours in their tissue of origin, the brain, we will shed light on the complex network of biochemical reactions that supply the tumour with energy and building blocks required for growth (i.e. cancer metabolism). Identifying the specific nutrients needed by brain cancer cells, could lead to the design and development of diagnostic and therapeutic interventions for patients.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>In this study the majority of mice will act as hosts to grow tumours. The tumour cells will come either from cancer patients, or from other mice with cancer. These animal models of the human disease will be treated with environmental and behavioural interventions (such as diet and physical activity), physical agents (such as radiation), or chemical compounds (such as drugs), aimed to understand, detect, image, or impair tumour initiation and progression. We expect to use approximately 2000 mice in total over 5 years. Up to 1500 mice will host a tumour derived from an-other organism. Up to 300 mice will be used as dis-ease-free controls.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The majority of the mice in this study will host a brain tumour. We expect to achieve scientific results at early stages of tumour development. However, in some mice the tumour will be allowed to progress towards an advanced stage. In either cases, the illness will be carefully monitored and the animals will be humanely killed when prefixed endpoint are met. The injection of tumour cells into the mouse brain requires cranial surgery. The mice will be under anaesthesia throughout the surgery and will be given post-operative pain relief. After surgery, mice are kept warm using a heated cage rack, and returned to</p>

	<p>their home cage when fully awake and mobile. The most commonly expected adverse side effects caused by the tumour growing in the brain are weight loss and unresponsive behaviour. Seizures are common in brain tumour patients, and they are also expected at advanced stages of the disease. Other adverse effects may include mild neurological and behavioural symptoms. Mice treated with drugs, diet, radiation or other agents may suffer from reduced blood cell count, diarrhoea, paralysis of limbs, or abnormal behaviour patterns. At the end of each experiment mice are humanely killed and, when appropriate, tissues harvested for further testing. The described procedures have moderate levels of severity.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Animal-free models of cancer do not allow us to study the complex relations between a) cancer cells and healthy cells, b) tumour and hosting organs, and c) tumour and systems of organs (i.e. body). Animal-free models such as cells cultures live in a closed system (culture dish) which cannot autonomously exchange nutrients and metabolites with the environment. Whereas, a real tumour grows in an animal which is an open system able to exchanges nutrients and metabolites with the environment. Moreover, the metabolism of specific organs (for example drug metabolism in the liver) plays an important role in how well the anticancer therapies work. Finally, the metabolism of the animals influences the type and level of side-effects, such as loss of body weight. Therefore the requirement of studies on animals remains.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>This study will be complemented by experiments employing cells in culture, which will be the preferred experimental models for screening anticancer targets and drugs, and for understanding their mechanism of action. The size of each experimental group will be based on experience directly gained during this study, or previously reported in similar models. The minimal number of animals needed to detect statistically valid differences between experimental groups (power calculation) will be</p>

	<p>calculated under the advice of an expert in statistics. Pilot studies with fewer numbers of mice will be performed when using new models and therapies. The use of non-invasive imaging techniques (such as PET-MRI), will enable us to see tumours at an early stage of development, to follow tumour growth over time, and to evaluate its response to treatment. Therefore, these techniques will avoid unnecessary killing of animals.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The large body of knowledge about the physiology and pathology of the central nervous system of mice makes these animals amongst the most suitable experimental animal model for brain cancer.</p> <p>Generally this licence uses immunocompromised mice to grow tumours of human origin with procedures that are no greater than moderate severity. The use of immunocompromised mice allow us to grow human tumours successfully without rejection by the host. Mice which have a full, uncompromised immune system may also be used in studies where immunity is thought to play a key role in tumour biology. A condition needed to study the impact of the immune response on tumour growth is the obtainment of genetically similar individuals. The short life-cycle and high reproduction rates of mice favour this result. Protocols with minimal severity will be favoured. To minimise suffering, all mice on procedure will be frequently monitored and humanely killed when exhibiting a sign of substantially altered health status. To clarify to users when mice should be killed, a dedicated monitoring sheet that lists clinical signs and gives classifications of "A", "B", and "C" symptoms will be used. All users will be fully trained in monitoring tumour development for intracranial and subcutaneous models and users will be signed as competent prior to initiating their own studies. Pilot studies will be performed when using uncharacterized cell lines for xenografts to determine the take rate, and for the characterisation of tumours. This will determine if a full-scale experiment is merited and will help answer scientific questions more efficiently. Surgical procedures will be performed in a dedicated well-equipped surgical suite, using aseptic techniques. All mice on procedure will be</p>

	<p>frequently monitored, and will be humanely killed when reaching pre-set endpoints clearly classified in monitoring sheets. Painkillers and antibiotic therapies will be administered as advised by the veterinary surgeon. The animals will be homed in an enriched environment where fun tunnels and nesting materials will be provided. Non-aversion handling of mice is implemented in our units.</p>
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Project	Understanding and Treating Cardiovascular Disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>This project will investigate how the heart and blood vessels function in health and disease, and how we can use this knowledge to improve outcomes for patients. These include patients who have an inherited condition that results in blood vessel problems that can also affect heart function. In addition, we aim to develop treatments for patients with a heart attack. Patients now have an increased chance of surviving a heart attack, providing they reach a suitable clinic in a rapid time frame due to improved intervention at the acute stage. However, heart attack patients who are discharged from hospital have an increased risk of developing heart failure over subsequent</p>	

	<p>months and years. This (together with our ageing population) means there is a growing number of patients in the western world who develop heart failure. There are now more than 0.5 million patients living with heart failure in the UK. There is no effective treatment (other than heart transplant) and the disease will get progressively worse. Better treatments for patients at the acute stage of a heart attack will reduce damage to the heart and thereby reduce the risk of later progression to heart failure. The work on this licence aims to address this issue using mice to model myocardial infarction.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Studies to further increase our understanding of the disease mechanisms will underpin improved treatments for two groups of patients: (i) those with an inherited vascular disorder that affects approximately 1/5000 people; and (ii) those who survive a heart attack that subsequently progresses to heart failure. Based on our advances in understanding we will use drug treatments that can completely or partially rescue the inherited vascular disorder. We will also use small molecule inhibitors for delivery at an early stage following a heart attack to reduce heart injury. Our goal is to improve long term outcomes for both these patient groups.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice : approximately 3,000 adults and 300 neonates per year. The majority of the adult mice are used in breeding programmes, and because of the silent nature of the gene alterations, they are indistinguishable from wild type mice</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Inactivation of target genes may lead to appearance of clinical symptoms due to the development of abnormal blood vessels. Delivery of substances to rescue the clinical problems will be given via the least stressful method possible eg via the food or drinking water. Alternatively, where injection is required, multiple doses will be given using a surgically implanted minipump. On rare occasions the drug may be delivered locally in the eye of young mice, and implants may be placed beneath the skin to monitor blood vessel development. Ligating a coronary artery will be used to model a heart attack. The surgery is complex and on some occasions this can lead to</p>

	<p>respiratory distress or intra-operative bleeding. If this occurs the animals are humanely killed without recovery from anaesthetic. A small proportion of animals may later develop fatal disturbance to the heart rhythm or rupture of the heart which leads to sudden death. Some animals will be imaged using MRI or fluorescent methods, and these imaging methods are not normally associated with adverse effects. We keep within moderate severity limits and all animals are humanely killed at the end of the work.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Unfortunately there are no suitable cell culture systems that can be used to replace in vivo models of cardiovascular development and disease, due to the complexity of the processes involved. Some organs on a chip are in development, but are not sufficiently advanced to model a beating heart, mature blood vessels, inflammation, blood flow and tissue repair. Mice are being used in this project because this is the simplest organism that has a similar heart and blood vessels to human, and that can be used to investigate the roles of different genes in cardiovascular development and disease. Alternative less sentient animals such as Zebrafish are not suitable for this work because they are so evolutionarily distant from human that it would be difficult to translate any of our findings. For example they are cold blooded, they have only two heart chambers instead of four; they have no lungs; and they show endogenous regeneration of the heart following injury, a property that adult mammals do not possess. In some cases we use mouse embryos for analysis. All the work is complemented by cell culture work, for example when investigating processes that occur within individual cells, the effect of bioactive substances will be tested in culture prior to in vivo work.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of</p>	<p>We use the minimum number of animals required for our experiments and regularly consult a statistician for advice. We do pilot work so that power calculations can be done in advance of full experiments. We use online tools for power</p>

<p>animals</p>	<p>analyses (eg https://eda.nc3rs.org.uk/ as discussed recently in Nature. 2016; 531(7592):128) to predict group sizes needed to detect differences with statistical significance based on pilot data. This key feature of good experimental design makes analyses more efficient and minimises the risks of overpowered or underpowered (and therefore wasteful) experiments. Group sizes, gender, strain and age are matched for control and experimental groups. Sources of variability will be identified and minimised wherever possible. With this aim in mind, we have made particular effort to establish a priori exclusion criteria for mice undergoing a surgical myocardial infarction by measuring infarct size by blanching area of the myocardium. This allows us to exclude animals with small infarcts, reducing variability and allowing us to use smaller group sizes for studies. In another example, variability in the matrigel plug experiments is minimised by using small syringes to generate equal plug sizes. In addition, variability in vascular phenotypes following gene activation with tamoxifen is minimised by ensuring the optimised tamoxifen dose is used. Data is collected by researchers blinded to treatment wherever possible and with experimental details recorded following the ARRIVE guidelines.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>In terms of general mouse handling we have moved to the tunnel method to reduce stress when removing a (post weaning) mouse from its cage. When pups are removed (eg for injection), they are removed within their nest and returned the same way, ensuring mother returns to feed them. The genetically modified mice that we use generally carry 'hidden' mutations, such that almost all of animals in protocol 1 are completely healthy until they are given the inducer (eg tamoxifen) to activate the mutation, reducing any clinical effects to the absolute minimum necessary for the project.</p> <p>As this is a continuation of a project licence that has already been running for over 9 years, protocols are already established for the majority of the work described in this application, and numerous refinements that we have introduced</p>

	<p>are summarised in the sections at the end of each protocol. For example 17 refinements have been added to the work in protocol 8. LASA surgical guidelines are carefully followed in all surgical procedures, and LASA guidelines are followed for blood sampling and injection volumes. Delivery of bioactive substances is now a major focus to rescue disease symptoms and to this end osmotic pumps are used instead of multiple IP injections. By using appropriate anaesthesia and analgesics in the procedures to alleviate pain and discomfort, the protocols cause the minimum possible discomfort to the animals. All PIL holders working under this PPL will only work independently for any single procedure after their competency has been confirmed by the named competency officer or deputy (see supporting document describing training and competencies). A body condition scoring system (Hickman and Swan JAALAS 2010) will be used to check animals following procedures with recovery and is accompanied by twice daily checks. A more detailed follow up of twice daily checks and daily weighing is used where a short term loss of weight is anticipated following the procedure and therefore it is important to establish that normal weight is regained. Where animals are to be killed for reasons unrelated to the scientific endpoints of the work described here, the NACWO will be consulted to establish whether the animal tissues would be of value in other studies.</p>
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Project	Understanding barrier manipulation influences the transport of molecules across the skin	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Maintaining good skin health and robust protective immunity is important for healthy ageing. However, skin barrier dysfunction (or damage) is a common feature in many skin conditions such as eczema, psoriasis as well as in skin ageing. In the presence of skin barrier dysfunction, the body release materials that promote inflammation both locally in the skin and the blood circulation and these are associated with the development of various age-related diseases (e.g. cancer).	

	<p>Healthy skin can be achieved by avoiding disease promoters, seeking early interventions for skin problems such as initial stages of skin cancer, and administering effective treatment once skin diseases are firmly established. However, to facilitate each of these processes it is important to be able to extract information from the skin to determine its condition and deliver medicine into the skin in order to treat arising problems. This is not easy as the skin is a highly effective barrier and thus approaches need to be developed to encourage chemical interchange across the skin.</p> <p>At present manipulation techniques which are used to try and extract molecules from the skin damage the skin. It is possible that the natural route by which molecules are secreted from the skin, such as hair follicles and sweat glands, could be enhanced. One means to do this is to stretch these appendages by subjecting them to a controlled vacuum.</p> <p>The project aims to gain a greater understanding of how skin stretching, using a controlled vacuum, influences the movement of molecules across the skin. To facilitate this, healthy skin as well as models of skin inflammation, skin barrier dysfunction and superficial tumour will be utilised. This information will help to develop efficient systems to deliver medicines (e.g. vaccines and skin cancer treatment) directly into the skin without the use of needles and without causing pain or skin damage. It will also allow information to be collected from the skin to detect the onset of disease and define disease status.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Benefit to humans: It is anticipated that upon the completion of this project, a new device will be developed which can both help to administer medicines into the skin and extract information across the skin without the use of hypodermic needles and without causing pain. The device will apply suction onto a small area of the skin and this will allow the movement of chemicals across the skin. Clinicians and scientists could use this device in their practice. Benefits for animals: Once established, the device developed in this project could be used in animal scientific research as a pain-free and needle-free</p>

	alternative to taking blood samples using needles (both as single or repetitive sampling).
What species and approximate numbers of animals do you expect to use over what period of time?	The study requires no more than 900 rats and no more than 1500 mice over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	<p>In this study, therapeutic agents will be administered through topical application to the skin using the novel suction device. The efficiency of therapeutic delivery will be investigated against more traditional routes of administration such as injection at different sites (e.g. intravenous, subcutaneous and intradermal). The application of the device will be completed under brief general anaesthesia (typically 30 min). The animals will be monitored closely to ensure normal behaviour is regained after the anaesthesia sessions. There would be no tangible adverse effects foreseen for most of the studies as the application of the suction to the skin is pain-free and does not cause damage to the skin and the therapeutic agents to be used are well-characterised prior to use in live animals.</p> <p>Two types of inflammation models will be used in this project. The first model consists of inducing minor skin surface damage by applying consecutive tape strips. This is a well-known technique widely used in skin research in animals and humans. It does not cause any pain nor any visible changes to the skin. However, it will cause changes in the biomarkers released in the skin and the blood. The second model is of local inflammation (i.e. swelling and redness) to either the paw (by injection to the footpad) or the ear (by topical application to the ear's skin) but never both. The inflammation symptoms are expected to occur rapidly and recede after 6-8 h. The animals are expected to experience some discomfort and hence close monitoring will be in place to ensure that the animals are not in distress. To minimise any discomfort or suffering, only well-characterised doses will be used, extra bedding will be added to the cages, during the paw inflammation model, and the duration of the experiments will be kept at the minimum required to obtain the required information (typically 6 h). If any animal displays signs of excessive distress or</p>

	<p>pain, it will be humanely culled.</p> <p>Superficial tumour animal models (e.g. skin cancer) will also be used. These models are well-characterised and will allow us to understand the changes that occur in the tumour local environment at the different stages of the progression. The tumours in these models are superficial and can be monitored for growth in a non-invasive manner by measuring their size. The animals may experience moderate discomfort but close monitoring and fastidious process of maintaining welfare will be put in place to ensure there no excessive distress or pain. If any animal displays signs of excessive distress or pain, it will be promptly humanely culled.</p> <p>Several studies will be conducted under non-recovery general anaesthesia which would reduce the animal's distress or discomfort. Any adverse effects that may arise during the study will occur under anaesthesia hence the animal will not experience any pain and if necessary, the animal will be culled before recovery. Whenever recovery is necessary, then multiple procedures (e.g. administration of medicines using injection, application of suction to the skin) will be completed under the same general anaesthesia session, the session is kept to the minimum required (typically 30 min) and the animals are allowed to fully recover and are closely monitored to ensure that natural behaviour is regained between anaesthesia sessions. At the end of the study, all the animals will be humanely culled and skin biopsies and major organs may be obtained for further analysis.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Over the last few years, work has been completed using synthetic membranes and using excised pig skin (obtained from butchers and abattoirs) to understand the effects of skin stretching on biomarkers profiling and therapeutic agent administration across the skin. This work was very useful but is limited as skin models or excised skin do not replicate the complex environment found in a living animal and do not</p>

	<p>account for the flow of the body fluids in the tissue (e.g. blood and interstitial fluid) which have an important role in molecules transfer. This can only be studied in living animals.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Prior to starting this new project, work has been completed using artificial membranes and isolated skin and the protocols have been refined in previous studies using rats in the laboratory. This has enabled us to optimise the suction device and define the best parameters to further the work using laboratory animals. The study design has been and will continuously be optimised and refined in order to reduce the number of animals required for a given scientific purpose. For example, in the cancer model, bi-lateral induction of tumour per animal and the use of non-invasive imaging techniques will allow us to significantly minimise the number of animals to be used while enable us to gather high quality scientific information. The number of animals to be used in each group has been established using statistical calculations based on the findings from our previous project licence. Further consultation with a statistician will be sought at the start of the project in order to obtain meaningful results using the minimum number of animals necessary in new studies using different protocols</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Porcine skin is the most suitable animal model in the absence of human skin to be employed in transport studies across the skin as rodent skin is more porous. However, rather than pigs, we have chosen rodents as the experimental model due to the species being less sentient, ease of handling and keeping of rodents in the laboratory and relevance to the studies to be undertaken. Despite the reported rodent's skin higher permeability, its anatomical and structural similarities to human skin will allow us to understand how manipulating the skin barrier through stretching, under controlled suction, could influence molecular transfer across the skin in humans.</p> <p>For the local inflammation studies, rats will be more suitable than mice as the small size of the mouse's paw and ear does not allow local</p>

	<p>application of the suction device. The ability to apply the device directly onto the inflammation site enable us to investigate biomarkers extraction across the skin and allows the assessment and monitoring of therapeutic interventions. Whenever possible, mice will be used. For example, mice will be preferentially investigated for the cancer studies as the site of the superficial tumours (e.g. dorsal skin) allow efficient application of the suction device.</p>
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Project	Understanding cortical plasticity	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our aim is to understand cortical plasticity sufficiently to manipulate it safely for therapeutic benefit.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The future benefits of our research are that we will gain an understanding of how synaptic plasticity works in the cerebral cortex, how it goes wrong in disease conditions and thereby learn how it can be manipulated for therapeutic benefit in neurological and mental health conditions such as schizophrenia, Alzheimer's, stroke and mental retardation.	

What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate using approximately 1,000 mice per year, plus a further 1,600 in breeding colonies.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Some of the protocols do not have adverse effects such as those for breeding and maintenance or some of the techniques such as behavioral testing. The protocols that involve surgery are conducted under anaesthesia and could potentially result in infection. The expected level of severity of the adverse effect might rise to moderate. The animals will be killed humanely at the end of the protocol or if the severity limit is exceeded despite remedial action.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are studying the biology of learning, memory and adaptation to sensory experiences. Only whole animals have these biological processes and therefore non-animal alternatives are not possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the appropriate numbers of animals to achieve statistical significance. Where possible we will use longitudinal studies and within animal controls to achieve as much efficiency as possible from the statistical design.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is the lowest sentient laboratory species with a cerebral cortex akin to humans. In addition, it is one of the most commonly genetically modified mammalian species, thereby allowing researchers to understand genetic models of human disease. Measures are taken to keep the duration brief and to administer anaesthetics where pain might otherwise occur.

Project	Understanding disease biology to identify new treatments for lymphoma	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Key specific objectives:</p> <ol style="list-style-type: none"> 1. To determine the mechanisms by which the tumour's surrounding environment, called the tumour microenvironment provides pro-tumour signals in lymphomas using established mouse models of disease. 2. To develop and test novel therapeutic strategies against lymphoma tumour cells or the tumour's surrounding environment, using established mouse models of disease. This 	

	objective will use knowledge generated from objective 1.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Identifying effective combination immunotherapies and the mechanism of action of relevant therapeutic agents would greatly benefit other research groups and clinicians by facilitating the planning of the future direction of their research as well as the design and conduct of clinical trials with the collaboration of pharmaceutical companies. Characterising novel therapeutic targets will also expedite the design and development of new drugs and cell-based therapies by the pharmaceutical industry. In addition, determining the efficacy of these new therapeutic approaches, could lead in the long term, to the development of medicinal products for human use with the ultimate goal of achieving longer remissions and better overall therapeutic outcomes in patients with B cell cancers which will lead to an undoubtedly better outcome in terms of both patient quality of life and healthcare economics.
What species and approximate numbers of animals do you expect to use over what period of time?	We have confined our experiments to mice. It is estimated that fewer than 5000 mice will be used during the 5 years of this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Anticipated adverse effects, caused by induction/development of cancer and/or anti-cancer treatment, include loss of appetite, weight loss, hunching, piloerection (fur standing up), lethargy, difficulty moving, difficulty breathing, skin rash, looking pale, diarrhoea and effects on specific organs, e.g. bone marrow, spleen or/and liver. In all cases, the effects are not expected to be more than moderate in severity and mice developing adverse effects during the course of the study will be humanely killed. All remaining animals will be killed by a schedule 1 method at the end of the study.
Application of the 3Rs	
1. Replacement	We have developed laboratory-based co-culture models of cancer/immune/non-malignant cells

<p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>that allow us to study many aspects of their interactions without needing animal-based studies. However, these experiments cannot accurately model all aspects of the tumour's surrounding environment, (physiological tissue environment) in a living organism, such as the dynamics of interactions between cell types, migration within tissues and the lifespan of tumour B cells. Moreover, the protective effect of the tumour's surrounding environment, is most likely a balance of many aspects that cannot be reproduced in the laboratory. Furthermore, since cancer immunotherapies are designed to work in conjunction with a patient's immune system to facilitate anti-tumour responses, a fully functional immune system is necessary for determining the full therapeutic potential of a cancer immune-based therapy.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Many aspects in our development of safe and effective therapies will be sufficiently addressed by laboratory-based investigations, without requiring animal studies. Experiments will only progress to live animal studies if favourable results are first obtained from laboratory experiments. Toxicity and suitable doses of used agents or cell-based therapies will be assessed in a small number of mice for keeping the resultant harm to a minimum. Where appropriate, pilot studies using small numbers of 3-5 mice will be used to establish feasibility and perform initial optimisations before proceeding to studies with larger numbers. Statistical considerations will feature prominently in the design of animal experiments to ensure single experiments are adequately powered to obtain all required data and thus performing multiple experiments will be avoided.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>All our animal experiments will be performed using mice, some of which may be genetically modified. To the best of our knowledge, appropriate animal models for Chronic Lymphocytic Leukaemia and Diffuse-Large-B cell Lymphoma do not exist in any other species than mice. Moreover, the mouse immune system has been extensively studied and where differences with humans exist they have been documented. All mice will be closely monitored for disease</p>

	<p>symptoms allowing analysis before progression of evident pathology. Humane endpoints are set and animals reaching these or displaying unexpected side-effects will be euthanised using Schedule 1 methods to prevent unnecessary suffering.</p>
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Project	Understanding energy balance in health and disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Health and wellbeing is dependent upon an optimal amount of body fat and muscle. Obesity, defined as an excessive storage of energy as fat, causes significant medical and socioeconomic problems. At the other end of the energy balance spectrum, cachexia, defined as unregulated breakdown of muscle and fat, is a clinical problem that reduces survival in patients with malignant and inflammatory disease.</p> <p>Both conditions have unmet clinical need with meaningful intervention requiring an understanding of the processes involved.</p> <p>This project aims to investigate how these</p>	

	<p>disorders of energy balance can result from disruption of the critical pathways functioning in us all that control how we eat, how we metabolise fuel and how we store excess energy in our tissues.</p> <p>The basis of these studies come primarily from studies of human disease, including preliminary findings from rare genetic forms of obesity, data from clinical intervention studies and also larger population based genetic studies.</p> <p>We intend to extend these findings and use animal models to help gain a more mechanistic understanding of how the pathways that have been highlighted can go wrong and result in metabolic problems.</p> <p>We will also examine how these pathways function in the face of different external stressors such as different ambient temperature and different diets because we know that conditions such as obesity are the result of complex interplay between genes and environment.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>In undertaking these studies, we will generate new scientific knowledge around the role of brain-centred pathways that control body composition. We will gain insights into the as-yet-undetermined causes of a severe wasting syndrome which, to date, acts as a barrier to successful therapy in cancer. We will also study the effects of a number of drugs that are being used in metabolic disease to better understand their site of action. These studies will benefit future interventions, being able to signpost potential strategies for therapeutic regimens with less side effects. Finally, we anticipate that we will expand our understanding of the evolving scientific field of the role of so-called “imprinted genes” and their impact on metabolic disease.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use mice. We anticipate the need to use up to 10,000 mice over 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the</p>	<p>Most experiments proposed will lead to no more discomfort than that experienced by any rodent bred in captivity and residing in a modern animal</p>

<p>likely/expected level of severity? What will happen to the animals at the end?</p>	<p>facility. Some animals will experience moderate but transient discomfort when given injections or when having blood samples taken. The injections will often be of naturally occurring hormones, or compounds closely related to them, and are needed as a way to stimulate and study the workings of crucial metabolic pathways. On occasion, animals may be given compounds that are recognised to produce circulating levels of inflammatory markers that are seen in acute illness. A minority of animal will also undergo surgery. This will involve placing small cannula into specific regions of the brain and placing bespoke drug delivery devices under the skin. Animals may undergo moderate, limited discomfort in the immediate perioperative period. However, this will be minimised by administration of painkillers. All animals will be humanely killed at the end of the experiments and tissues taken for further analysis.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Human metabolic disease is the end-result of a complex interaction between multiple external environmental factors and internal hormonal, chemical and neuronal messengers. This cannot be meaningfully replicated in anything other than animal models and although we increasingly use non-vertebrate animals which are of lesser sentience than rodents to help in our studies, none have the necessary complexity in organ structure or wider networks to adequately address the scientific questions posed.</p> <p>However, we continue to replace animals whenever possible and have successfully done so using yeast assay systems and neuronal cell culture lines as alternative methods to animal models. We have also begun studies in fruit fly models and developed a screening method to further study genes relevant to metabolic disease that have been identified in human population genetic studies. These will provide invaluable data to provide focus in future work and replace the need to undertake such screening in mouse models.</p>

<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>To avoid wastage of animals, appropriate background research will be done prior to all experiments. Whenever possible, we will look to work with existing colonies of animals rather than breed new colonies. We allow other trained researchers to work with the colonies in our unit rather than moving mice, reducing the number of mice that are both bred and transported. Studies will be of appropriate size to detect significance. Protocols will include a series of analyses and steps on a single animal, rather than single analyses on multiple animals. We aim to balance impact upon an individual animal with scientific output but reason that this approach significantly reduces the number of animals used.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rodent models allow access to metabolically relevant tissues (like the brain and the pancreas) that remain, due to ethical and practical considerations, inaccessible in human studies. Rodents have well defined pathways that both match those in humans and are readily amenable to genetic manipulation. To minimise impact on welfare we will</p> <ul style="list-style-type: none"> - use enriched, size appropriate housing - use refined standard methodologies in experiments - use non-invasive techniques whenever possible - embed in study plans clear steps for monitoring and early detection of potential side effects, enabling us to apply early humane end-points. <p>To enable accurate measurement of food intake, sometimes mice may be single housed. During this period, there will be appropriate steps to enrich the environment . In addition to shelters, nest boxes and nesting material, tubes to act as hiding tunnels, shredding toys and wooden chewing toys for animals to gnaw on will also be supplied. When not having food intake actively measured, food may also be hidden in bedding and floor covering to give the animals the opportunity to forage.</p>

Project	Understanding how lymphotropism effects tumour metastasis
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
What's the aim of this project?	The overall aim of this project is to identify which genes/cell types are involved in tumour lymphotropism (movement to, and transit via the lymphnodes) and how these impact cancer metastasis.
Why is it important to undertake this work?	Lymphotropism is an important prognosis factor relating to a variety of different cancers, from Oral squamous cell carcinoma, to lung cancer. Once cancer has been detected in the lymph nodes (LNs) it is thought to be an indication of how progressed the tumour is and how aggressive – the more cancer cells found in the LN, the more aggressive the cancer is deemed to be. Often the removal of local LNs is associated with improved survival – this has been found in both lung and endometrial cancer for example. We have chosen to focus primarily

	<p>on breast cancer. With this type of cancer, LN metastasis is frequently seen (30 %) and the 5 year survival rate goes from 99% to 85% if cells are detected in the LN. Once they have travelled to distant organs that falls to 27%, and because of this the LN are often removed as a course of treatment if caught before distant metastasis is found. It is unclear how the LN invasion truly contributes to distant metastases, but it is considered to be an early event in the fatal spread of cancer, so if we can find out what influences this, we may be able to find a way to reduce distant metastasis even after LN invasion has been observed.</p>
<p>What outputs do you think you will see at the end of this project?</p>	<p>Objective 1 will provide a novel reporter mouse model and a new method to visualise and track tumour cells, and the routes they take as they spread throughout the body. Completion of Objectives 2-6 will provide a greater understanding of the mechanisms involved in metastasis and how these may affect treatment. We foresee publications in peer reviewed journals to arise from the project proposed here.</p>
<p>Who or what will benefit from these outputs, and how?</p>	<p>The tool to visualise and track how tumour cells spread throughout the body could have many applications that our lab or other research groups may benefit from.</p> <p>By identifying and profiling lymphotropic cancer cells in both the primary and metastatic sites (Objective 2 and 3), in the short-term we will identify genes involved in promoting lymph node metastasis. In the long-term, these will be validated in vitro and in vivo and could be used as potential biomarkers and targets for therapies aimed at predicting/ preventing lymphatic spread of cancer cells.</p> <p>The completion of Objective 4, as a short-term, will help pin-point which immune cell type may promote lymphatic spread and which genes in that cell type are responsible for this effect. Therefore, it will provide new molecular targets that could be used. In the long-term, to design better informed immunotherapies to stop breast cancer spread via the lymph nodes.</p> <p>Objectives 5 and 6 will provide a greater understanding, in general of how the progression of cancer metastasis is influenced by transit route. The hope is that this will benefit the wider community and ultimately those patients in the clinic.</p>

Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	We will be open to collaborate with other laboratories that have interesting models, data or questions where our methods can be applied. Moreover, all the knowledge and expertise generated with this project will be spread through talks, conferences and publications.
Explain why you are using these types of animals and your choice of life stages.	Mice are the least sentient, and most understood (in terms of gene expression and gene modification and cell labelling) animals that represent mammalian biology. They allow us to recapitulate human disease in a way that other, less sentient, animals can not do. We have many years of experience working on breast cancer in this animal model and have developed tools and strategies that are most suited to mice. We are working with adult mice as they are more tolerate of the methods we wish to use and they are more developed in terms of their mammary gland / breast tissue system (similar to a human who develops breast cancer would be).
Typically, what will be done to an animal used in your project?	An animal enrolled in our project will typically be injected into their breast tissue (mammary fatpad) a solution of cells which will go on to form a primary breast cancer. The cells may have certain genes modified to increase or decrease expression. None of these will be anticipated to create harm to the mouse. The mouse may under go a surgical procedure to remove lymphnodes close to where the primary tumour is expected to grow, this will be done to limit the access of the cancer cells to the lymphatic system and to measure the effect of this on the spread of the cancer cells from the breast tissue to the rest of the mouse organs. Mice may be enrolled in a treatment program to remove or influence certain cells that we believe may play a role in cancer spread. These will be done by administering compounds, via injection typically, the duration of this will be dependent of the experiment but all procedures will typically be finalised within a week or two. Where surgery is required to remove the lymph node, we are proposing a 2 week recovery period before the mouse is given the cancer cells. Mice may also be imaged to look for developing cancer cells in organs that are not externally visible, this will help guide the duration of each experiment.
What are the expected	The mice will develop a tumour in their mammary fat pad

<p>impacts and/or adverse effects for the animals during your project?</p>	<p>, this will be on the underside of the mouse between the midpoint and the back legs. The tumour will in most cases not impede the mouse and normal behaviour, such as feeding and grooming will still continue. They may experience some periods of ill health if they respond poorly to any substance we give them to study/ influence the behaviour of the cancer cells. This should be transient, but some mice may experience some weight loss or digestive abnormalities but this should not persist. The substances we give the mice are not intended to create adverse effects and should we observe this we modify the experimental design. Some mice may experience pain/ discomfort should they develop cancer cell spread to organs. Typically growth in distal organs can be tolerated for a period of time (depending on how aggressive the cancer cells are) and should we observe the mouse is showing signs that the disease has progressed to an intolerable stage we will finish the experiment.</p>
<p>What are the expected severities and the proportion of animals in each category (per animal type)?</p>	<p>We expect the mice will reach a moderate severity in almost all cases where we are using them for experimental purposes. Mice used for breeding purposes (~25%) we expect will be mild or sub-threshold.</p>
<p>What will happen to animals at the end of this project?</p>	<p>killed</p>
<p>Why do you need to use animals to achieve the aim of your project?</p>	<p>Metastasis is a multi-step process involving different systems and environments (i.e. entry into a vascular system, immune-evasion in circulation, extravasation into a secondary organ and invasion of the new tissue). We need the interaction of multiple cell types and systems in order to study the whole process. it is not possible to truly study these interactions in an in vitro setting.</p>
<p>Which non-animal alternatives did you consider for use in this project?</p>	<p>We plan to use many non-animal alternatives within this project, namely cell to cell interactions carried out in tissue culture dishes, and the selection of optimal genes to modify in cells tested within dishes. We also considered using 3D cultures with multiple cell types (tumour cells plus immune cells), in vitro 3d culture systems that model vessels, and ex vivo lymph node cultures.</p>
<p>Why were they not</p>	<p>We do use cancer cell cultures and complex mixed</p>

suitable?	<p>cultures, but once we have identified the best therapy options we have to test them in the context of the whole animal. It is very easy to modify cancer cells in a dish, but we have to identify genes that can be used as potential therapeutic targets that function as suspected within the complexities of a whole body.</p> <p>For all those experiments where the model can be reduced to study a specific aspect of metastasis (i.e. invasiveness, lymphangiogenesis) or the interactions of two cell types (i.e. the immune cell type of interest and tumour cells), we will use in vitro culture assays. In order to reach this level, however, we need to first investigate the in-vivo model. We will also need to return to an in-vivo setting to validate whether our findings would properly translate to a real organ environment (i.e. whether inhibition of a gene that proved effective in culture can really modulate lymphatic trophism in vivo)</p>
Enter the estimated number of animals of each type used in this project.	mice: up to 10,000
How have you estimated the numbers of animals you will use?	<p>For each experiment in each of our objectives, we have carried out a statistical sample size calculation which is detailed in the project plan. In general, we have based our estimate of the size and how variable the data might be that we expect to acquire on similar experiments performed by our laboratory in the past. Where this has not been possible (for instance for methods which are still in development) we have clearly specified so in our project plan, and we will perform a pilot study to better characterize the data which will inform a proper statistical calculation of the sample size.</p> <p>In general, we have designed our experiments using power calculations to achieve a high level of confidence in our results. In all cases in which multiple treatments or multiple testing are used, we have applied statistical methods to take into account the combination of anticipated variability.</p>
What steps did you take during the experimental design phase to reduce the number of animals being	We have attempted to follow the best available guidelines on experimental design, including these listed on the NC3R website. In addition, we screened the existing literature to inform ourselves on the best

<p>used in this project?</p>	<p>standards currently applied in terms of sample size determination.</p> <p>Wherever possible, we have attempted to perform multiple measurements from the same dataset (applying the appropriate corrections) and to compare multiple treatments to the same control, reducing the number of control animals required.</p> <p>We based our sample size calculations on estimates of effect size and variability from previous data of our laboratory. However, this is just an initial estimate, and we fully expect that our understanding of the data will increase over the project. As this happens, we will refine our sample size calculations and either use less animals, if possible without reducing scientific significance, or seek permission to enrol more animals in the study if we realize that a higher number of replicates is needed.</p>
<p>What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?</p>	<p>Pilot studies may be used to establish novel methods and/or to generate preliminary data prior to a large scale experiment. This will also help with statistical guidance. Some tumour-bearing mice with no treatment at all may be used for tissue collection for pilot studies testing novel analytical methods (e.g new flow cytometry / IMC panels). We may also use a pilot study to establish cell depletion assays, on non tumour bearing mice.</p> <p>Pilot studies will also be used to establish the dosing of substances where dosing information is not already available from our experience or from the literature. We will perform a small pilot screen with 2 animals per dose, and determine the minimum dosage leading to the desired physiological effect and the maximum dosage that can be administered without significant adverse effects.</p> <p>mouse colonies will also be maintained using efficient breeding.</p>
<p>Which animal models and methods will you use during this project?</p>	<p>We will use laboratory mice of several different strains, some of which genetically modified in order to either tag certain parts of the body with a fluorescent molecule or to make the immune system less active, in order to study its interaction with tumours. None of these modifications will cause harm to the animals, as they will be housed in a highly clean environment where even immune-depressed</p>

	<p>animals have a very low risk of infections.</p> <p>In order to study the role of the immune system on tumours, we will need to give mammary tumours to the animals and let them progress until they start forming metastases. However, the primary tumours will be in a location that will not severely hinder the activity of the mouse. While metastases are more dangerous, we aim to not let these progress to a clinical disabling stage.</p>
<p>Why can't you use animals that are less sentient?</p>	<p>Mice are the least sentient , and most understood (in terms of gene expression and modifying and cell labelling) animals that represent mammalian biology. They allow us to recapitulate human disease in a way that other, less sentient, animals can not do. We have many years of experience working on breast cancer in this animal model and have developed tools and strategies that are most suited to mice.</p>
<p>How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?</p>	<p>Our institute routinely circulate advances in the 3Rs and we will always seek to identify ways these can be incorporated in our project, while ensuring they do not effect the (statistical/ biologically relevant) consistency of our data collection</p>
<p>How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?</p>	<p>We will use several strategies, refined over the course of our previous work, to minimize suffering for the animals. While producing transgenic animals, we will use methods and breeding schemes that maximize the number of animals with the correct hereditary traits that are produced, this will ensure that no transgenic animal is born unnecessarily. The facility creating the transgenic mice now use a more refined methodology with far improved success rates over those used previously. When creating tumours in the fat pad, we will, where possible, use a non surgical method of entry into the fatpad, that we have refined over the years. While the tumour develops, we will check each animal daily, or in some circumstances twice a day, to ensure that we detect any sign of pain immediately. We will also call on the extensive experience of the animal care staff. We will use a new (live) imaging analysis method based on bioluminescence which is 100-times more sensitive than the conventional one and can visualise metastases when they are much smaller, this will help us to find a humane end point.</p>

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	Our institute has a series of guidelines regulating many of the procedures routinely done to animals, for instance how often an animal can receive an injection, where, and how much, or how often it can give a blood sample. We will follow all of these, as well as several other standard operating procedures that were developed by a team of specialists at our institute for the explicit purpose of minimizing animal suffering, and are periodically updated. While designing experiments. we will follow a series of guidelines existing in the literature to design and report our experiments, and will consult with an on-site statistician to ensure that we're using as few animals as possible for our study.

Project	Understanding how neutrophil migration behaviour is fine-tuned during inflammation	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Inflammation is a natural response of your body to injury or harmful agents that permits rapid defence against infection. All of us experience the bothersome symptoms of inflammation at some point or another, which include local redness, swelling and pain. The redness and swelling are in fact associated with the increase of blood flow and the infiltration of white blood cells, which are crucial for fighting harmful bacteria that exploit the opportunity to enter your body upon injury.</p> <p>A key type of cell that infiltrates inflammatory sites is the neutrophil. It is believed that by</p>	

	<p>controlling the infiltration of neutrophils in tissues it should be possible to increase or decrease inflammation on demand. Why would we want to control inflammation? Excess inflammation is associated with numerous debilitating diseases such as rheumatoid arthritis or chronic obstructive pulmonary disease. Conversely, increasing neutrophil infiltration can be beneficial in some cases of cancer. Current anti-inflammatory drugs have side effects due to their broad mechanism of action. Drugs that specifically target neutrophil infiltration are considered a promising alternative. However, such strategies require careful approaches to manipulate neutrophil migration to balance the trade-off between undesirable excess inflammation and insufficient immunological defence. The overarching aim of this licence is to gain a better understanding of how neutrophil behaviour at inflammatory sites is controlled.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This work would improve our basic understanding of how immune cells sense and respond to damage and how they move and accumulate in tissues. Work from this licence is likely to unravel new ways to manipulate the behaviour of these cells and inflammatory responses therapeutically. Given the pervasive roles of inflammation in disease and the unmet need to fine-tune this process pharmacologically, the long-term benefit of our work is thus considerable. Examples include chronic inflammatory diseases (such as rheumatoid arthritis, asthma or chronic obstructive pulmonary disease) or cancer (such as pancreatic cancer, which has been linked to neutrophil migration and remains one of the most incurable cancers). Our work will also generate new, non-mammalian animal models that could partly or largely replace mouse models for the purpose of drug discovery research.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>12525 adult zebrafish over 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity?</p>	<p>There are no specific adverse effects expected in relation to breeding the animals or the genetic modifications used. In limited cases (up to 5%) we will need to anaesthetise fish to collect eggs</p>

<p>What will happen to the animals at the end?</p>	<p>and sperm for In Vitro Fertilisation (IVF) or for verifying the genetic status of the animals (up to 40%). The latter entails either observing the fish for manifestation of the genetic alteration or direct assessment of DNA from small tissue biopsies, causing minimal temporary discomfort. Our procedure will be carefully monitored and continuously refined to eliminate or minimise any pain or suffering. In the unlikely event that something unexpected occurs, affected animals will be immediately killed. Fish produced under the authority of this project will either be used on this licence or excess stock will be supplied to other projects with authority to use genetically altered fish of this type.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The key aspect of our programme is to visualise neutrophil behaviours in situ by advanced microscopy techniques, to discover new mechanisms regulating this process. This is because the cell behaviours we study are difficult to recapitulate outside the body. We propose to use the zebrafish larva, under 5 days post fertilisation, which is a much simpler organism, not capable of independent feeding and complex cognitive functions but complex enough to recapitulate the neutrophil behaviours in question. Zebrafish larvae are least likely to feel pain and experience distress as they are at an immature life stage. This represents the simplest organism in which we can perform such studies because invertebrates (e.g. fly or worm, which are typical invertebrate models) lack neutrophils. The zebrafish larva is transparent, allowing non-invasive visualisation of neutrophil behaviour by microscopy (the equivalent process in mammals requires surgical exposure of tissues). Genetic modification is also simpler and less invasive than in mammals. Thus, working with this relatively simple organism entails less invasive methodologies.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of</p>	<p>We intend to perform manipulations only on embryos/larvae younger than 5 days post fertilisation (not protected under The Animals (Scientific Procedures Act) 1986. Adult animals (wild type or genetically altered) will be used only</p>

<p>animals</p>	<p>for breeding purposes. The limiting factor in the number of animals used is their breeding performance. The quality of breeding activity is continuously monitored and optimised in our facility (for example through keeping a record of breedings, avoiding repeated use of breeders in small time intervals and performing regular outcrosses). This ensures that we don't over-breed fish. To ensure minimal numbers of fish bred we will carefully consider experimental design to have enough animals to answer a scientific question but not more than necessary.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We are always looking for ways to refine our breeding protocols and keep what we do constantly under review. One way we achieve this is through our Zebrafish User meetings, where users, animal welfare experts and veterinarians meet to discuss and exchange good practice ideas. In this meeting, users of the shared zebrafish facility report issues on survival or breeding rates and we discuss and implement better ways of breeding the fish in a consensus manner. A key regulated procedure as part of breeding genetically modified animals is the required verification and screening of zebrafish for the genetic modification. This entails either observing the fish for manifestation of the genetic alteration or direct assessment of DNA from small tissue biopsies. The former will be the preferred method. We are currently testing environmental enrichment as a potential improvement in our practices.</p>

Project	Understanding inflammasome dependent inflammation	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Here we aim to understand the regulation of an organisms inflammatory response to infection or injury. The inflammatory response is how our immune system reacts to a stress or danger. Inflammation in the absence of infection (sterile inflammation), during injury or disease, can often be damaging and is increasingly implicated as a important factor in many human diseases such as Alzheimer's disease, stroke and metabolic diseases. There are particular components of the inflammatory response that are now known to contribute to disease (called the NLRP3 inflammasome) but we do not know fully how this is works and we do not have good ways of stopping its actions. The objectives of this work</p>	

	are to understand how the inflammasome (in particular NLRP3) works, to identify molecules that can stop it working.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We aim to develop a better understanding of the pathways regulating inflammasome-dependent inflammation and through this understanding, develop new therapeutic interventions for inflammatory disease. In particular we hope to identify new drugs to modify dangerous inflammatory responses. This work could benefit humans as well as animals where inflammation plays a key role in the disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (1800) and rats (350) will be used over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The severity is moderate and the animals will only experience temporary discomfort. We will cause an inflammatory reaction in the animals by injecting them with agents that mimic an infection or a sterile injury. The animals will experience some sickness and the impact will be similar to what humans experience when sick with an infection and symptoms could include lethargy, fever and reduced appetite. However, the symptoms will usually only last for a few hours before the animals are sacrificed to take tissues for ex vivo analysis. In those tissue we will then look at the expression of immune cells and the agents that they release.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Inflammation is a complex vascular and cellular response that cannot be modelled accurately in <i>in vitro</i> systems. An inflammatory response also produces symptoms such as sickness behaviour which is due to an interaction of the immune system with the nervous system and the brain. Thus, the whole body is involved in an inflammatory response and as such whole animals are needed to understand this complexity. The proposed studies could also not be undertaken in lower species because they do not show such similarities to humans including their immune system, and importantly they do not

	have NLRP3, which is a key complex that we are interested in.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Several factors lead to a reduction of animal numbers, including reducing variation and good experimental design involving the use of appropriate statistics. In particular statistical tests will be used to ensure that we use the minimum number of animals possible to reliably interpret our data. Whenever we get new data we will always re-do our calculations in order to make sure we are still using an appropriate animal number to achieve our aims.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice and rats are the lowest vertebrate species that share common pathways to humans with respect to this pathway. We will use well-established methods to cause inflammation without causing severe or long lasting harm to the animals. We understand what doses cause a response in the animals without making them too sick, so we will always use the lowest dose possible to give us an answer. Sometimes we will test the behaviour of the animals but the tests we will use do not cause any distress or lasting harm and usually rely on natural behaviour of the animals (exploration, social interaction) However, all animals will be constantly monitored to ensure that they suffer minimum distress.</p>

Project	Understanding lung injury, inflammation, airway remodelling and pulmonary fibrosis	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Lung injury, inflammation, airway remodelling and fibrosis are complex processes that occur when wound repair goes wrong. generation of the pro-scarring molecule Transforming Growth Factor beta (TGFβ) is a key event in their development. This project will investigate how TGFβ is generation in lung cells, identify the pathways and molecules controlling this process and determine how lack of regulation of TGFβ in the lung leads to the development of these chronic fibrotic lung diseases.	

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>These studies will increase our understanding of how lung injury, inflammation, airway remodelling and fibrosis occur. They will increase our knowledge of fundamental wound repair principles in the lung, and will ultimately lead to the development of desperately needed therapies to treat fibrotic lung diseases that are currently incurable and are amongst the most severe conditions a patient can develop.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice and rats will be used and we would estimate that approximately up to 6,600 mice, and 300 rats, will be used over the 5 year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Each of the animal models requires induced lung injury resulting in development of lung fibrosis and/or airway remodelling. Previous experience suggests that all of these animals will experience weight loss and increased breathing rates and that they may also have a hunched appearance with their hair standing up on end. In aging studies, genetically modified mice may spontaneously develop lung diseases including emphysema or fibrosis, resulting in the development of symptoms similar to those exhibited by animals following lung injury. The overall level of discomfort for both the lung injury and aging studies is expected to be moderate and progress will be carefully monitored to ensure the well being of all animals during the course of these studies. All animals will be humanely killed at the end of the studies.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Where possible we have replaced screening or “blue sky” experiments in animals with experiments performed in test tubes, or using techniques that involve use of tissue samples eg lung slices. However, the processes which control the scarring of the lungs seen in patients with fibrosis are complex and appear to involve interactions between cells of the lung with the cells which circulate in the blood. As it not possible to model all of these interactions in the test tube or tissue samples due to the lack of blood supply, some studies will require live</p>

	<p>animal experiments.</p> <p>These experiments will only be performed when there is initial evidence that these studies will lead to meaningful data that may change the way we approach patients who suffer from lung injury and fibrosis.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>To reduce the number of animals used in experiments we use inbred strains of animals to reduce any genetic variability; perform appropriate power calculations to avoid excessive sample sizes; measure endpoints which are reliable and have the lowest variability; use non-invasive measures of injury and fibrosis, such as CT, CT/SPECT or CT/PET, where possible; and measure as many different endpoints as possible from a single animal.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice will be used for the majority of these studies as they represent the lowest vertebrate species that develops lung injury, lung inflammation and pulmonary fibrosis in response to various challenges. Rats offer some advantages over mice in terms of size and development of fibrosis in response to certain stimuli and will also be used in some studies. We have refined our laboratory procedures to minimise variability in the measurable outcomes and this has allowed us to utilise the smallest effective dose of injurious agent or therapy. As a result in many of our studies we have been able to shorten the duration of experiments, reduce the severity of the symptoms and minimise the suffering that might be experienced by the animals whilst still obtaining meaningful data. Within the scope of these studies we will further refine our methods including developing further novel non-invasive imaging strategies to measure real-time changes in fibrosis and inflammation in a single animal both during disease progression and in response to therapy.</p>

Project	Understanding mechanisms of fibrosis	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Fibrotic diseases are increasing and a major cause of morbidity and mortality worldwide. In some cases, end-stage diseases can be treated by transplantation; however, there is a huge shortage of donor organs; significant side-effects from immunosuppression; and focus on end-stage disease is too late. Urgent development of novel diagnostics to determine stage of disease and anti-fibrotic therapies are needed. This requires a better understanding of the underlying mechanisms of fibrosis to develop hypothesis based approaches to identify novel dynamic markers of disease and targeted strategies for therapeutic intervention. The aim of this project is to provide a greater understanding of the	

	<p>molecular mechanisms underlying chronic fibrotic diseases to instruct identification of novel diagnostic and therapeutic targets that can be used for patient benefit.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Fibrosis is a common step in the progression of the majority of chronic diseases. However, there are no approved anti-fibrotic drugs and diagnosis remains poor. Our work in this area has already uncovered novel mechanisms implicated in broad organ fibrosis that are currently under discussion with pharmaceutical companies as novel diagnostic / therapeutic strategies in fibrosis. There are clear implications for patient benefit and this has only been achieved by proof of principle using both in vitro and in vivo models of disease.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use rat but more often mouse, particularly because of the ability to use genetically modified strains. Over a period of 5 years, with funding and staff / students working on these projects, I would expect breeding numbers to reach approximately 10,000 mice using several different genetic strains and for experimental protocols ~1,500 rats and ~18,500 mice (a mix of wild type background and genetically modified animals drawn from those bred under this licence or other appropriate licences). Where possible we will try to use both sexes from our transgenic breeding, but this may not be appropriate as females can be resistant to developing liver fibrosis. However, we always aim to include any appropriate females in cell preparations for in vitro studies as support for the in vivo work.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In most instances, tissue from animals will be removed for studies in the laboratory. In some instances, animals will be treated with agents that cause an imbalance in regeneration and/or fibrosis. Although transient discomfort may occur at the time of administration the animals appear normal soon afterwards. Similar to humans, animals can sustain fibrotic injury for a long period of time with no apparent symptoms. In the rare scenario that an animal shows signs of organ failure the animal will be put down to ensure the animal does not exceed the severity limits set out in the project. Some animals will</p>

	undergo surgery to induce fibrosis, but these are not life-threatening procedures. In general, animals will suffer moderate adverse effects from this, which are similar to and primarily associated with the surgical procedure, the effects of which will be alleviated with pain-killing drugs.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Despite progress in understanding the biology of fibrotic diseases, these discoveries have been unsuccessfully translated into the clinic. Fibrotic diseases are complex which develop and resolve over many weeks; involving the organ, immune system, and cell-cell interactions. For this reason, it is not possible to study these events in isolation in an <i>in vitro</i> / <i>ex vivo</i> system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power calculations performed based on an important component of fibrosis (collagen deposition) indicate 6 animals per group are required to analyse the fibrotic processes. For example our experience of biological variability shows fibrotic livers of 6 weeks CCl ₄ treated rats have a mean collagen (hydroxyproline) content of 1.45 ± 0.25 (SD) mmol/g liver. Based on these data, accepting an 80% chance of detecting this difference at the level of p≥0.05, gives a sample size of $16/(1.74)^2 = 5.3$ animals per group. Where possible we will make use of archived material and importantly make use of human cells and tissue to reduce animal use. Through refining our technical skills, see below, we are also able to reduce animal numbers. Animal breeding will take into account the power calculations required for the experimental protocols.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms)	In the case of cellular studies, particularly for liver fibrosis, we will use rats as this allows a greater analysis of the mechanisms associated with the disease process compared to mouse. However, for <i>in vivo</i> studies, mice will be necessary based on the use of genetically modified strains. To investigate the therapeutic potential of our

<p>to the animals.</p>	<p>findings in fibrotic disease in different organs from multiple etiologies, it is necessary to use more than one model of injury. We have chosen established models of organ fibrosis that have good comparison with human disease and have been refined over many years in labs worldwide.</p> <p>As evidence of limiting animal experimentation through refining our models, improved technical skills and post-operative care we have reduced the mortality of bile duct ligation from 30% to ~10% on our current liver fibrosis models. We will ensure similar refinement in all protocols (which are much less severe).</p> <p>As further refinement, and in agreement with our resident statistician, we will seek additional statistical assistance as required to refine experiments.</p>
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Project	Understanding mechanisms of inherited heart disease (cardiomyopathy) and exploring treatment options	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to better understand a group of human inherited heart diseases called cardiomyopathies. These diseases are caused by genetic “spelling mistakes” (i.e. mutations) in the genetic blueprint of cardiac proteins. Cardiomyopathies contribute substantially to the burden of heart disease in the UK (overall costs of heart diseases are £11 billion to the NHS annually), as they can lead to heart failure. Clinical management of heart failure so far is general, there are no specific therapies (e.g. specific drugs to cure). A major risk of the disease are heart attacks	

	<p>("arrhythmias") which can lead to Sudden Cardiac Death. A recent example of undiagnosed cardiomyopathy featured in the media was the professional football player Fabrice Muamba, who collapsed during a match in 2012 and was lucky to be resuscitated successfully.</p> <p>The majority of cardiomyopathies are caused by genetic "spelling mistakes" in proteins directly involved in cardiac contraction, however this project will focus on a novel group of proteins, which helps the heart muscle to sense and respond to stress the organ experiences under demand (and is called "biomechanical stress signalling proteins") e.g. during exercise and in pregnancy. Biomechanical stress signalling is a complex network of signalling cascades and proteins involved and we are just beginning to understand which proteins are involved, but our understanding of "how it works" is very limited. We hypothesise that genetic mutations in this group of proteins cause the heart to "adapt" (e.g. to grow bigger, i.e. undergo hypertrophy) in the absence of appropriate triggers.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This research will help to gain insights in how mutations in a group of proteins can cause heart disease: The project will generate mouse models for human disease and our experiments will tell us which cellular processes "go wrong" in this condition. Based on the findings and our better understanding, we will test options to treat or prevent disease, e.g. by manipulating the steps which "go wrong" in the disease. In the short run, our research will be disseminated to other researchers (e.g. through scientific publication and conference abstracts). It will also help to inform Geneticists in the NHS how to interpret certain findings of genetic testing in human patients and how to advise patients and clinical practitioners on the findings. Ultimately, we hope that our project will contribute to the development of novel, specific therapies (e.g. new drugs) for this group of diseases. In this project, we aim to generate mouse models reflecting the mutations found in human patients and to perform experiments helping us to understand mechanisms of disease. In particular we are interested how the disease develops and what the earliest measurable signs of the condition are (long before the mice are visibly</p>

	sick). We will also explore how the disease can be ameliorated or treated, e.g. whether substances derived from Green Tea may have a protective or therapeutic effect. This will be tested in a mouse model for Hypertrophic Cardiomyopathy and help us to understand the molecular action of the substances. If safety and efficacy can be documented in a mouse model, this will pave the way for clinical studies.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use primarily mice (6,950) and some rats (250) over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	From previous experience we assume that the introduction of the mutations will have little effect on the majority of mice. However, in some cases, the mice may develop heart failure similar to humans. A small proportion of mice may die suddenly of arrhythmias and sudden cardiac death or develop severe heart failure. Wherever possible, we will avoid death by using humane end-points, the most important one being deep irregular abdominal breathing. We will monitor these mice vigorously (some up to three times daily) to spot early signs of heart failure. The animals may have several imaging sessions or other investigations, under general anaesthesia where appropriate. Some animals will have ECGs or implanted ECG monitors (telemeters) to identify those at risk of arrhythmias. At the end of the studies the animals will be killed and their organs be used for molecular studies. Animals who do not show signs of cardiac disease will be challenged either with a surgical procedure called “trans-aortic constriction”, or by the administration of drugs. Both interventions will mimic the effects of high-impact endurance training on the heart and will help to reveal defects in signalling pathways, or test how drugs and/or novel compounds may ameliorate disease.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use	We use bioinformatics predictions, biochemical experiments and cellular models to understand the impacts of the genetic “spelling mistakes” on heart cells. While these experiments are informative on

<p>non-animal alternatives</p>	<p>certain aspects of disease (e.g. they can show binding to other proteins is affected, mouse models are needed to understand the effects of the genetic “spelling mistakes” on the whole organ level, e.g. how these genetic “spelling mistakes” can cause arrhythmias.</p> <p>In our in vitro experiments, we use human cardiomyocytes derived from blood or skin biopsies via an exciting novel technology called "induced pluripotent stem cells". These cells are human to reflect best real human heart cells and future work will show which aspects of animal work can be replaced by these cells.</p> <p>Over the course of the project we will review and potentially incorporate alternatives as they became available.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use a novel technology (called “CRISPR/Cas9”) to introduce the genetic “spelling mistakes” into the mice. This new method needs less animals than conventional approaches to generate new lines. We generate all mouse models in one particular strain of mice (C57bl6) to reduce variability and preferentially use males only in in vivo investigations, as we know there is less variability among males. However, the corresponding females will be included in molecular analyses. We will design our breeding strategies in a way to avoid the production of unwanted genotypes. We will use power calculations for planning experiments and use blinded, randomised block design wherever possible.</p> <p>We will use small trial studies (pilot studies) before embarking on full scale experiments.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We will use very few rats. Mice will be used for the majority of experiments, as methods are available to introduce genetic “spelling mistakes” easily and mice are similar enough to humans in terms of cardiac physiology, e.g. their hearts have 4 chambers like the human heart. Animals are kept in a modern and well-resourced facility in individually ventilated cages, and being offered enrichment and group housing (where possible).</p> <p>The team has long-standing expertise and</p>

	<p>experience in all experimental procedures; we will constantly refine techniques and apply best practice to all animal work.</p> <p>We will use non-invasive imaging techniques (such as echocardiography or MRI scanners) to monitor the development of heart disease in the models. Once we have established when the animals develop symptomatic heart disease, we will perform subsequent studies at an earlier time-point (when there is some measurable change in the heart, but before the animals become symptomatic), to avoid unnecessary suffering. We have defined clear humane end-points to minimise the suffering of animals and will adhere to monitoring regimes (documented in observation sheets) when adverse effects are expected. We will use small implantable ECG monitors (telemeters) to identify those mice at risk of arrhythmias and potentially sudden cardiac death so we can monitor such animals more closely.</p> <p>Where scientifically justified, we will use drug induced models of challenging the animals instead of trans-aortic constriction. The latter is the equivalent of open heart surgery, hence has quite an impact on the animals, while in the first case the drugs will be delivered via small devices (similar to insulin pumps), which require only minimal surgery to be implanted. This slow release device also means we can avoid twice-daily injections of the animals, hence reducing stress and discomfort in the mice. Moreover, this method can be tailored to certain substances and hence provide a better understanding of the signalling cascades being affected by a particular genetic “spelling mistakes”.</p>
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Project	Understanding mechanisms of inherited heart disease (cardiomyopathy) and exploring treatment options	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to better understand a group of human inherited heart diseases called cardiomyopathies. These diseases are caused by genetic “spelling mistakes” (i.e. mutations) in the genetic blueprint of cardiac proteins. Cardiomyopathies contribute substantially to the burden of heart disease in the UK (overall costs of heart diseases are £11 billion to the NHS annually), as they can lead to heart failure. Clinical management of heart failure so far is general, there are no specific therapies (e.g. specific drugs to cure). A major risk of the disease are heart attacks	

	<p>("arrhythmias") which can lead to Sudden Cardiac Death. A recent example of undiagnosed cardiomyopathy featured in the media was the professional football player Fabrice Muamba, who collapsed during a match in 2012 and was lucky to be resuscitated successfully.</p> <p>The majority of cardiomyopathies are caused by genetic "spelling mistakes" in proteins directly involved in cardiac contraction, however this project will focus on a novel group of proteins, which helps the heart muscle to sense and respond to stress the organ experiences under demand (and is called "biomechanical stress signalling proteins") e.g. during exercise and in pregnancy. Biomechanical stress signalling is a complex network of signalling cascades and proteins involved and we are just beginning to understand which proteins are involved, but our understanding of "how it works" is very limited. We hypothesise that genetic mutations in this group of proteins cause the heart to "adapt" (e.g. to grow bigger, i.e. undergo hypertrophy) in the absence of appropriate triggers.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This research will help to gain insights in how mutations in a group of proteins can cause heart disease: The project will generate mouse models for human disease and our experiments will tell us which cellular processes "go wrong" in this condition. Based on the findings and our better understanding, we will test options to treat or prevent disease, e.g. by manipulating the steps which "go wrong" in the disease. In the short run, our research will be disseminated to other researchers (e.g. through scientific publication and conference abstracts). It will also help to inform Geneticists in the NHS how to interpret certain findings of genetic testing in human patients and how to advise patients and clinical practitioners on the findings. Ultimately, we hope that our project will contribute to the development of novel, specific therapies (e.g. new drugs) for this group of diseases. In this project, we aim to generate mouse models reflecting the mutations found in human patients and to perform experiments helping us to understand mechanisms of disease. In particular we are interested how the disease develops and what the earliest measurable signs of the condition are (long before the mice are visibly sick). We will</p>

	<p>also explore how the disease can be ameliorated or treated, e.g. whether substances derived from Green Tea may have a protective or therapeutic effect. This will be tested in a mouse model for Hypertrophic Cardiomyopathy and help us to understand the molecular action of the substances. If safety and efficacy can be documented in a mouse model, this will pave the way for clinical studies.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use primarily mice (6,950) and some rats (250) over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>From previous experience we assume that the introduction of the mutations will have little effect on the majority of mice. However, in some cases, the mice may develop heart failure similar to humans. A small proportion of mice may die suddenly of arrhythmias and sudden cardiac death or develop severe heart failure. Wherever possible, we will avoid death by using humane end-points, the most important one being deep irregular abdominal breathing. We will monitor these mice vigorously (some up to three times daily) to spot early signs of heart failure. The animals may have several imaging sessions or other investigations, under general anaesthesia where appropriate. Some animals will have ECGs or implanted ECG monitors (telemeters) to identify those at risk of arrhythmias. At the end of the studies the animals will be killed and their organs be used for molecular studies. Animals who do not show signs of cardiac disease will be challenged either with a surgical procedure called “trans-aortic constriction”, or by the administration of drugs. Both interventions will mimic the effects of high-impact endurance training on the heart and will help to reveal defects in signalling pathways, or test how drugs and/or novel compounds may ameliorate disease.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use</p>	<p>We use bioinformatics predictions, biochemical experiments and cellular models to understand the impacts of the genetic “spelling mistakes” on heart cells. While these experiments are informative on</p>

<p>non-animal alternatives</p>	<p>certain aspects of disease (e.g. they can show binding to other proteins is affected, mouse models are needed to understand the effects of the genetic “spelling mistakes” on the whole organ level, e.g. how these genetic “spelling mistakes” can cause arrhythmias.</p> <p>In our in vitro experiments, we use human cardiomyocytes derived from blood or skin biopsies via an exciting novel technology called "induced pluripotent stem cells". These cells are human to reflect best real human heart cells and future work will show which aspects of animal work can be replaced by these cells.</p> <p>Over the course of the project we will review and potentially incorporate alternatives as they became available.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use a novel technology (called “CRISPR/Cas9”) to introduce the genetic “spelling mistakes” into the mice. This new method needs less animals than conventional approaches to generate new lines. We generate all mouse models in one particular strain of mice (C57bl6) to reduce variability and preferentially use males only in in vivo investigations, as we know there is less variability among males. However, the corresponding females will be included in molecular analyses. We will design our breeding strategies in a way to avoid the production of unwanted genotypes. We will use power calculations for planning experiments and use blinded, randomised block design wherever possible.</p> <p>We will use small trial studies (pilot studies) before embarking on full scale experiments.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We will use very few rats. Mice will be used for the majority of experiments, as methods are available to introduce genetic “spelling mistakes” easily and mice are similar enough to humans in terms of cardiac physiology, e.g. their hearts have 4 chambers like the human heart. Animals are kept in a modern and well-resourced facility in individually ventilated cages, and being offered enrichment and group housing (where possible).</p> <p>The team has long-standing expertise and</p>

	<p>experience in all experimental procedures; we will constantly refine techniques and apply best practice to all animal work.</p> <p>We will use non-invasive imaging techniques (such as echocardiography or MRI scanners) to monitor the development of heart disease in the models. Once we have established when the animals develop symptomatic heart disease, we will perform subsequent studies at an earlier time-point (when there is some measurable change in the heart, but before the animals become symptomatic), to avoid unnecessary suffering. We have defined clear humane end-points to minimise the suffering of animals and will adhere to monitoring regimes (documented in observation sheets) when adverse effects are expected. We will use small implantable ECG monitors (telemeters) to identify those mice at risk of arrhythmias and potentially sudden cardiac death so we can monitor such animals more closely.</p> <p>Where scientifically justified, we will use drug induced models of challenging the animals instead of trans-aortic constriction. The latter is the equivalent of open heart surgery, hence has quite an impact on the animals, while in the first case the drugs will be delivered via small devices (similar to insulin pumps), which require only minimal surgery to be implanted. This slow release device also means we can avoid twice-daily injections of the animals, hence reducing stress and discomfort in the mice. Moreover, this method can be tailored to certain substances and hence provide a better understanding of the signalling cascades being affected by a particular genetic “spelling mistakes”.</p>
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Project	Understanding mechanisms that regulate tumourigenesis and metastasis	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Almost 1 in 4 people in the UK will develop cancer at some point in their lives. Although great advances have been made in cancer biology with many patients now being cured, it is still a devastating disease that can be hard to treat for some cancer types, especially if the cancer cells have spread to other organs of the body. Critical to improved patient care is a deeper understanding of the biology of cancer, which can pave the way for the development of new therapies.</p> <p>The specific aspects of cancer we are focussing</p>	

	<p>on are ‘tumourigenesis’ (the initial formation of the tumour) and ‘metastasis’ (the tumour cells ability to grow at a secondary site). Both these events are multi-step processes that depend on the accumulation of mutations within the cells that allow them to become cancerous. Thus knowledge of the key genes that control this processes is critical – genes that when mutated result in a cancerous cell.</p> <p>However, this is only part of the story, as factors ‘outside’ of the tumour cells, i.e., what is going on in the body, also have a key role to play in both tumourigenesis and metastasis. This can include the normal cells around the tumour cells and critically the immune system, as well as factors such as the age of the person. Thus understanding of the way the body can ‘control’ the ability of the tumour cells to grow and spread to other organs provides avenues for potential therapies, as highlighted by the success of “Ipilimumab” – a drug that works to activate the specific cells of the immune system that are able to kill off cancer cells.</p> <p>The aims of this work are:</p> <ol style="list-style-type: none"> 1. To identify genes found in cancer cells that when changed/mutated can affect tumour growth and metastasis. 2. To identify how factors such as the patient’s immune system influence tumour growth and metastasis.
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>An understanding of the genes that are altered in cancer cells, and how they work to ‘alter’ the normal functioning of the cell, is critical if we are to have any hope of identifying ways in which we can ‘kill’ the cancer cells, i.e., the development of drugs/therapies that are able to target these ‘altered’ cells and leave normal ‘healthy’ cells alone. Similarly, if we can find ways in which the body is able to control the growth of tumour cells or prevent their growth at new tissue sites, then this information can be utilised by pharmaceutical companies to develop drugs that target the tumour cells or help the ability of the body to fight them.</p>

<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>It is expected that a maximum of 150,000 mice will be used over the course of 5 years, with 50,000 of these being used solely for breeding to generate mice for analysis.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>In our licence, 60% of the mice we propose to use will experience no more than at most a transient feeling of pain, such as when they are administered substances via an injection into the tail vein or when a blood sample is taken and are considered to be 'mild' in terms of severity. The remaining mice (40%) will undergo more experimental procedures and thus are classified as 'moderate' severity. These mice may carry an altered gene and are monitored to see if they develop a tumour. Alternatively mice can be administered tumour cells and their ability to control the tumour or the spread of the tumour is investigated. When administering a 'new' tumour cell line, pilot studies (on 2-3 mice) will be performed to determine the size to which the tumour may grow to allow sufficient time for metastasis (spread of the tumour cells to another tissue site) to occur. Tumour cells may also be administered to 'aged mice' (~1 year old) to determine the effects of age on tumour growth and spread. We also characterise the immune system of the mice. All mice are monitored daily for any signs of a developing tumour or signs that the animal may be starting to experience abnormal clinical features that suggest it is no longer able to tolerate the presence of the tumour (be it a swollen spleen due to the development of lymphoma or metastatic tumour cell growth in the lungs starting to make breathing laboured). Since animals can also develop tumours internally (i.e., where the tumour growth/mass cannot be directly observed), we use other signs to indicate the mouse is starting to become unwell, such as coat condition, pale and cold extremities, reduced movement and/or social interaction. At the point when the mouse starts to display these clinical signs, it will be humanely killed and the mouse examined to determine why it was displaying these symptoms, as well as tissue samples collected for further analysis.</p>
<p>Application of the 3Rs</p>	

<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Humans and mice share many similarities, both in terms of our basic organs (as we are both mammals) and our genetic make up (there is a very high degree of similarity in the actual genes that both mice and humans share). When mice develop cancer (either due to our alteration/mutation of their genes or due to the administration of tumour cells to them), their tumours are very similar to that seen in humans (in terms of the way they develop and their actual characteristics). Also, using the mouse means we can look at the way the body reacts to the cancer cells and how factors such as the immune system try to control them. This is something that simply cannot be performed by growing cancer cells in a dish in the laboratory.</p> <p>Thus mice are a very good model for human cancer, and allow us to perform studies that cannot ethically be done using human subjects. Importantly, mouse studies have enabled the development of clinically relevant agents in cancer treatment, such as the development of targeting antibodies that are currently being used to cure patients with advanced melanoma (antibodies that target two proteins on the surface of the immune cells that are able to kill the cancer cells). Indeed, although these are only two examples virtually every compound used in the oncology clinic was developed or validated using mouse model systems and mouse models have also contributed significantly to our fundamental understanding of the mechanisms of cancer.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Where possible we shall always import existing mice rather than generating new ones.</p> <p>In some circumstances, such as when certain mouse lines have been only recently created, less published data will be available and in these instances we propose to perform small pilot experiments to determine the final experimental design.</p> <p>All mouse lines will be archived so that they may be distributed to other researchers worldwide. This will reduce the number of animals used globally, as fewer animals will be required to re-generate these archived lines.</p>

	<p>Data will be generated from the statistically determined minimum number of animals, and wherever possible, experiments will be designed to avoid the known sources of variability that can arise.</p> <p>Wherever possible, multiple experiments will be performed on the tissues collected from an individual mouse so as to maximise the use of the mouse.</p> <p>All data generated from our research on the mice will be published in scientific journals available to the whole scientific community, reducing duplication of production resources and phenotyping procedures elsewhere. Wherever possible, the results of experiments that involve large datasets will be made publically available to serve as a resource for other scientists and clinicians</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We are constantly refining our experimental processes to minimise harm and reduce adverse effects on the mice without affecting the experimental data. For example, when taking blood samples we have refined our protocols such that only a drop of blood is needed to be able to complete our analysis, thus minimising the distress to the animal.</p> <p>When mice are to be irradiated (given gamma-radiation to wipe out their bone marrow prior to transplant of donor bone marrow), we have instituted a new policy whereby the mice must be weighed 24 hours beforehand and their condition thoroughly observed. This allows us to avoid irradiating mice that may be rather small in body weight and/or may have started scratching (for example) and would be less likely to tolerate the irradiation procedure. We also ensure the mice are placed on antibiotics for 2 weeks after the irradiation, whilst their immune system is compromised, and we also provide mash in dishes on the cage floor for the first week after irradiation to ensure that should they feel slightly weak/tired (as some patients can feel after irradiation therapy), they are still easily able to access food.</p> <p>Mice are social animals and thus wherever possible we try not to house them on their own.</p>

	<p>However, in cases where we observe fighting in a particular cage of mice, the aggressive mouse will be removed and solo-housed, so as to prevent further harm to the rest of the cage.</p> <p>We have highly trained technicians looking after the mice, and the mice are checked every single day to ensure they are healthy. Those that are being observed for the development of tumours are observed twice daily and humanely sacrificed if they are starting to show any signs of discomfort.</p> <p>The implementation of a sophisticated database system and animal tracking system ensures that data on procedures and welfare assessments can be readily accessed and analysed.</p>
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Project	Understanding pathogen behaviour in relation to the immunity, vaccines and antibiotic treatment	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim of our research is to understand how infections caused by bacteria can be defeated or prevented by making better vaccines and antibiotics. Bacterial infections are a big problem because they cause about 6 million deaths in the whole world. Many bacteria are becoming resistant to antibiotics and many of the vaccines that we use are not sufficiently good. Therefore we do not have optimal weapons to fight infections. We primarily work on bacterial diseases that affect and kill children in poor areas of the world. For example, one of these diseases,	

	<p>invasive non-typhoidal salmonellosis (iNTS) causes about 680,000 deaths every year, 68% of which are in children less than 5 years old in Africa and South East Asia. <i>Streptococcus pneumoniae</i> and <i>Neisseria meningitidis</i> also cause very severe infections in children and immune-compromised adults worldwide. Currently there are no vaccines against iNTS and an increasing number of iNTS bacteria are becoming resistant to the antibiotics that doctors use to fight them. Better and more affordable vaccines against <i>Streptococcus pneumoniae</i> and <i>Neisseria meningitidis</i> are also a priority. Furthermore, we do not understand how these bacteria spread in the environment and how they infect people. Therefore better vaccines and antibiotics remain the main weapons to fight these infections in poor countries.</p> <p>Our research will study how and where the bacteria hide in the body to resist to vaccines and antibiotics and will test new innovative vaccines. . This will enable us to produce new vaccines and antibiotics that can reach the bacteria in the locations where they hide and persist and kill them more efficiently.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Our work will create scientific knowledge that will guide a better use of the vaccines and antibiotics that are currently available to us and will make it easier to produce new and better antibiotics and vaccines. Doctors and patients will benefit from this research that will improve the treatment of sick people, especially children and will also reduce the spread of diseases in the community. These benefits will be stronger especially in developing countries where there are many conditions that weaken the immune system especially in young children (for example, viral infections, gut parasites, malaria, malnutrition). In fact a weak immune system makes vaccination and treatment of an infection a lot harder to accomplish. In the long term, better use of antibiotics and vaccines will reduce the disease burden and slow down or stop the emergence of bacteria that are resistant to antibiotics. Our work will also impact on disease prevention in the veterinary field and in food-animals where vaccines and antimicrobials are widely used often</p>

	with suboptimal results.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 13000 mice over five years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals will be infected with live bacteria via several possible parenteral routes, intranasally or via oral gavage and then may be treated with antibiotics or molecules that affect the immune system. In some experiments, new vaccines will be tested, selected and optimized by immunisation schedules followed by reinfection with pathogenic bacteria and monitoring of the immune responses. In the majority (> 90%) of experiments no animals will show signs of infection. However, it is possible that very occasionally a small number of animal show clinical signs. These animals will be closely monitored and assisted via careful and skilful husbandry that is typical of the culture of care present at our establishment. If signs persisted for more than a few hours the animals would be killed to avoid further suffering.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	We perform many preliminary experiments in systems that do not involve animals. However, these systems cannot reproduce the complexity of the body of a whole animal where the blood transports the bacteria between different sites and each organ influences the functioning of other organs. Therefore to ensure that our research has a real future impact on human health, it is necessary also to study infections in a whole animal where we can capture the impact of medical treatments and new vaccines on the behaviour of bacteria in an environment that closely resembles the human body.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	We greatly strive to reduce the numbers of animals that we use in our experiments. Whenever possible we perform preliminary studies in systems that do not require animal experimentation so that we can improve our protocols and use smaller numbers of animals only for the final validation of our results. We

	<p>combine several experiments in one so that, for example, we can compare the effect different vaccines or treatments using just one untreated (control) experimental group. We use the smallest possible experimental number of animals for each experiment being very careful that this does not affect the accuracy of our results. To determine the smallest number of animals that we can use in our work we use calculations bases on advanced statistics and mathematics. Statisticians and mathematicians have become an important part of our research group.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We use infections in adult mice. This model captures the essential traits of many bacterial infections of humans and other animals. Mouse models are a reliable tool to study vaccines and antibiotics before these are used in humans and domestic animals. The availability of genetically altered mice allows us to mimic model human conditions and immune-deficiencies such as malaria, AIDS, congenital absence of components of the immune system that predispose to infection. The model therefore enables refined studies on the interaction between bacteria and the immune system in the course of vaccination or medical treatments.</p> <p>Most animals do not show any signs of infection during our experiments. We minimise suffering via careful and skilful handling of the animals, use of the smallest possible size of needles, minimal numbers and frequency of repeated procedures and light anaesthesia for some procedures. Whenever possible we use less infectious bacterial strains for our studies to minimize the signs of infection that may occur. We progressively refine our protocols to ensure that the smallest possible doses of bacteria are administered to the animals and we perform observations at time points before the occurrence of signs of infection. To achieve this we are making use of new technology to increase the sensitivity of our assays that detect bacteria, bacterial genes/proteins, or immune parameters triggered by low numbers of bacteria in the body of the infected animal. This has also the scientific advantage of looking at infections when bacterial numbers are relatively low and more closely related to what happens in the human infections</p>

that we model.

Project	Understanding Synaptic and Network Dysfunction in Neurodegenerative Diseases	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		<input checked="" type="checkbox"/> Basic research <input checked="" type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Dementia is one of the leading causes of death in the UK, and a growing problem for our society as we are living longer. Dementia is a devastating, progressive decline in mental function that is caused when the brain is damaged by diseases such as Alzheimer's disease (AD) and related neurodegenerative disorders. In the UK, we spend over £26 billion per year caring for people with dementia, and currently, we do not have any treatments that can stop the devastating progression of the underlying diseases. The symptoms of dementia result when the cells in the brain are damaged and can no longer communicate	

	<p>effectively. Currently, we do not fully understand these changes in the brain, which is why we do not have effective treatments. In this project, we aim to better understand the brain changes that cause Alzheimer's and related diseases in order to develop effective ways to prevent or treat these devastating conditions.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>We will use rodent models of dementias to study changes in the brain and to try and reverse damage. The short term and highly likely benefits of this project include: - The advancement of knowledge about dementias - Knowledge shared with other scientists and drug companies that they can then use for further advances - Scientific paper publications which are freely shared with everyone - Data about dementia which are freely shared on open web based systems for others to use The longer-term potential benefits include; - Development of medicines that will help people with dementia - Influences on government policy about how to help people with dementia and how to fund the best types of research.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We will use mice in this project over the 5 year programme. Many of these mice have genetic modifications to either reproduce the brain changes observed in dementias or changes in the brain called reporters that allow us to ask specific scientific questions. Most of these genetic modifications do not cause overt symptoms that affect the daily lives of the mice. Over the 5 years, we expect to use approximately 20,000 mice.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The vast majority of the mice on this licence will not undergo potentially harmful procedures as they will be used for breeding or post-mortem tissue collection after humane killing. Some of the genetic modifications can cause seizures, which are rare but could be severe. We also plan some moderate procedures including recovery surgeries to induce models of dementia and to observe brain changes and recovery with treatments over time. We plan some mild experiments to examine behavioural changes and treat mice with drugs that might help the dementia like symptoms and brain changes. We do not expect any common adverse effects from these procedures. Rarely, our procedures may have adverse effects such as infection after surgery or</p>

	<p>side effects from treatments. Any animals experiencing adverse effects will be examined by a veterinarian, and if the effects cannot be alleviated, the animals will be killed humanely. At the end of experiments, animals will be killed humanely. Some of our genetically modified mice may be provided to another Project Licence if appropriate.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Animal experiments are essential to meet our goal of better understanding of brain changes in dementias and how to develop effective treatments. We are studying brain function and degeneration, which requires a system with an intact network with all of the types of cells that are needed to make a healthy brain. The diseases we study occur over many years and involve clumping of toxic proteins in the brain. Currently, there are not cell models that can make entire brain networks that develop age-related disease like our mouse models. Nor are computer models advanced enough to test the questions that we need to in order to help people with dementia. It will be impossible to develop effective dementia treatments without using experimental animals at this point, although we are continually evolving both cell and computer models that we hope will replace even more animals in future. Mice are an ideal species as their brains share with humans the basic structures involved in memory. They are also amenable to genetic manipulation, which allowed the introduction of genes that cause human dementias into the mouse resulting in brain disease and memory impairments.</p> <p>The mouse work in this programme is part of a wider effort incorporating experiments in human post-mortem brain and human stem cell derived neurons, which replace some mouse experiments. Only by taking multiple approaches will we be able to come to a better fundamental understanding of disease that will lead to effective treatments.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We have taken several measures to reduce animal numbers:</p> <ul style="list-style-type: none"> • Studying animals while they are alive with advanced techniques that let us look at the brain before and after dementia changes and

	<p>before and after treatments will reduce animal numbers needed for each experiment. This is because the brain is assessed at the first time point in each animal allowing it to serve as its own baseline, lowering the numbers needed compared to needing large cohorts.</p> <ul style="list-style-type: none"> • Wherever possible, an individual animal will be used for investigating brain function when the animal is alive and also for looking at brain changes after it is killed. This practice will reduce numbers, and increase power of the data. We routinely share brain tissues from individual animals between multiple experimenters in order to maximise the data collected from a single animal. • We will use best practice for designing experiments. This will avoid having either too few animals to answer the question (which wastes the whole group), or too many animals (which adds unnecessary mice).
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The majority of experiments will be carried out in models of dementias. The mice exhibit many of the brain changes seen in dementia patients and so provide an appropriate model for these studies. We have chosen particular dementia models based on how well they mimic the aspects of the disease we are trying to study, and we participate in international scientific initiatives to refine our models.</p> <p>We also refine the procedures we use on the mice in order to minimise harms, for example, administering the drug in jelly instead of other more stressful methods. The mice love receiving their jelly and get the entire needed daily dose in a stress-free manner.</p> <p>We propose some surgical procedures, which will all be carried out using appropriate anaesthetic and analgesics. We continually interact with our veterinary team to be sure we use the most refined methods.</p>

Project	Understanding the fundamental biology of dangerous pathogens	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Infectious diseases REDACTED pose a serious threat to life. The aim of the work detailed in this project licence is to i) develop accurate models of disease to support our experiments; ii) determine which environmental conditions affect pathogen infectivity to allow us to understand the hazard posed by the disease; iii) determine the effectiveness of existing and novel therapeutics to advise physicians of the most appropriate response in emergency situations; and iv) use animal models to assist in the discovery of factors involved in disease which can be used as the targets in drug discovery programmes to	

	generate the next generation of antimicrobial agents.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The most basic output of this work will be an increased understanding of these pathogens and their interactions with their hosts. This knowledge forms the basis for efforts to combat infections caused by these pathogens by indicating effective medical countermeasure strategies in the short term and providing potential diagnostic and therapeutic targets to be developed in the longer term.
What species and approximate numbers of animals do you expect to use over what period of time?	The expectation is that this licence will use no more than 10,000 adult mice, no more than 700 adult rats and no more than 450 adult hamsters over the 5 year course of the project
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	One aspect of the work on this licence involves understanding the disease process for a range of serious pathogens. This is to establish accurate models of disease and identify targets for the development of medical treatments. Animals will be exposed to pathogen either by injection (e.g. intra-peritoneal, sub-cutaneous) or by intra-nasal instillation under general anaesthesia or by inhalation whilst physically restrained in an aerosol exposure tube. The procedures by themselves are expected to result in few adverse effects. However, animals exposed to pathogens are at risk of developing disease; they may become ill and without intervention could die. Clinical signs of disease may include piloerection, hunched posture, reduced activity and responsiveness. They might also experience laboured breathing or neurological signs such as circling behaviour, twitching or tilting of the head and progressive limb paralysis; such animals would be classed as experiencing severe suffering and would be killed. In parallel, another aspect of the work on this licence will examine the effects of potential therapies such as antibiotics or vaccines in non-infected animals. These animals will receive these treatments by the same routes as described above for the pathogens. The procedures by themselves are expected to result in few adverse effects and the expectation is that the therapies will have few adverse effects. These animals would be classed

	<p>as undergoing mild to moderate suffering. Finally, the therapies will be tested to see if they work against the disease. The expectation based on information from previous licences is that the majority of medical treatments tested would be successful to an extent so that animals receiving these therapies would be protected from disease and its consequences, either fully or in large part. However, some of the medical treatments may not work well and these animals would be expected to develop disease with all of its consequences. All animals will be killed at the end of studies.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The interactions between a disease-causing microorganism and its host are extremely complex and not well understood. This is especially true because the primary interface includes the host immune system, an incredibly interconnected network of host responses involving virtually every cell type in the body. Although some isolated aspects of these host-pathogen interactions can be recapitulated in non-animal systems in the laboratory, only an animal allows the complexity of the host-pathogen interactions to be fully expressed and studied.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>Studies will be designed with the advice of a team of statisticians to ensure each study has suitable levels of power without unnecessary use of animals. Pilot studies will be conducted where appropriate to provide information to inform these power calculations. Where possible, studies will be run in parallel to utilise the same control groups. To reduce inter-animal variation, animals within a study and within a series of studies will be matched in age and weight and studies typically use only male or female animals from a consistent supplier. Similarly, inbred strains of animals will be utilised in order to reduce the inter-animal variability.</p>
<p>3. Refinement Explain the choice of species and</p>	<p>The animal models we use in place of humans to study disease are scientifically appropriate because they reproduce the disease seen in</p>

<p>why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>humans in terms of severity, eventual outcome and clinical features of disease. There are other animals which could be used in place of mice; these would also be suitable models because they also reproduce the disease in humans. However, these would be higher order animals such as non-humans primates which are perceived to have a higher neurophysiological response than mice and rats. Since disease would manifest with the same severity in these higher order animals as in mice and rats, the rodent models offer the more refined option. This also allows advantage to be taken of the numerous commercially available murine reagents and genetically modified mouse strains which will increase the amount of data that can be obtained from each animal.</p> <p>Animal suffering during the infection will be minimised by close monitoring of animals during study to allow the rigorous application of established humane endpoints. The success of the humane endpoints will be assessed after each study and will be continually refined during the course of this licence to alleviate suffering. Unfortunately, keeping the animals sedated for the duration of the studies, which is weeks at a minimum, is not practical, but brief periods of anaesthesia will be used for specific procedures which would otherwise be stressful.</p> <p>Environment enrichment will be provided appropriate to the species and will include nesting materials, plastic and cardboard dome houses, chew block and transfer of own scented material following routine cleaning of cages etc. Where appropriate animals will be familiarised to the procedure (e.g. handling, environment) to reduce stress during the actual procedure.</p>
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Project	Understanding the mechanisms of learning and attention during sensory-guided behaviour	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Our senses are constantly sending information from our environment to the brain. However, the capacity of our brain is limited. Our brain therefore needs to select information from our senses that is most important for our behaviour. This selection is changed in multiple mental illnesses including schizophrenia and autism. This causes problems in attending relevant information and ignoring distractions.</p> <p>However, how our brain selects sensory information is not well understood. The aim of our</p>	

	<p>research is therefore to understand how we learn what is relevant, where in the brain information is selected, and how we can flexibly select dependent on our current goals.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Selecting sensory information in our daily lives is a crucial skill to make sense of the world and make good decisions. We will study both successful and unsuccessful selection of sensory information in mouse models of mental illness and investigate methods to treat impaired selection. This can help improve diagnosis and treatment of mental illness. This is important because we currently do not understand the brain mechanisms that cause impaired selection and no effective treatment is available.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We use the mouse as an animal model to understand the brain. We will use as few animals as possible to achieve reliable conclusions. We expect to use approximately 8300 mice over a 5 year period.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We need to measure how activity in brain cells is linked to behaviour and therefore need to perform surgeries in 1800 mice. During the surgery, while the mouse is under anaesthesia, we will implant a head post, which is a small piece of metal connected to the skull that can be connected during behavioural testing to an external holder to fix the position of the head. Using a head post is the best way to stably fix the head without causing pain to the animal compared to directly clamping the head of an animal. Fixing the head is necessary in the majority of experiments to be able to control the sensory information that the mouse receives. Fixing the head is also necessary to allow for activity measurements in brain cells using microscopy. During the surgery, we additionally make small openings in the skull to gain access to the brain to allow for measurements of activity in brain cells. In the opening we can insert electrodes in the brain to measure electrical activity of cells or inject substances that label brain cells enabling us to optically measure cell activity using microscopy in later experiments. In some mice we inject a substance to make some brain cells sensitive to a specific light, or we implant a small tube in the</p>

brain. This allows us in a later experiment to use either light stimulation or add a substance in the brain via the tube to temporarily increase or decrease activity in some brain cells to test what function those cells have in selecting sensory information. These methods that we use to measure or modify activity in brain cells (once the animal has recovered from the surgery) are not painful or stressful for the animal. At the end of the surgery, the openings in the skull are sealed with either a transparent glass window to allow for microscopy or with a special type of non-transparent cement that adheres to the skull. The surgery is expected to cause moderate discomfort. Mice recover within a few days and they will be given painkillers and post-operative care (for example, they receive special food that requires minimal chewing). During recovery, mice are closely monitored. For example, we compare the animal's weight to the weight before surgery and we check whether a mouse is eating and drinking and moving around the home cage normally. After mice are fully recovered, we will train them in behavioural tasks. Mice will participate in experiments where they learn new associations between sensory features and rewards. In some experiments, we need to fix the head of the mouse using the head post. During head fixation, we also place the mouse on a treadmill. The treadmill allows the mouse to move more freely (apart from the head which is fixed) because the mouse can choose to either sit still or walk (the treadmill only moves when the mouse decides to move) instead of being forced to sit still without a treadmill. Mice become accustomed to head fixation within a few days, and the treadmill helps mice to get used to head fixation more quickly than without a treadmill. Mice will then be presented with sensory features, including visual objects on a screen, sounds from a speaker, or smells coming from a tube. The sensory features and behavioural testing procedures are not painful or stressful, and mice will learn within a number of days which features are associated with food rewards. For example, mice learn that when they see one type of visual pattern (e.g. vertical stripes) they can get a food reward when they lick a spout, and that they cannot get a food reward when they see another visual pattern (e.g. horizontal stripes).

	<p>Sometimes we need to restrict the food of the mouse to motivate the animal to learn a new task. Mice are expected to only experience mild discomfort during periods with food restriction (and we closely monitor the mice and check for example their weight to ensure they stay healthy). In some of our experiments, head fixation is not needed and animals learn the associations between sensory features and food rewards while freely moving inside a training cage in which visual features, sounds and smells are presented to the animal. In these experiments we usually attach temporarily lightweight sensors to the head post in order to be able to measure the behaviour of the mouse (for example, the position of the animal in the training cage and the eye position of the mouse by using a miniature camera). We found that mice are not bothered by these lightweight sensors, for example, we find that the sensors do not change how mice move around. We also study natural behaviours that do not require training or food deprivation such as exploration of new environments. After all experiments are completed, mice will be killed by a humane method and brains will be studied to obtain additional details about the brain cells that were recorded including their location and cell type.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The research is only possible with the use of animals. Human studies allow us to also measure brain activity, but these studies do not allow us to measure brain activity in as much detail as in mice. We achieve much greater detail in mice by placing electrodes in the brain under anaesthesia and subsequently using them to measure the electrical activity of single brain cells during perception and behaviour. We can also genetically label brain cells so that we can use microscopy to measure activity of these cells through a small transparent glass window placed in the skull. This is necessary to understand which precise brain activity patterns cause specific behaviours. Computer simulations are sometimes used but cannot replace animal use because the modelling of brain activity during behaviour is not yet advanced enough to provide</p>

	the required level of detail to answer our research questions.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We are committed to using the minimum number of animals required to obtain reliable scientific results. We use statistics to estimate the number of animals required. We also design our experiments to maximize the amount of results obtained from each animal (by using long-term measurement to collect multiple data points from the same animals) to reduce animal numbers.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>This research is only possible with the use of mice, since they have a brain comparable to humans, they are capable of behaviours that are similar to important aspects of human behaviour, and there are unique methods available in mice such as methods to genetically alter mice that allow us to model mental illness and measure brain activity with great detail. We take the welfare of our animals very seriously: our mice take part in long-term experiments in which they typically learn during behavioural tests associations between sensory inputs such as specific visual objects and food rewards, and it is therefore necessary that the animals are not stressed and in good health. To reduce stress, animals are acclimatised to experiments by handling and giving food rewards. Animals are monitored closely and if we observe any unexpected adverse effects we will consult specially trained staff and vets. If the animal cannot be treated effectively then animals are humanely killed to avoid any potential suffering that may otherwise arise.</p>

Project	Understanding the mechanistic origins and sequelae of epilepsy at all life stages	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	In a certain proportion of adults, especially those that are untreatable with current medications, there is a risk of sudden unexpected death in epilepsy (SUDEP) in adults. The aetiology of this condition is not fully understood, but there is the possibility that neurological dysfunction associated with the epileptic fits may lead to cardiorespiratory failure. Our aim is to understand the mechanisms by which epileptic fits could compromise the function of cardio respiratory neurons which in turn could lead to SUDEP, and to look at new treatments that could prevent it from happening.	
Why is it important to		

<p>undertake this work?</p>	<p>In the UK, around 600,000 people – almost 1% of the population – have epilepsy. Of these people with epilepsy approximately 600 die every year from SUDEP. Many more people have to live daily with the risk of SUDEP hanging over them. Whilst it is known that seizure-induced cardiorespiratory autonomic dysfunction is a potential cause of SUDEP, identification of effective therapies to prevent SUDEP is hampered by poor understanding of the underlying neural mechanisms that lead to seizure-induced cessation of breathing and/or circulation. Improved knowledge of microglial activation or inhibition in regulating cardiorespiratory function and seizure threshold/frequency will help to inform the future development of therapeutic interventions. Developments in therapeutic care will ultimately benefit the patients, clinicians and the health care system. It will reduce the duration of hospitalization and disabilities due to uncontrolled seizures in patients with epilepsy and reduce suffering of their family members, and improve the quality of life in lessening concerns over seizures and SUDEP. We hope that in the longer term, the UK health care system will benefit by saving money and resources spent on epilepsy patients.</p>
<p>What outputs do you think you will see at the end of this project?</p>	<p>Sudden unexpected death in epilepsy (SUDEP)</p> <ul style="list-style-type: none"> • Identification of the key cardiovascular and/or respiratory autonomic neurons that are impacted by seizures. The knowledge of functional changes in neurons in specific cardiorespiratory nuclei will help us to understand how these changes may lead to SUDEP. • Potential for development of functional biomarker tests for people with epilepsy who are at higher risk of SUDEP. • Establishing whether inhibition or activation of microglia could be effective in preventing seizures and maintaining the activity of cardiorespiratory networks during acute or chronic seizures. • High impact publications and generation of new intellectual property.
<p>Who or what will benefit from these outputs, and how?</p>	<p>Our project may lead to direct patient benefits in the short to medium term:</p> <p>Non-invasive testing of the responses of cardiorespiratory</p>

	<p>autonomic neurons to CO₂ (biomarker test) at different phases of epilepsy might identify people with epilepsy who are at higher risk of SUDEP. This would allow adjustment of lifestyles (e.g. supervision, nocturnal breathing support) to mitigate risk. Minocycline is a commonly prescribed antibiotic, that crosses the blood brain barrier. There is evidence to suggest this drug also blocks microglial activation, and if we can identify that microglial activation could lead to SUDEP then this drug might be a potential intervention to lower the risk of SUDEP.</p> <p>Longer term</p> <p>The identification of the mechanisms that underlie SUDEP - specifically the alterations to brainstem neural circuitry may suggest new strategies to lessen the risk of SUDEP e.g. use of stimulants that might act specifically on certain key circuits. Additionally these new therapeutic targets could lead to new drug development or repurposing of existing drugs that already act on these targets.</p>
<p>Will this work be offered as a service to others?</p>	<p>No</p>
<p>How will you look to maximise the outputs of this work?</p>	<p>In addition to disseminating our findings through high quality scientific papers and conference presentations, we shall have a systematic program to engage with a wider audience.</p> <p>This wider engagement will involve:</p> <ul style="list-style-type: none"> • Dissemination through funders and relevant charities (Redacted) • Engagement with relevant patient groups • More general public engagement e.g. with communities local to the University, Science Fairs, Schools.
<p>Explain why you are using these types of animals and your choice of life stages.</p>	<p>We use mice throughout the project as they are an excellent genetic model. This gives the advantage of a rich suite of genetic strategies to target specific cell populations in the brain either to specifically record their activity, manipulate their activity, or alter their gene expression. These strategies are essential to allow rigorous analysis of</p>

	<p>underlying mechanism.</p> <p>To study the potential pathways that lead to sudden unexpected death in epilepsy (SUDEP) in man we will use adult mice. Mice have proven to be an excellent model for the acute and chronic states of epilepsy which permit rigorous investigation of underlying mechanisms and are used by many labs around the world.</p>
<p>Typically, what will be done to an animal used in your project?</p>	<p>A small hole will be made in the skull to enable precise injection of virus particles to give expression of a protein that will report the activity of the neuron that it is expressed in by its intensity of fluorescence. The mouse will be allowed to recover and 2 weeks later another hole made into the skull and a special lens implanted into the brain so that the fluorescence can be measured. At this time electrodes to allow the recording of brain waves and heart signalling, and a cannula to allow delivery of a substance to induce seizures, will also be implanted. The mouse will be allowed to recover and a further week later a special baseplate will be glued to the head in final minor surgical procedure. This baseplate allows mounting of a mini-microscope that will allow recording of neural activity as the mouse behaves normally.</p> <p>Following this preparative work we will record activity of neurons in these mice for up to 4 months. Seizures will then be induced by brief infusion of kainic acid through the cannula. This will cause acute seizures, followed by the establishment of a chronic epileptic state where the mouse will exhibit occasional spontaneous seizures. We will also study neuronal activity in this chronic state.</p>
<p>What are the expected impacts and/or adverse effects for the animals during your project?</p>	<p>The mice recover well from the surgical procedures and show minimal adverse signs either during or between recording sessions. Following kainic acid treatment, the mice will experience acute seizures for up to an hour. This will produce longer term changes in animal behaviour such as increased anxiety, memory/learning deficits and chronic epilepsy.</p>
<p>What are the expected</p>	<p>For about 75% of animals the expected severity is severe.</p>

<p>severities and the proportion of animals in each category (per animal type)?</p>	<p>For the remaining animals on the protocol it will be moderate.</p>
<p>What will happen to animals at the end of this project?</p>	<p>killed</p>
<p>Why do you need to use animals to achieve the aim of your project?</p>	<p>This project seeks to understand phenomena that arise in the whole brain, and rely on interconnections within the brain such as: the mechanisms that underlie acute and chronic seizures; and how seizure activity can disrupt autonomic activity -specifically the neural circuits in the brainstem that control breathing and cardiovascular regulation. Catastrophic disruption of the activity in these neural circuits is likely to underlie SUDEP. These phenomena can only be studied in whole animals and tissues taken from animals, in which seizures have been previously induced. It would be unethical to use human subjects in these experiments.</p> <p>Brain organoids replicate to a very limited extent some of the circuitry of the brain. While these are unlikely any time soon to give detailed recapitulation of brainstem cardiorespiratory networks suitable for our aims, we shall monitor developments in this area, in case advances may make them suitable for investigating cellular and molecular mechanisms within the relevant neuronal subtypes and subcircuits.</p>
<p>Which non-animal alternatives did you consider for use in this project?</p>	<p>Induction of seizure like activity in isolated brain tissue</p>
<p>Why were they not suitable?</p>	<p>Seizure induction in isolated tissue:</p> <p>These models are acceptable for investigation of rather general phenomena related to the mechanisms of seizures. Brain slices are typically cut from one region. Our investigation of the mechanisms that could lead to SUDEP requires an interconnected brain whereby a seizure initiated in the cortex can invade, through many interposed steps, the key brainstem nuclei involved in cardiorespiratory control. It is impossible to achieve this</p>

	type of interconnectivity in vitro. In vitro models are inadequate to progress the aims of the project.
Enter the estimated number of animals of each type used in this project.	mice: 300
How have you estimated the numbers of animals you will use?	<p>We have performed power calculations based on expected effect sizes and consulted statisticians to optimize our experimental program.</p> <p>Power calculations from our previous experiments suggest that we will require around 100 mice to achieve our aims. However, if we find that particular subsets of neurons are affected by seizures we may need to perform further analysis with genetic targeting of these neurons. To allow for this we have anticipated that this may be the case for 2 additional populations and adjusted total numbers accordingly.</p>
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	We took statistical advice on the design of the experiments. We examined the NC3Rs Experimental Design Assistant, but concluded that we did not need to use it for our studies at this stage. We have attended NC3Rs 2019 Symposium to keep abreast of new thinking in the field. We used the best estimate of effect size based on pilot data or published literature to inform our power calculations.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	<p>Planning and execution of our experiments will be according to best practice as exemplified by the PREPARE guidelines (Lab Anim. 2018 52:135-141. doi: 10.1177/0023677217724823). https://norecopa.no/PREPARE</p> <p>Pilot studies will be performed to get good estimates of effect size. Should our program demonstrate that our estimates of effect size are wrong (either too big or too small) we shall reperform the power calculations to ensure we arrive at a rigorous outcome. If it appears that the effect size is much smaller than anticipated for a particular experiment, we shall consider abandoning that part of the program. We shall routinely harvest tissue to enable biochemical, genetic, cellular and morphological analyses</p>

	<p>and share this tissue with our collaborators.</p> <p>The in vivo microscopy permits repeated imaging sessions from the same animal and this reduces the number of animals required in the study as the repeated measurements will give greater statistical certainty and will allow study of the acute and chronic stages of epilepsy in the same animal.</p>
<p>Which animal models and methods will you use during this project?</p>	<p>Rodents are a well-established model for studying the neural mechanisms underlying epilepsy. This gives a robust literature and wealth of potential data obviating the need to repeat past findings. Rodents in general are seen as a simplified and experimentally tractable model for far more complex mammals such as humans.</p> <p>Wherever possible, physiological testing of phenotypes will be achieved by using non-invasive methods such as: whole body plethysmography for detection of breathing movements. More invasive procedures will be used only when there is good reason (from the non-invasive experiments) to expect mechanistic insight.</p>
<p>Why can't you use animals that are less sentient?</p>	<p>Immature life stage:</p> <p>Mouse pups cannot be used because the cardiorespiratory control circuitry is still developing and will not model the adult circuits. As SUDEP occurs in adulthood, we need to study the effects of seizures on the mature brain stem circuitry.</p> <p>Sentience:</p> <p>Lower vertebrate species such as fish, lamprey, frog, while being able to generate seizures are not a good model for epilepsy and its effect on cardiorespiratory control networks as their cardiorespiratory control networks do not correspond to those of mammals. By choosing mice, we have selected the least sentient model to study the relevant underlying mechanisms that could lead to SUDEP</p> <p>Terminal anesthesia:</p> <p>Terminal anaesthesia alters the operation of the brainstem networks by for example, disinhibiting certain networks and generally depressing respiratory drive. Thus, use of</p>

	terminal anaesthesia will not allow us to uncover the relevant mechanism of altered neuronal signalling that may lead to SUDEP.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	We subscribe to the NC3Rs newsletter and we receive update Emails from this organisation. We make efforts to attend their symposia. We network with colleagues from other universities to ensure that we stay abreast of any developments. As an example of our openness to best practice, we have implemented improved mouse handling procedures designed to minimize stress as a result of coming from advice from NC3Rs.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	<p>We have considerable experience with the <i>in vivo</i> imaging methods and have considerably refined them already over the past 2-3 years. We have implemented standardised post-operative monitoring methods to ensure that outcomes and adverse effects from our surgical procedures are carefully assessed. We use this information to optimise post-operative care and pain management.</p> <p>We shall use rigorous aseptic techniques to protect against infection of the animals.</p> <p>We train the animals with a dummy camera to acclimate them to the imaging procedure and to the imaging cage to minimize stress.</p> <p>As imprecise targeting of viral injections can increase animal usage, we shall be very careful in this aspect of our procedure. We shall ensure we use calibrated stereotaxic frames, check the alignments thoroughly and regularly use the injection of fluorescent beads (in a non-recovery terminal procedure) to ensure that correct targeting is achieved prior to commencing the lengthy procedures required for the SUDEP part of the project.</p> <p>We continually look to refine and improve our experimental models and procedures. This is a regular topic of discussion in weekly lab meetings.</p>
What published best practice guidance will you follow to ensure	We shall take careful note of advice from collaborators and researchers highly experienced in these methodologies to ensure that we refine our experiments as much as

<p>experiments are conducted in the most refined way?</p>	<p>possible. We shall follow the Laboratory Animal Science Association guidelines on performing aseptic surgery to ensure best practice for the recovery surgery involved in all surgical procedures. We shall follow the guidelines published in J Neurosci Methods. (2016) 260:2-25. doi: 10.1016/j.jneumeth.2015.09.007 which comprises a NC3Rs-sponsored metareview of models of epilepsy and has comprehensive advice on best choice of mode and refinements.</p> <p>Planning and execution of our experiments will be according to best practice as exemplified by the PREPARE guidelines (Lab Anim. 2018 52:135-141. doi: 10.1177/0023677217724823). https://norecopa.no/PREPARE</p> <p>To ensure effective and rigorous reporting of our results, we shall write papers according to the ARRIVE guidelines which are recognised as providing excellent transparent standards for reporting of research using animals and were developed by the NC3Rs.</p>
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Project	Understanding the neural circuitry of somatosensation and how it changes following nerve injury	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Nerve injury can result from trauma or occur as a complication of common conditions such as diabetes or treatment with drugs such as chemotherapeutic agents. Unfortunately nerve repair is usually incomplete and patients often have to live with long lasting weakness and pain. There are currently no effective clinical therapies available. We will determine what happens following a nerve injury both within the nerve itself but also its connections for instance to the spinal cord. Using this knowledge we will develop and	

	<p>test potential treatments to enhance neural repair.</p> <p>Despite recent advances we still have a limited understanding of the sensory nervous system and how it changes following trauma to a peripheral nerve. We propose to study injuries to the peripheral nervous system in order to determine how the circuits carrying sensory information change after injury and find molecules that drive this change which could be targets for treatment.</p> <p>We will also study the different types of sensory nerve fibre which are activated by particular types of stimulation for instance cooling, warmth, touch and pain. We don't yet understand how these different sensory nerve fibres code for the different types of sensation and how this changes after injury. We will use genetically modified mice and rats which allow us to investigate how the different types of sensory nerve fibre react to nerve injury and to study the effect of silencing specific types of sensory nerve fibres for instance just those that normally respond to cold or high temperatures. The aim is ultimately to develop treatments which are based on silencing particular types of sensory nerve fibre that can be used to reduce pain after nerve injury but leave other types of sensation (such as touch) intact.</p> <p>Brain regions have been found in humans that become active following the application of a stimulus that causes pain. Just because a brain region is activated however does not mean that it has a role in the generation of the sensation of pain. It is important to determine the brain regions that are critical for pain as then they could be targeted in humans either with drugs which are likely to be effective in reducing the activity of that brain region or even using magnetic fields to silence that region non-invasively in humans. By correlating brain imaging results in rodents with existing human data we can then specifically silence brain regions in the rodent and demonstrate their importance in pain related behaviour. We can then focus efforts in therapeutic targeting in humans.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced)</p>	<p>Peripheral neuropathy and pain are common problems. Peripheral neuropathy affects 6% of the elderly population and chronic pain up to 20% of</p>

<p>or humans or animals could benefit from the project)?</p>	<p>the population. Unfortunately there is no treatment yet available to help nerves regenerate and although there are a number of treatments for pain these are limited in their effectiveness and associated with side effects such as addictive potential. Neuropathy and pain therefore have a major negative impact on quality of life. The ultimate aim of this project is to develop treatments for these conditions which would be of major benefit to humans and animals.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We estimate that we will use up to 800 rats and 15500 mice with the majority of the latter being generated through the breeding of transgenic mice. The duration of this project will be 5 years. Transgenic technology means that genes can be manipulated in mice to study very specifically the role of individual genes. Mouse and rat were chosen for this work plan because the sensory nervous system in these animals is similar to human and genetic manipulation can be undertaken in these animals. Careful experimental and statistical design will be employed to minimise the number of animals used to generate robust results. For instance the minimum number of animals will be used in each group to give robust statistical results.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Under general anaesthesia animals will undergo surgery in which a peripheral nerve will be partially injured in a controlled fashion. This will produce focal weakness for instance of a hindlimb and altered sensation such as numbness and pain. Pain killers will be given at the time of the operation. Alternative models of neuropathy will include administration of drugs which induce neuropathy or using genetic models which develop diabetes and neuropathy. To test the response of animals to sensory stimuli mechanical stimuli eg. bendy hairs (von Frey hairs), thermal stimuli (eg. warming or cooling) or chemicals (eg. capsaicin an extract of chilli peppers) will be applied to the paw. We may also manipulate activity of the sensory nervous system with light (switching it on or off). These stimuli are predicted to evoke a brief sensation of pain as we are mostly determining the point at which the animals first detect the stimulus. By using stimuli from which animals can withdraw suffering is minimised. Measurements of electrical</p>

	<p>changes in neurons as well as imaging to determine brain activity will be used to assess repair and function of the sensory and motor nerves and their connections within the brain. For these procedures animals will undergo general anaesthesia. Animals will be humanely killed at the end and tissue taken after death.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Techniques using cultured neurons are not yet sufficiently advanced that they can fully model the nervous system. For example pain arises due to the complex interaction of millions of different types of neurons. Although we have a long list of brain regions that become activated following administration of a stimulus that causes pain in humans we do not know which regions are actually important in generating the sensation of pain. This is an important question to answer so that we can correctly target treatments for pain. This is due to the complex connectivity of the nervous system and the multiple cell types involved. Behavioural analysis of gait and sensory function requires the use of awake animals. We have pioneered the use of human induced pluripotent stem cells which can be differentiated into sensory neurons. During this project licence these will enable the investigation of molecular interactions and electrical properties of these neurons hence ultimately reducing the use of animals. These are helpful in understanding the molecular means by which sensory neurons detect stimuli however head to head comparison shows that they do not yet fully match sensory neurons in humans or rodents furthermore they can't be used to model sensory circuits as we can't yet use them to look at connections between neurons. Wherever possible we do test normal sensory function in humans however some models require gene manipulation, creation of experimental nerve injury, or modulation of specific brain regions which can't be performed in human.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of</p>	<p>Throughout this project we will reduce the number of animals used by using rigorous experimental design in each experimental paradigm to use the minimum number of animals to generate a statistically meaningful result and have taken</p>

<p>animals</p>	<p>statistical advice in order to do this. We will use careful experimental design to reduce any bias in our experiments and to reduce variation in the data. We will randomise our allocation of animals to treatment groups and the experimenters undertaking measurements will not know which animals have received the active treatment versus the inactive control treatment (ie. experimenters will be blind). By reducing bias we will generate robust and reproducible results. We will examine sensation in male and female animals and build this into our experimental design because both in humans and experimental animals there can be differences in the response of the nervous system to injury and in pain sensation.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rodents are the most commonly used animals for the study of neural injury and persistent pain because there is vast knowledge of the rodent peripheral nervous system. The sensory and motor neurons in the rodent have comparable features to those seen in primates, including man and both species show a similar response to nerve injury. Other animals which can be manipulated genetically such as fruit-fly have a sensory nervous system which is very different to mammals and so are not appropriate for this project. The models used attempt as far as possible to mimic human neuropathies and persistent pain states. Our intention is for the different animal models to represent different underlying mechanisms generated following nerve injury in patients in order to translate findings to clinical benefit. We will use models which recapitulate the human condition of neuropathy as closely as possible but are associated with the minimum suffering for the animals. Our MRI imaging experiments will help to correlate our findings in animal models with those derived from human patients. We will minimise the severity of models to reduce suffering. The most common test of sensory function is measurement of reflex withdrawal to threshold stimuli rather than subjecting animals to the most intense stimuli. Animals will be closely monitored following surgical procedures which will be performed efficiently by well trained staff using aseptic technique and peri-operative analgesia will be administered. Any drugs administered will be done so at the minimum dose to be effective whilst minimising side effects. We will make sure that there is sufficient time</p>

	between drug treatments so that they do not interact and have combined effects.
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Project	Understanding the neuronal basis of learning and memory disorders	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Common CNS disorders including schizophrenia, depression, Attention Deficit Hyperactivity Disorder (ADHD) and Autistic Spectrum Disorders (ASD) share symptoms including cognitive dysfunction. Although drugs such as antipsychotics, antidepressants and psychostimulants, exist to treat the primary symptoms, no treatments rectify cognitive defects or prevent disease development, which is the focus of this project. Development of new medicines to treat cognitive dysfunction requires a better understanding of the neurobiological basis and aetiology of these disorders and	

	<p>requires rigorous testing in well-validated animal models. The NIMH introduced the Research Domain Criteria (RDoC) to provide a research framework to develop new approaches to research on mental disorders in which core common symptom domains (like cognitive dysfunction) shared across the current diagnostic disorders are used to group patients in clinical trials and provide a new translational approach to understand the neuropathology, neuroscience and behaviour in animal models. The risk of developing these common disorders involves a complex gene/early-life environment interaction that will be modelled in this project. This project will utilise rodent models of these common CNS disorders, including rats subject to early-life drug, surgical and/or environmental manipulation; factors known to be risks in man, to gain this information. These models will be used to determine how selected drugs can improve dysfunction and identify the specific neurotransmitters, receptors and molecular targets involved in mediating the beneficial effects, and where appropriate, compare these effects on the same learning and memory processes in the normal rodent.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This project will improve our understanding of the underlying neurobiology, neurotransmitter pathways and molecular mechanisms contributing to learning and memory disorders in rodent models of common human CNS disorders. With continue collaboration and support from pharmaceutical industry a tangible outcome is development of improved therapy for learning and memory disorders. However, the development of any new therapy through to phase III clinical trials and eventual use in patients is likely to require at least 15 years. Staff working under this project licence will continue to produce high quality research publications advancing our understanding of the neurobiology of learning and memory in high impact journals and make regular presentations at international scientific meetings to disseminate findings to a broad audience. The PPL frequently communicates findings at University departments, pharmaceutical companies and international conferences, such as being an invited speaker at the Swiss Lab Animal Science</p>

	Association meeting (SGV, a member of FELASA) in November 2018.
What species and approximate numbers of animals do you expect to use over what period of time?	Experiments will use rats (5000 over five years) and mice (1000 over five years) at all stages of development.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most experiments performed under this licence will involve acute drug administration and monitoring of behavioural and/or neurotransmitter release and function, some will occur with indwelling intracerebral cannula or peripheral implants previously implanted under anaesthesia. In most cases the drugs administered will produce mild transient changes in behaviour with no long-lasting effect. In some cases pregnant dams will be administered drugs to modify neuronal development of offspring without causing any gross developmental abnormalities to either the dam or offspring. None of the behavioural paradigms involve exposure to long lasting painful stimuli, some involve exposure to a transient load noise to induce a startle or to a transient mild aversive foot shock to enable training. Each procedure has been evaluated for severity. The drug studies combined with behaviour in the absence of any surgical intervention are likely to be associated with only mild clinical signs but several can be graded as moderate due to, for instance, the use of mild electric shock or desired and expected drug-induced changes in behaviour, so these, like all procedures involving surgery, will be likely to be rated as moderate. It is anticipated that no more than 30% of the experiments will involve surgery in some form, so an overall grading of moderate for the licence is expected. All animals will either be humanely killed under terminal anaesthesia or by a schedule 1 procedure to allow collection of brain and selected other tissues for further analysis and correlation.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-	Due to the nature of the project there is no suitable <i>ex vivo</i> alternative to assess complex behavioural functions of the CNS. <i>In vitro</i> slices and cell preparations may be used to examine

<p>animal alternatives</p>	<p>molecular mechanisms underlying specific components involved in learning and memory such as long term potentiation, but this cannot be extrapolated to human behaviour. As the underlying causes of cognitive dysfunction are unknown, computer modelling techniques are of limited use. It is also not ethical or possible to use patients to test novel experimental agents proposed for use in the current animal studies. In rodent models it is possible to perform invasive procedures that will establish specific neuronal circuits, neurotransmitters and molecular mechanisms underlying learning and memory that can't be directly measured in man.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>For each study undertaken many parameters will typically be measured and careful consideration is given to incorporating suitable controls, often both for drug treatment and experimental condition. Almost all data is analysed by ANOVA, often with two or three independent factors. Personal licence holders undertaking this work attend statistical workshops as part of their graduate training and all are encouraged to undertake the preclinical experimental studies programme run by the British Association for Psychopharmacology that covers experimental design and data analysis. Where required, advanced statistical knowledge will be obtained from Statisticians within the University or specialists employed by sponsors from the pharmaceutical industry. Typically a power analysis has been performed on the primary outcome measure for each behaviour, to ensure that sufficient animals are included in the design and that there is a robust likelihood of achieving significance (P less than 0.05 with a power of 0.8) and guidance also obtained from previous publications with the technique.</p> <p>For each experiment, as required by the BSU, and according to the ARRIVE guidelines, we will write an experimental protocol which includes: a statement of the objectives, description of the experiment, covering experimental treatments, the size of the experiment (number of groups, number of animals/group), the experimental material, and an outline of the method of analysis of the results.</p>

3. Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

This project will utilise rats and mice because many aspects of rodent learning and memory are similar to that in man. Behavioural tasks assess multiple learning and memory domains relevant to human psychiatric disorders. A wealth of data is available on mouse and rat behaviour and excellent stereotaxic atlases enable CNS implantation of probes and injection guides. For brain lesion and microdialysis studies rats are the most suitable small species where a wealth of previous literature exists.

Rodents have been selected over less sentient vertebrates, such as zebra fish, because it is not possible to perform complex learning and memory tasks in these animals, and few such tasks have recognised translational predictive validity to the cognitive domains affected in human CNS disorders. However, the well-characterised learning and memory paradigms to be utilised in rodents have proven relevance to cognitive dysfunction seen in man, which means that a higher species of animal is not required to meet the objectives of this study.

The most severe procedures used in this project involve surgery that is carried out under anaesthesia, using aseptic surgical techniques practised by experienced licence holders and followed by high standards of post-operative care including analgesia as advised by the NVS.

Refinement will advance and improve neurochemical markers and biosensor probes to improve measurement of GABA and glutamate both in terms of sensitivity and temporal resolution.

Improvement in the predictive validity of animal models of common neurodevelopmental learning and memory disorders is also being actively pursued, by combining early-life adversity with chemical or immune activation that may produce 'dual-hit' models with better translational relevance to schizophrenia and depression.

The project uses rodents at all stages of development including exposure of the dam and/or neonatal pups to environmental and/or drug treatment to produce a programmed change in neuronal development with consequent

	<p>alteration in adolescent or adult behaviour required to replicate the human neurodevelopmental CNS disorder. It also uses adult rodents to examine drug-induced alterations in behaviour and relate these to the underlying neurotransmitter or anatomical pathways involved. Pilot Studies will also be used where essential to develop a new technique.</p>
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Project	<p>Understanding The Pathogenesis Of Diabetes And Optimising Islet Transplantation In Diabetes</p>	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Diabetes is a condition characterised by high sugar levels which can lead to multiple health complications. People with diabetes are usually dependent on medication to control their sugar levels. In the case of Type 1 diabetes patients are dependent on insulin injections and in the later stages of type II diabetes most common form of diabetes patients can also be dependent on insulin injections. The pancreas contains islets, a group of cells that under normal conditions can secrete insulin. However after many years of diabetes and repeated episodes of low glucose concentrations</p>	

	<p>secondary to too much insulin, the hormone secreted by the islets that can usually rescue a low blood glucose termed glucagon can become inappropriately low during these low glucose episodes causing further problems. The aims of the project are to understand how diabetes progresses and why the hormone glucagon becomes abnormally regulated; we could therefore find ways in which to normalise this hormone. The second part of the project involves improving a technique called islet transplantation. This technique involving placing islets from a donor pancreas into a patient with type 1 diabetes and is a treatment for extremely poorly controlled diabetes. However, there is a shortage of donor pancreases, secondly, more than 60% of the islets which are transplanted into the liver fail to engraft in the first three days following islet transplantation. Therefore most patients require two or more islet transplantations to achieve a beneficial clinical response. Furthermore in the longer term islet function diminishes and little is known about the effects that cause these changes. In addition as the adult population is becoming more obese consequence of this is that more people are developing fat in their liver which may progress to become inflamed with a degree of fibrosis. We will therefore aim to develop techniques to optimise islet engraftment in the context of a normal, fatty and fibrosed liver. We will also directly visualise the islets by microscopy by transplanting into one of the eyes (enabling the mouse to see) and examining these islets over time. This ultimately means that the same mouse can be used for these experiments and saves therefore on numbers of mice used to track islet function. The second purpose is to evaluate the function of “manufactured” cells made into insulin secreting cells.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The main benefits are scientific/knowledge based in the first instance leading in the longer term to clinical benefits. This project would mean that potentially only one donor pancreas would be sufficient per person. Therefore more donor pancreases would be available for more people. Furthermore, alternative strategies to achieve insulin secretion would be evaluated.</p>

<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Rat (including BB rat) mouse including humanised mouse models and other immunodeficient (NOD SCID) mice <3700 mice over 5 years <1300 rats over 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The potential adverse effects in this project are mainly of surgery, and medicine administration. Deaths resulting from anaesthesia or surgical complications are uncommon (<1%) and will be minimised by correct dosing of anaesthetics and analgesics, by accurate weighing and by maintenance of body temperature during and post-surgery e.g. use of heat pads. Pain will be controlled during and after surgery by general anaesthesia and analgesics. Surgical infections are rare and the risk minimised by good surgical and aseptic techniques. Surgical sites will be monitored for signs of infection and medicines including pain relief given as appropriate. Within any experimental procedure administering/transplanting insulin producing cells, there is a risk animals may go hypoglycaemic, with the potential for fitting, seizure or death. Careful monitoring of blood glucose levels, and therapeutic administration of dextrose, should allow reversal of hypoglycaemic events. With respect to specific models the main adverse effect with respect to: 1) the diabetes model is weight loss and excessive diuresis and thirst – animals will be closely monitored. All animals that have diabetes will be treated with some form of insulin and therefore we expect side-effects to be extremely low; 2) carbon tetrachloride model – this may cause drowsiness initially and the animals may appear unwell for 24-48 hours afterwards however their condition will be closely monitored during this period. Over 95% make a full recovery. At these doses, we expect a mild form of fibrosis in the liver but we do not anticipate ascites however if this does develop then animals will be killed humanely. 3) Partial hepatectomy: After the surgery there may be an increased risk of blood loss although the group here has much expertise in this technique and therefore this is seldom seen here. 4) Methionine choline deficient diets– these diets generally cause increased fat in the liver but they may cause rapid weight loss (this may occur in <10% of cases). Weight will be monitored regularly. Animals will be humanely killed at the end of the study so that</p>

	<p>tissues can be analysed. 5) Kidney ligation/nephrectomy: removal of a kidney may cause hypertension, or expansive remodelling of the remaining kidney. During the time frame of our experiments we don't expect these to be problematic</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>It would not be appropriate to use humans for these experiments as this would involve multiple liver samples taken surgically removed, a technique that is associated with a high risk of bleeding. Mice that have been transplanted with islets into the liver reflect key aspects of the transplant process in humans. The use of genetically modified animals as well as interventions that are not possible in humans allows us to dissect the different contributions of hormones, nutrition and immune cells to islets engrafting into the liver. This research can provide vital data to enable treatments in humans.</p> <p>Our investigations in live experimental animals are supported by extensive analyses of tissues taken once the experiment is complete and are complemented by investigation of isolated cell systems.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The number of animals used in our investigations is based on power calculations to determine optimum group size and statistical power. Where possible, a multi-factorial design is used to increase power and reduce the overall number of animals required. The use of inbred mice reduces experimental variability and thus overall numbers required. Imaging techniques (similar to those used in humans) in live animals allow sequential non-invasive measurements, providing repeated measures within a single animal, increasing statistical power and reducing the number of animals required for experiments.</p> <p>The effects of treatments are based on comparison with appropriate control and/or sham treated groups. Study design is based on current best practice and, where necessary, following discussion with statisticians.</p>

<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>In all our experiments we are mindful of the need for refinement to reduce suffering, and appropriate modifications to protocols will be incorporated where possible. In carrying out experiments in rodents, we will always seek to incorporate these refinements. We will observe carefully for signs of stress in the animals.</p>
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Project	Understanding the perturbation of innate immunity in vascular inflammation	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We would like to understand how common features of a 'Western' diet (e.g. high fat) and poor kidney function impact on the organs and processes of the body that fight infection and protect against toxins in 'cardiovascular disease' (disease of the blood transport system). By increasing our knowledge of how these common mechanisms can lead to increased risk of infection and worsen infection outcome, we hope to ultimately identify new treatments.	

<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Other diseases can exist at the same time as cardiovascular disease, including long-term kidney disease. Together these two diseases account for the majority of deaths in the UK. In all cases, a high fat diet, elevated levels of cholesterol in the blood and poor kidney function is known to worsen these conditions. This research will help provide more information about 'cardiorenal disease' (a disease which effects both the heart and kidneys together) and how the body's infection defence system contributes and responds to this disease. Increased knowledge about how this happens will advance research in this area and could help identify new ways of treating the disease.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Approximately 6000 mice and 3000 rats over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>We will mainly use animals which have been engineered in the laboratory ('genetically modified') to either change how likely they are to get vascular diseases and/or to make it easier to follow the progress of disease. Most of the procedures involve giving these animals a diet which is high in fat in order to study cardiovascular disease. Some animals may undergo surgical procedures and a small proportion may have bone marrow transplants. The maximum severity for all animals is moderate. Any adverse effects from procedures will be carefully monitored. All animals will be humanely killed at the end of the study.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Inflammation and vascular disease are complex processes that cannot be reproduced in a lab, because the responses of the body involve different organs. Nevertheless, there are some parts of these processes which can be studied in the laboratory and which often provide more clear-cut conclusions than are possible in an animal model, mainly because we can control the conditions to focus on specific parts of the process. Our approach is to therefore use animal</p>

	<p>and laboratory experiments in a complementary manner and to use laboratory based experiments in place of animals wherever possible. An example of this is where we use cells which we grow in the laboratory, exposing them to different forms of blood flow in a specialist chamber, to mimic the conditions found in a vein or artery.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We design our experiments so that we only use the minimum number of animals in order to gain meaningful results. We use cutting-edge technology to track the process of disease in animal models of disease using whole body imaging without any requirement for surgery. This means that the same mice can be anaesthetised and imaged many times with minimal stress, greatly decreasing the number of animals required. We will use laboratory systems in place of animals wherever possible, as explained above.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice and rats are used as they share many processes with humans and their responses to disease accurately model human responses and biology. All animals will receive pain relief to treat any apparent discomfort, and pain relief drugs will always be given prior to any surgery. Surgery will be carried out under anaesthesia and using sterile techniques. Veterinary advice is available if needed and animals will be carefully monitored for signs of distress following interventions.</p>

Project	Understanding the role of inflammation in the development of cardiometabolic diseases	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Chronic inflammation has been associated with development of cardiovascular disease (atherosclerosis, heart attack, stroke) in humans. Blood vessels are lined by specialist cells, endothelial cells, which regulate how blood vessels respond to stimuli which narrow or widen the vessels to control how much blood can flow through. We know that inflammation can affect how well the endothelium can work, but the exact mechanisms are unclear.</p> <p>Our project aims to investigate how molecular</p>	

	<p>regulators of inflammation in the endothelium impact on cardiovascular disease. By altering which molecular regulators are present or functional in the endothelium of mice, we will test how well their blood vessels respond to stimuli and compare these results to normal mice. We will also give mice a diet which resembles that of western society that is high in fat, which we know is a risk factor for developing cardiovascular disease.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Cardiovascular disease is a major human health issue in the western hemisphere and is become one more globally too. Identifying why and how inflammation contributes towards the development of cardiovascular disease will allow future research to target this inflammation in humans and reduce the chances of people suffering heart attacks and stroke. By studying the effects of very specific molecular regulators of inflammation, we hope to identify opportunities for new medicines to be developed.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Less than 4000 over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The proposed experiments are not expected to produce any severe adverse effects in the animals. Mice will carry alterations in specific genes that are strongly suspected of playing a role in inflammation. We shall measure the properties of their blood vessels non-invasively in their skin. It is necessary to shave and depilate a patch of skin for this purpose, but otherwise no significant adverse effects are expected. Animals fed a high-fat "western" diet may become obese, but not to the extent that this, in itself, will interfere with normal welfare. Animals will be humanely culled at the end of the study in order to harvest tissues for further detailed analysis.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use</p>	<p>Cardiovascular disease is a complex condition which involves both the endothelium and a variety of other cell types, including many components of</p>

<p>animals and why you cannot use non-animal alternatives</p>	<p>the immune system. Currently, there are no cell-culture based alternative which can accurately mimic this environment and thus, we need to perform these experiments on living organisms with a working vascular system, similar to that of humans</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We will use strict, calculated breeding strategies to ensure we produce just sufficient animals for our research programme. We also use careful statistical calculations to determine the optimum number of animals to be used in each experiment</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We are using mice to study cardiovascular disease and inflammation. As these animals do not normally develop frank cardiovascular disease, we use a line that has inherited high cholesterol levels and therefore develops the early signs of cardiovascular disease. We also use mice that have specific alterations in genes encoding inflammatory regulators, to determine how altered regulation affects the properties of blood vessels. In most cases, we expect these genetic alterations to protect against cardiovascular disease. Some animals will receive a high-fat diet but, as we are using a line that is already susceptible to cardiovascular disease, this diet will not be administered for very long periods of time. We do not expect it to cause significant additional harms. Animals which do become obese and mildly diabetic will be cared for appropriately, including regular health checks and clean bedding to maintain good hygiene. Animals that have had patches of skin shaved for the vascular measurements will also receive appropriate care, for example the application of moisturising cream to areas of dry skin, should they have any.</p>

Project	Understanding the role of vascular mimicry (VM) and tumour heterogeneity in metastasis and response to therapy.
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research <input type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim to gain a greater understanding of the process of vascular mimicry in cancer and it's role in how tumours respond to drugs or spread to other parts of the body (metastasise). Spread of blood, carrying nutrients and oxygen, around the body occurs through blood vessels made up of the bodies professional blood vessel cells called endothelial cells. Tumours have an

	<p>increased need for nutrients and oxygen because they grow quickly and need to make lots of proteins. As such tumour cells send out signals that instruct endothelial cells to grow into the tumour to supply it with blood and nutrients, a process called angiogenesis. Several anti-cancer drugs have been developed to block the signals that tumour cells send to endothelial cells with the expectation that this would starve the tumour and lead to its death. However, these anti-angiogenic agents have been disappointing in the clinic. This may be in part because tumours have alternative ways to supply themselves with blood. One of these is vascular mimicry (VM) which involves the tumour forming its own blood vessels by some of the cancer cells changing themselves to become more like endothelial cells, creating blood vessels lined with tumour cells. Patients whose tumours show evidence of VM have poorer survival than those whose tumours that do not show evidence of VM, and our previous work has shown that VM-proficient tumours do not shrink when treated with anti-angiogenic therapy.</p> <p>The work described in this license seeks to understand the process of VM further by using state-of-the-art imaging technologies to look at VM vessels in tumours grown in mice, to understand what can be transported through these vessels. We also are trying to find out what genes are important for VM and whether we can target any of these cellular pathways to inhibit VM.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>It is expected that this work will benefit the field by generating an in depth picture of how VM occurs and the pathways that are important to make it happen. Ultimately we hope to find new or existing drugs that can inhibit VM and could be used in combination with anti-angiogenic agents, with the idea that blocking the body's professional blood vessels and the tumours DIY blood vessels will leave the tumour unable to nourish itself and lead to its demise.</p>
<p>What species and approximate</p>	<p>We are planning to use up to 7200 mice over a</p>

numbers of animals do you expect to use over what period of time?	period of 5 year. We will attempt to reduce this number further if that is possible while maintaining the benefits of this research.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Due to the nature of the projects the mice will undergo surgical procedures and/or develop tumours, however these are anticipated to cause only mild discomfort, and pain relief will be given when necessary. The animal's welfare will be closely monitored to ensure they do not suffer unduly beyond expectations. All animals will be humanely killed at the end of the experiment, or transgenic mice will be kept alive in the authorised establishment or transferred to alternative authorised protocols or establishments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Vascular mimicry involves the formation of tumour cell lined blood vessels that transport oxygen and nutrients to the tumour. As such its study requires a circulatory system and an animal host. There are certain aspects of VM that can be measured in tumour cells grown in a dish and we endeavour to always go down this route first before involving animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our in vitro experiments with tumour cells grown in a dish allow us to perform much more focused experiments in animals thus reducing the overall number of animals necessary for our research. We also endeavour to use the minimum number of animals in each experiment required to achieve meaningful data. Our genetically modified animals will also be bred using an efficient breeding strategy to minimise the number of mice used to obtain the desired genotype.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having	We have chosen to do this work in mice. These are the best suited for this project as they capture human disease relatively faithfully. In addition, there are a large number of models available to us, and there is compatibility with the commonly

<p>regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>used cell lines of mouse tumours.</p> <p>We will minimise the animal suffering by monitoring the growth of the tumour and ensuring it does not extend beyond recommended guidelines. The surgeries will be performed under published best practise guidelines, or where we have modified these to reduce suffering further. Preliminary studies show that the mice recover well after surgery and are fully active upon waking, however we will additionally monitor the mice even after this to look for any (unexpected) clinical signs that may develop within the first 24 hrs.</p>
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Project	Understanding the signals that regulate liver development, repair and cancer.	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The liver houses a network of tubes that moves bile from your liver into your intestines where it is needed to help digest food. There are lots of questions though around how these tubes are formed in the embryo as the liver grows and also how, following damage to these tubes in an adult, they regrow to ensure the liver continues to function normally.</p> <p>The first two aims of this license seek to address these questions. Cells do not just happen to form into ducts – it requires the cells to talk to each other and understand where they are and</p>	

	<p>importantly who their neighbours are – cells do this using special proteins. By understanding these proteins we can alter them to see if we can change the way a liver develops (which is important for patients who’s liver doesn’t develop in the right way, such as children who have a disease called Alagille Syndrome) or we can try and help the liver regrow in the adult following injury.</p> <p>Over the last five years, my lab has found that lots of the proteins that allow the liver to grow normally in the embryo or in the adult are used by liver cancers to help them grow. The final aim of this license is to see whether we can find out how these proteins help a liver cancer to grow and by working with other scientists and companies who develop medicines, figure out whether we can alter these proteins and what they do to slow down or stop liver cancers growing.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>There are an increasing number of people in the UK with liver disease and as a consequence of this there is an increasing number of people in the UK with liver cancer. The work we will do during this license will help us understand 1. How liver disease develops in adults 2. How liver disease can become cancer and finally, whether we can develop medicines with other universities and with companies that can help people with liver disease.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>In this study, we will use both mice (approximately 3000 over the five years) and rats (1000 over five years), some of which will be genetically modified. Most mice will be used for relatively short studies to understand how liver diseases progress and cancers form; however, some mice and the majority of rats will be used longer (for around 1 year) to understand how cancer grows.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>For 8 in 10 of the animals used in this study, most animals will experience moderate severity procedures and might lose 20% of their bodyweight; however for many of these animals they will not reach a 20% weight loss and will only experience modest loss of weight. Throughout, we will closely monitor animals and</p>

	<p>have developed a number of interventions that can support animals if they become unwell. For 2 in 10 animals During this license, 2.5% of animals (5 in every 200 animals) will be subject to a severe protocol (mainly rats) we will use a severe protocol, where animals will develop progressed and advanced liver cancer. These animals will experience weight loss and could progress to a point where their livers begin to fail, we will closely monitor animals for signs of this and mice will be provided with support. Following the study animals will be culled using a recognised procedure and we will study the livers and other tissues from these animals.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Organs are made up of lots of different types of cells and we want to understand how these cells talk to each other in disease and cancer. We do use human tissue and human cells wherever possible, and we have also developed ways of growing these cells in the lab so that they behave more like adult tissue. But a limit of this is still growing lots of different cell types together which are coordinated into a tissue. For this, we require animals and even lower organisms, such as flies, do not have the same level of tissue complexity that we find in mice and human.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We use minimal animal numbers throughout all experiments by performing calculations (known as power calculations) which predict how many animals we need. We also use the very best models available that mean each animal gives us essential information, with little wastage. Tissues from the animals we use in research are stored and kept for future analysis, meaning that we do not need to repeat the experiments to generate further tissue.</p> <p>A major limitation of using genetically modified animals in cancer research is that we must generate large numbers of mice that do not have the right genetic changes in order to breed enough to use in our studies. We have developed a new model, where we can use wild-type mice and change cancer genes directly in the liver without having to breed lots of mice. This has</p>

	<p>saved us having to breed ~250 mice in the last year that would not have been usable in our studies.</p> <p>One of the changes that we are beginning to make now is to use new small animal approaches to watch diseases form and cancers grow. These approaches are similar to MRI or PET imaging that are used in hospitals.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Over the course of our previous license we have tried to make the liver disease and liver cancer models we use the very best we can. We have introduced a high protein diet to mice and rats that have established cancer as we have found that this prevents some of the weight loss seen in these animals. We have also established a score to look at how animals' appearances have changed over the time of disease and cancer. This way we have a record of any changes and know what to look out for in future experiments.</p>

Project	Understanding Thymus Function and Immune Reconstitution	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The thymus is an essential organ of the immune system. The primary role of the thymus is to support the development of T cells that are act to provide immune protection against infection and tumour formation. In addition, the thymus plays a vital role in preventing the emergence and activity of T cells that would otherwise drive autoimmune disease. Despite the critical role of the thymus in ensuring protective immunity, the basic cellular and molecular interactions that control thymus development and function remain unclear. This project aims to investigate the identity of the cells and molecules that control T cell development	

	<p>within the thymus and understand how these impact upon T cell-dependent immune protection. These studies will inform further work where we will seek to use identified regulators of thymus function to enhance thymus activity and T cell driven immune protection in settings where this is defective.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Continued output of T cells from the thymus is required to establish and maintain a peripheral T cell pool of sufficient size and diversity necessary to provide effective protective immunity. In addition, correct thymus function is required to delete those T cells capable of inducing autoimmune disease. Thymus function however can be disrupted in a variety of settings, including: inherited genetic deficiencies, chronic age-associated loss of thymus tissue (thymus atrophy) and acute loss of thymus tissue in response to damage e.g. irradiation or infection. In all instances, loss of thymus activity leads to reduced T cell development and a corresponding increase in susceptibility to infection and potential for tumour formation. This project seeks to advance our fundamental scientific knowledge of the cells and molecules that control the development, maintenance and loss of thymus tissues. The identification of novel regulators of thymus function will provide potential routes that may allow manipulation of T cell development with the ultimate longterm goal of improving health and wellbeing through improved immune protection.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Approximately 18,000 mice will be required to breed and maintain colonies of mice, and perform the planned experiments over the five year duration of the project</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The majority of experiments will reach a sub-threshold level of severity which will involve the generation and breeding of genetically-modified mice for the isolation of tissues after they have been humanely killed. In some cases however, a moderate level of severity will be reached due to the bone marrow and tissue transplantation approaches used to precisely identify the cell types and molecules involved in thymus function. To assess the cells and signals involved in either</p>

	<p>loss or recovery of thymus tissues some animals may be subjected to low dose irradiation in order to induce acute (transient) loss of thymus and T cell development. Following loss and recovery of thymus tissues, it will be necessary to assess the function of the T cells generated by testing their ability to provide protective immunity. In some cases the T cells that develop may possess the potential to drive autoimmune disease when thymus function is defective. In all cases, animals will be carefully assessed and any suffering will be kept to a minimum. All mice will be killed humanely at the end of the protocol or should pre-defined humane end points be reached prior to the end of the protocol. In the course of these experiments, animals will also necessarily be subjected to injections, blood sampling and/or modification of their diet. Any adverse effects to animals will be minimised by ensuring the rigorous use of the most refined approaches by skilled staff</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>This project requires animals as the study of thymus function and T cell development must be examined in live animals due to the complex dynamics and cellular interactions involved. For example a key part of T cell development is not only the development of such cells in the thymus, but their subsequent export into the blood circulatory system and entry to peripheral tissues to provide immune protection. At present no in vitro experimental systems exist that allow this to be accurately reproduced in non-animal models</p> <p>We will conduct continued review of the scientific literature in order to identify any newly emerging models that may provide the opportunity to replace existing animal models wherever possible.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>Rigorous experimental design and use of statistical analyses will be performed to ensure that we are able to accurately and robustly generate meaningful experimental outputs whilst ensuring the minimum usage of animal numbers. We have extensive experience of breeding and using mouse models to investigate thymus</p>

	<p>function, and have published extensively in high-impact scientific journals. We will ensure that we use such existing experience and experimental frameworks to ensure the use of minimum numbers of animals. Further, in order to reduce the numbers of live animals undergoing experiments, we will use in vitro culture of thymus tissue within a petri dish to identify candidate molecules capable of influencing T cell development prior to performing experiments in live animals. Using such systems will help ensure that non-functional candidates do not progress to live animal models thereby keeping the number of animals undergoing such experiments to a minimum.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The mouse replicates the placental pattern of human development in a short (21 days) gestation time and provides an accurate model of the development of the human immune system, including thymus. It is also the only species that provides a range of natural and induced mutants with defined genetic alterations that allow the study of target molecules crucial to the function of the immune system. A large body of published data has been generated characterising the immune system in mouse models, and the vast majority of knowledge regarding thymus development and function is based on the use of murine models. Using genetically-modified mice we are able to precisely identify the role of specific cells and molecules and define their role in thymus function. The approaches described are established and every effort has been made to develop refined techniques causing minimal adverse side effects. An example of specific refinements include the development of new tissue transplantation approaches that involve the placing donor tissue under the skin of the ear. Such techniques can potentially reduce the use of more invasive transplantation approaches and may aid in reducing the risk of infection</p>

Project	Unravelling the molecular regulation of atypical cell division in malaria parasite	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Malaria is a major health problem that is especially prevalent in tropical/sub-tropical regions. The causative agent is <i>Plasmodium</i> , a single-celled parasite transmitted by mosquitoes. It multiplies both in humans and mosquitoes using two distinct modes of cell division regulated by highly unusual mechanisms that are quite different from those described in the textbook. One of these atypical divisions occurs in humans and is responsible for the symptoms of the disease, while the other takes place in the mosquito and is required for disease transmission. The project here will explore the mechanics and regulation	

	<p>of these unusual cell divisions</p> <p>(parasite proliferation) using rodent malaria model propagated and transmitted using mice or rats and mosquito as vector. This fundamental research will study in depth some unique protein molecules identified that can shed new light on their role most unusual aspect of cell biology. This research may identify new targets that could be exploited to kill the parasite and support the fight against malaria.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This is a basic research project that will unravel the regulation of atypical cell division process in malaria parasite using rodent malaria model. By following this we will have increased knowledge of the dynamics, mechanics and regulation of key parasite multiplication machinery genes in malaria parasite that can help us to identify the new intervention targets. It will use cutting edge live cell imaging, proteomics and genetic approaches to decipher the unique mechanism of cell proliferation and propagation of malaria parasite in mice and rats. The data generated during this project will provide novel information about how the some of molecules kinases and phosphatases protein molecules modulate the process of cell proliferation and multiplication in malaria parasite.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We plan to use 8000mice 200 rats during the course of five year period in this project licence</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>No adverse effects, exceeding the moderate severity limit, are expected in this work. Transient mild clinical symptoms of malaria (partial piloerection, reduced motility, transiently hunched posture, mild pallor), may be observed when the parasitemia reaches 20-30%. The animals will be monitored for parasitemia on a regular basis (usually once a day from day 2), with monitoring tailored to a particular experiment taking into consideration the parasitemia and the condition of the mouse. However if the limit is reached the mice or rat will be immediately terminated with Schedule 1.</p>

	<p>In most protocols the animal will be monitored for parasite load on a regular basis and parasite load will not be allowed to exceed 30%. Sometimes the antimalarial administered through drinking water may not be palatable to the rodents but these will not last longer than 1 hour. All such agents will be used at appropriate low concentrations that are well tolerated by mice. If a parasitemia greater than 30% (i.e. 30% of the total erythrocytes contain parasites) is detected or if the animal displays the following symptoms: A) marked (more than partial) piloerection, (B) subdued behaviour patterns even if provoked, (C) more than just transient hunched posture (D) pallor of eyes, nose, ears and foot pads, the experiment will be terminated and the animal will be killed by a Schedule I method or by exsanguination under terminal anaesthesia and the health record sheet for the rodent updated to record the symptom seen and action taken.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The use of rodent malaria model is required to investigate and enhance our understanding of role of particular malaria genes throughout the parasite life cycle in the in vivo context. It is the best robust genetically tractable system. This is crucial when we want fundamental knowledge on genes involved in cell division or polarity these can have function both in the mammalian and mosquito host. It provides the best model system for human malaria <i>P. falciparum</i> where only the asexual stages can be studied in in vitro culture system. More recently the sexual stages and their dynamics can be studied in the rodent model and we can get complete array of molecular, cellular, physiological interaction necessary to have complete understanding of parasite multiplication and how it interact and propagate in the host.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We will maximise the use of any material collected from rat or mice. We will draw upon the knowledge from in silico and in vitro studies and smallest number of mice or rat per batch will be used whilst still remaining statistically significant. Each experiment will use control to</p>

	<p>have proper validation of the data. Sometime many transgenic lines will be analysed with one control so that we can reduce the number of times the control is used and it also gives the power to the experiment.</p>
<p>3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The rodent malaria model <i>P berghei</i> shares many feature close to human malaria <i>P falciparum</i> especially the clinical symptoms of cerebral malaria. This offers the robust in vivo model for understanding and to produce data of significance for any future drug targets and drug screening programme. We very closely monitor the animals infected with parasites and new refinement are implemented on our ongoing experiences and new technological developments in the field. All of the animal work will have a moderate severity limit mice, mainly due to the possible effect of high parasitemia, although this is unlikely to be reached. If the animals do reach (or are likely to) these limits, the experiment will be terminated immediately. In most experiments the parasitemia will not be required to reach 30%, which will reduce the likelihood of malaria related symptoms. This will be monitored by smearing tail blood every day (2 days post infection) as well as monitoring the general health of the animals. Only in case of experiments involving mosquito bite back the parasite are checked after 3-4 days as it takes 48-72 hours incubation of the parasite injected by mosquito bite to develop blood stage infection.</p>

Project	Use of mechanical stimuli to enhance drug and vaccine delivery and therapy	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Many drugs and vaccines could be made more effective and safer if more of the delivered dose reached the target sites within the body. We have developed technology that will help push drugs into tumours so that they are treated more effectively or push vaccines across the skin so that they are more effective. This technology uses mechanical stimuli such as ultrasound, or the shockwaves generated by the type of machine used to treat kidney stones or magnetic force to achieve this pushing.</p> <p>We have shown this can work for a range of cancer drugs and some example vaccines but</p>	

	<p>there are still many classes of new drug and vaccine we need to test and improvements to make to our technology. Ultimately if more drug is delivered to where it needs to go we hope treatment will be more effective. Notably all the technologies we are developing (ultrasound, shockwaves, magnetic force) can be applied non-invasively i.e. without the need for surgery or in the case of vaccine delivery without the need for needles, making the treatments more acceptable to patients. There is also the possibility that combining the new technologies with the old methods of delivery (needles) may be even more effective so this will also be explored. In addition to cancer the work may help us address the perennial problem of flu as well as increasing our preparedness and response to emerging human corona viruses.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Applying a non-invasive, safe, low cost external stimulus, such as ultrasound, magnetism or shock waves similar to those used in treatment of kidney stones, to improve the movement of cancer drugs into tumours and vaccines across the skin will make these treatments more targeted and more effective. It may mean less drugs can be used lowering toxic side effects. It may mean that drugs that previously did not work because they did not previously reach their target can be used. This work could therefore potentially help in the treatment of cancer and the effectiveness of vaccines.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We expect to use 4975 mice over the next 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The systems we are developing are non-invasive and so although drugs will still be administered via their normal routes no surgical procedures are involved. The mice will never be exposed to levels of severity higher than moderate and in the majority of cases will be exposed to mild levels. To test whether anti-cancer agents are effective the mice will be implanted with tumour cells and to test whether they are protected by vaccination they will be exposed to pathogens, but the effects of their health will be closely monitored and controlled.</p>

	<p>We do need to use in-vaccinated control mice to compare the effectiveness of our approach against. These animals will experience some of the symptoms of flu (leading to weight loss) and will be closely monitored to ensure these do not become too severe (more than 15% weight loss), and we will endeavour to make these groups as small as is possible to still produce a scientifically valid result. Mice will also undergo anaesthesia, perhaps on several occasions, and this is associated with distress and aversion. Tumour growth in mice will on the vast majority of cases be subcutaneously and will not impeded welfare or movement. In a very small % of mice cancer cells may be injected intravenously and may grow to impede liver or lung function, this will be closely monitored. Mice will be killed at the end of the studies.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>We are currently working on developing model systems to replace animals. These involve growing cancer cells in a jelly like substance called agar. We have even put holes through these 'phantoms' to try and represent blood vessels so we can try and mimic intravenous delivery of drugs to the cells. These models help us reduce the parameter space for our subsequent animal studies but as yet they can not accurately reproduce the complexity of the real intratumoural environment, especially with respect to the suppression of the immune system which takes place within a tumour or the complexities of the entire immune system when trying to test vaccination. We have tried to use discarded pig skin from abattoirs for our transdermal work but the quality and supply of the samples is too variable.</p> <p>There are species such as zebra fish which have been used to characterise the genetic complexity and development of tumours but the routes of drug delivery that are feasible in fish (dissolution in the water, oral dosing) do not mimic the route we are interested in and the complexity of the tumours and the vasculature that feeds them does not provide a good mimic</p>

	of the mammalian situation.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Careful planning and design of our experiments and the use of statistical modelling will help us minimise the number of mice needed. Animal models and techniques will be used that allow information to be gathered from one mouse over a time-course and so a different mouse will not be needed at each time-point.</p> <p>Good practice such as randomisation (where animals are randomly assigned to treatment or control groups) and blinding (where the person taking the measurements is unaware of which group received which treatment) will be appropriately applied. This ensures valid reproducible results are obtained and animals are not wasted trying to reproduce flawed experiments. Furthermore, control groups will be kept to the minimum size required to provide significance as informed by pilot studies. Where appropriate studies will be combined to share control group. When the amount of delivery achieved is being measured several areas can be exposed on one mouse rather than using separate mice. We also hope that the passive cavitation methodology we are developing will allow confirmation of successful delivery without the need for killing of mice and sectioning tumours, once confirmed this will allow the same animal used in testing delivery to then be left for testing of efficacy, reducing the total needed.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Mice are the most suitable model in terms of creating tumours for our cancer work and modelling the immune system for our vaccination work. Tumour sizes will be prevented from exceeding a defined limit (1000mm³). Weight loss will not exceed 15% or more. Pain relief will be provided at site of injection with the application of cream which contains anaesthetic. Careful training of all staff and electronic record keeping of their competency ensures that the health and welfare of the mice is well regulated and recorded.</p> <p>Combining simple procedures during periods of anaesthesia, for example microchipping whilst</p>

	<p>anaesthetised for tumour cell implantation reduces the exposure to the anaesthetic and the number of times the mice experience unconsciousness.</p> <p>We hope to continue to develop our technology so that prototype lab based ultrasound transducers can be replaced with more clinically relevant ultrasound probes allowing a move away from exposure performed in a waterbath toward those using ultrasound gel. This will reduce the time taken for the procedure, reducing exposure to anaesthetic.</p>
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Project	Use of next generation sequencing to identify the role of zinc oxide within the gastrointestinal tract of pigs and in seeking out appropriate alternatives to support the health and performance of the young pig at weaning.	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim is to understand why pigs fed a diet containing higher than required levels of zinc oxide (ZnO; 3.1 g/kg feed), have better growth and are healthier than pigs fed lower levels of ZnO (~0.15 g/kg feed), immediately after they are weaned. It is thought that the main influence of	

	<p>ZnO is within the gastrointestinal tract of the pig, but it is currently unknown exactly what this effect is and how ZnO is leading to the improvements seen in the pigs. The aim will be fulfilled by sampling pigs at numerous time points from weaning, to 28 days post-weaning, as this is considered the most critical time in a pig's life. Gut digest, tissue samples, blood samples, rectal swabs and rectal temperature will all be taken to allow for several analysis techniques to provide a greater chance of identifying the mode of action of ZnO.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The current use of high levels of ZnO has reduced diarrhoea in pigs after weaning, and improved their growth performance. However, ZnO has provided concern as a result of high levels of Zinc, found in slurry, causing environmental problems. As a result, the EU has placed a phasing out ban of the current use of ZnO in weaner pig diets, by 2022. Currently, the mechanism by which ZnO improves health and performance of pigs is unknown, and has made identification of successful, more sustainable alternatives, problematic. Identification of alternatives before the ban is essential to prevent a sudden decrease in growth performance and an increased incidence of diarrhoea and ill-health in newly weaned pigs. This would impact on the majority of the EU pig industry.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Approximately 600 production pigs would be used under the entire licence (across maximum of 5 years). This would allow for a maximum of 200 pigs to be used in 3 separate trials under licence.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Within each trial run under this project licence, blood samples will be taken from ~176 pigs at any one occasion. Approximately 96 pigs will be sampled from a maximum of 5 times. No adverse effects are expected to be seen from these pigs as this is considered mild level of severity. Rectal swabs will be used to collect faecal samples as this is the most efficient method of collection. Samples will be taken on 11 separate occasions, with ~96 pigs sampled on all occasions. This will be completed quickly, with no adverse effects expected. Alongside the first 8 rectal sampling points, rectal temperature will also</p>

	<p>be recorded from the piglets, this will not cause any adverse effects and is considered mild in severity. Approximately 72 pigs within each trial will be euthanized using Schedule 1 methods and used for dissection. This will leave ~96 pigs after 28 days post-weaning, to progress through the standard commercial farm before going to market as normal.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The programme of work aims to determine the use of zinc oxide in pig diets and therefore the only species appropriate for this line of research is pigs.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Given 32 pigs will be euthanized at D14 and D28 of the trial, and allowing for some pigs to be removed from the trial if treatment is required due to ill-health, all pigs will be sampled from at each time point. This will be a maximum of 5 times for blood sampling and 11 for rectal samples. The same pigs are required to be followed through given significant differences seen between pigs, and to assess long-term effects within the pig gastrointestinal tract and on their immune system response.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>This research has the overall aim to benefit the pig industry and its current high use of ZnO, therefore, for results to be most applicable to the industry, work has to be carried out using commercial production pigs.</p> <p>Blood samples will be taken by a trained technician to reduce any pain and suffering. Rectal swabs allow for limited disturbance to the pigs as it minimises contact time. Daily checks will be carried out on all pigs within the trial, and where required, pigs will be removed from the trial if medication or euthanasia is needed.</p>

Project	Using in vitro generated cells to improve haematological disorders
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research <input checked="" type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Blood Services in the UK provide life saving products for patients with blood disorders. However, it can be difficult to get enough donations for patients with rare blood groups who require blood transfusions at regular intervals. If these patients receive donor blood that does not fully match their own blood type, they are at risk of having a severe reaction. The ability to grow blood cells from stem cells in the laboratory offers a solution to this problem. We plan to investigate blood cells grown in the laboratory to test whether they can function as well as normal blood cells when transfused. We

	will also investigate whether it is possible to use blood cell proteins make individuals more tolerant to donated blood and consequently and whether we can use these cells to reduce the amount of harmful reactions in patients whose lives depend on receiving regular blood transfusions.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will allow us to test the safety and effectiveness of using culture blood, which is an essential step in progressing this treatment into a clinical setting. It will also enable us to test strategies to avoid the harmful side effects that occur in patients who receive regular blood transfusions. For patients with blood cancer, increasing the number of stem cells transplanted should result in replacement of malignant cells with normal blood cells and could improve survival and quality of life for these patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mouse strains that are suitable for studying human blood cells. The proposed work is expected to use around 2500 mice, including those used for breeding, over the 5 years of the project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The vast majority of animals used in these studies are not expected to experience any adverse effects beyond that incurred by giving an injection. In some studies, drugs will be used that may cause short term side effects, such as lethargy and/or lack of appetite, in some animals. These animals will be carefully monitored and should the adverse effect persist or exceed those expected the animal will be humanely killed. In very rare cases, animals could die following drug administration. This can be avoided by using mature adult animals. At the end of the study, the animals will be killed in order to collect tissue samples to confirm the effectiveness and safety of the treatment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use	In order for new developments in treatment to be adopted into clinical practice it is essential to first demonstrate that they are safe and effective using animals. Although studying blood cells in culture can provide a lot of useful information it is

<p>non-animal alternatives</p>	<p>only possible to determine how long they will survive after transfusion and whether they continue to mature into fully functioning blood cells by testing them in animal models and tracking their survival using surface markers that are specific to human red blood cells.</p> <p>Likewise, it is not possible to test if an individual has become more tolerant to red blood cell proteins using cell culture systems only. Studies in live animals are required.</p> <p>Likewise, it is not possible to test if an individual has become more tolerant to red blood cell proteins using just cell culture systems. Studies in live animals are required.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Our experiments have been designed to use the smallest number of animals needed. Much of our work is undertaken in the laboratory to generate cells that can be used for blood transfusion and stem cell transplantation. These laboratory-based experiments will provide essential information as to the suitability of the expanded cells and the best techniques to generate them. Only blood cells that can expand and start to mature in the cell culture systems will be used in animal studies, thereby significantly reducing the number of animals used.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>We will use mouse strains that are the suitable for studies of human blood cells. These strains do not recognise human cells as foreign and do not react to the human cells infused mice have an underactive immune system, so they do not reject human cells. The animals will be kept in a high health status environment to ensure that they remain healthy throughout the study. Only adult animals will be used for studies involving drug administration. Good experimental techniques by experienced staff will ensure that any stress is minimised. All cells and drugs used in the study will be prepared under aseptic conditions by expert staff and experimental studies are designed to run for the shortest possible time.</p>

Project	Using larval zebrafish to study nervous system development and function in health and disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Our Objectives are to use imaging of neural activity in the brain of larval zebrafish to:</p> <ol style="list-style-type: none"> 1. To understand how the visual brain works 2. To understand how genes and environment work together to shape how the brain develops 3. To how seizures develop in the brain. 	
What are the potential benefits likely to derive from this project	<ol style="list-style-type: none"> 1. Our research will provide insight into how the normal brain develops and how, once 	

(how science could be advanced or humans or animals could benefit from the project)?	established, neural circuits work. Understanding these processes in the healthy brain is an essential prerequisite for understanding how they are perturbed in the diseased brain. 2. Our research will also focus on how disruption of genes implicated in epilepsy cause seizures in the brain. This work will help us develop new treatments for epilepsy
What species and approximate numbers of animals do you expect to use over what period of time?	Over five years: Zebrafish: 10,250 Adults, 10,000 of which are used solely for the production of embryos which will be used in experiments. 24000 larvae.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For the vast majority of zebrafish they will live a normal life within the animal facility with no adverse effects and be used for breeding until they are humanely killed around 18 months of age. The vast majority of experiments will be performed on zebrafish larvae and are classified as mild i.e. they will not cause pain or distress.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Our aim is to understand how the brain develops and how it functions. We are also studying what happens to the brain when it develops abnormally- as a result of epilepsy, for example. Because the brain is so complex we have no alternative to studying it in intact animals</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Because the experiments we perform do not harm the animal we can perform repeated experiments on the same animal rather than having to use lots of different animals. In this way we dramatically reduce the number of animals we use in our research.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Most of our experiments will be performed on zebrafish. Because fish are small and transparent we can image the intact brain without the need for any surgery. Thus, our main experimental approach causes no pain or distress to the fish. Also the larval fish brain contains only 100,000 neurons (compared to the 80 million neurons in the mouse brain). Thus,</p>

	larval zebrafish are less sentient than mammals.
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Project	Using zebrafish to study neuroinflammation	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Leukodystrophies are severe neurological diseases affecting the brains of children. This proposal studies a disease called “ribonuclease T2 (RNaseT2)-deficient leukodystrophy”, which causes abnormalities on brain scans of affected children. There is little understanding of the mechanisms of the disease and no treatment is available.</p> <p>We published the first <i>maset2</i>-deficient model in zebrafish which mimics the human disease and develops brain abnormalities, like patients. Using our zebrafish mutant, I will study how those brain abnormalities appear, including the role of the immune cells of the brain and I will test new</p>	

	therapies.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will characterise the involvement of immune cells in the development of a childhood neurological disease. This will increase our understanding of the disease and will identify exactly which cells are deficient. This will help us to develop new therapy with potential use in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Mutant and transgenic fish are generated, which are used to generate the next generation, used almost exclusively for non-regulated procedures. These experiments will involve imaging immune cells in real-time. In order to see how immune cells function, they need to be in their natural environment. This cannot be established in vitro. The proposed study uses larval zebrafish rather than adult mice. The number of zebrafish used is determined primarily by the number of breeding adults required to supply the unprotected larvae for the studies suggested. 25,050 adults will be required over the course of the 5 year programme of work, and 16000 of these will all be healthy, and used for mating purposes only – no suffering is anticipated. 50 will be used to develop live MRI scanning and 9000 are anticipated to suffer from moderate neurological disorders and therefore undergo therapeutic interventions during larval stage.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most animals will be under a mild severity protocol. Some animals will be under moderate severity to accommodate for swimming deficit that some neurological mutant might develop. Our therapeutic regimes will endure to reduce those moderate symptoms for the fish to return to full health. Animals will be sacrificed before they suffer at the end of their natural lifespan, or earlier if indicated.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Brain development and inflammation is a complex process requiring interaction of multiple cell types and three dimensional tissue changes. This cannot meaningfully be modelled in vitro. Most experiments are performed on larval zebrafish under the age of

	<p>protection and a few experiments will use adult fish. Alternative models include mouse and rats, but these are of higher neurophysiological sensitivity than the zebrafish and do not have the advantages that the zebrafish can offer, such as ease of imaging and drug treatment. The zebrafish is a very suitable model for the experimental work we wish to perform.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Most experiments are performed on larval zebrafish. Therefore we have planned to keep sufficient numbers of adult fish to ensure a steady supply of embryos for our experimental work. The numbers of adults are under constant review to ensure that they meet this demand but not exceed it. The mature fish in this programme will be used multiple times to assess brain integrity using live MRI scans throughout their life. This will avoid having to kill a fish each time we want to assess their brain. We have extensive experience of the assays used, and are confident of our calculations of the minimum number of fish required.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Zebrafish are the model with the lowest neurophysiological sensitivity suitable to study a whole brain (a vertebrate immune system is sufficiently similar to humans to be useful, but insect or worm immune systems are not). It breeds well in captivity and is kept to the highest standard of welfare in our aquaria, with daily checks on fish health and water quality. All new experiments require an Individual Study Plan, discussed with the Named Animal Care and Welfare Officer, who will advise on refinements. We will aim to refine experimental design wherever possible and are routinely using anaesthesia to minimise harms.</p>

Project	Vaccine research studies in wildlife against Mycobacterium bovis	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	4 Years 5 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
	<input checked="" type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The British Government is aiming to achieve Officially Bovine Tuberculosis Free (OTF) status for England and Wales by 2038. The epidemiology of bovine tuberculosis (TB) is complex. It is caused by infection with Mycobacterium bovis bacteria and there is transmission of this infection between cattle, but transmission also occurs from wildlife to cattle and vice versa. Vaccination of wildlife against TB is considered as an option to reduce and control the disease alongside other measures such as vaccination of cattle and more efficient testing regimes. The objectives of this project is to	

	<p>develop a safe and effective oral vaccine against TB in wildlife and generate data suitable for submission to the Regulatory Authority to licence it for use. In addition it is to check that fertility control vaccines do not interfere with the BCG immunity The vaccine to be used is BCG, an attenuated form of M. bovis, which stimulates immunity but does not cause disease. BCG is used to vaccinate people against TB. The bait vaccine must be shown to be safe for wildlife species intended and other species that might come in contact with it, including people. In this project, bait with and without BCG will be developed, optimised and tested with the relevant wildlife species to achieve these requirements. Specifically the project will: 1) Confirm the palatability of baits to wild life, particularly during the scaling up process for production. 2) Determine the dose of oral vaccines necessary to protect wildlife. 3) Generate data to prove the safety of the final oral vaccine candidate. 4) Potential effects of fertility control vaccines on BCG immunity 5) Duration of Immunological response to BCG</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>Oral vaccination of wildlife against TB is a will contribute to the reduction and control of TB in cattle alongside other control measures, but also the reduction and control of TB in wildlife with the associated reduction of suffering in wildlife, who unlike cattle which are regular monitored for the disease, TB infection in wildlife leads to uncontrolled disease and death. Oral delivery of a vaccine in a bait holds the best prospect for vaccinating wildlife over a wide geographical area and has proved highly successful for mass vaccination of other wildlife species against rabies. In the short to medium term, the TB vaccine (BCG) represents the best available option for the vaccination. Additional work is to be done looking at the potential for injectable fertility control vaccines, which are used to reduce wild life human conflicts due to excessive numbers of wildlife in areas, to interfere with the protective effects of BCG vaccination .</p>
<p>What species and approximate</p>	<p>Approximately 150 wild animals will be used in</p>

<p>numbers of animals do you expect to use over what period of time?</p>	<p>total, over the 5 years of this licence.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The severity limit for the work is moderate, with the most likely adverse effects associated with general anaesthesia, which is necessary in order to undertake procedures on wild animals safely during the course of the study. The number of anaesthetic procedures will be minimised in this project and anaesthesia only used where data cannot be obtained by other means (e.g. using remote video surveillance). Initially there is a need to sample the animals to ensure they are suitable for the trial (not infected with TB already) and there are experiments to confirm the palatability of baits during the scaling up process for production. Subsequently animals will be used in work to establish the dose of the vaccine that will provide protection. As well as vaccination this experiment will involve infectious challenge. However, the slow progressive nature of TB infection means that 12 weeks after infection the animals can be humanely killed before any clinical signs develop and the experiment still meet its scientific objective of testing the efficacy of the vaccine. The final part of the experiment is a safety study which involved a larger dose of the vaccine than normal to ensure it does not generate any side effects. BCG is a very safe vaccine so no adverse effects are expected, but the experiment has to be done as there is not data for oral bait in wildlife.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The main requirement is to generate data for consideration by the Veterinary Medicines Directorate VMD for the granting of a licence for an oral TB vaccine for wildlife and for this purpose, no alternative to using wildlife is available. It is not possible to optimise bait or vaccine formulation in a surrogate species, as bait preference is peculiar to the species under evaluation, as is the response to vaccination.</p>
<p>2. Reduction Explain how you will assure the</p>	<p>Professional statistical advice to ensure that the minimum number of animals are used to generate sound and valid data. The protocol for each study</p>

<p>use of minimum numbers of animals</p>	<p>is scrutinised in the context of the whole R&D programme in order to focus on the most relevant questions to answer.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>As the principle aim of this programme of work is to generate data for consideration by the VMD for the granting of a licence for an oral wildlife vaccine, there is no alternative than to perform studies in the target species. The majority of data for bait development will be collected without direct intervention (using CCTV footage obtained at night under infrared illumination). For vaccine studies, as well as intramuscular and subcutaneous dosing, there is also oral consumption. We prefer to allow conscious animals to consume the bait voluntarily. If intervention is required to achieve the aims of the experiment, small volumes of the vaccine are placed directly into the mouth while the animal is under general anaesthesia. Experimental infection with <i>M. bovis</i> is not expected to cause overt signs of disease during the 12 week interval between challenge and necropsy. The infection model was developed prior to 2005 and has successfully been used for previous vaccine studies in wildlife. Since animals have to be anaesthetised for handling, care is taken to arrange procedures to minimise the number of anaesthetic events.</p>

Project	Viral and non-viral gene therapy	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>We are developing novel treatments for a range of diseases that currently have insufficient treatment options and are affected by high treatment burden and/or pre-mature mortality. These include cystic fibrosis (CF), alveolar proteinosis, thrombotic thrombocytopenic purpura and haemophilia.</p> <p>Over the last 20 years we have developed gene therapy for cystic fibrosis and have recently completed the world's largest clinical trial which showed that gene therapy can stabilise CF lung disease. However, we have to further improve the efficiency before gene therapy can be licenced as CF drug.</p>	

	<p>As part of the CF programme we have shown that gene transfer to the lungs and nose of mice can lead to protein release from the lungs and nose into the blood. This data opens the possibility of using the lungs as factories for proteins required in the blood.</p> <p>For example we envisage to produce FVIII, a protein that is lacking in haemophilia patients, in the lung.</p> <p>Importantly treatments for haemophilia are currently suboptimal and many patients don't have access to regular treatments.</p> <p>Furthermore, we aim to produce ADAMTS13, lacking or deficient in TTP individuals, in the lung of acquired TTP mice or ADAMTS13 knockout mice. Currently TTP individuals have a high treatment burden and rely completely on donor plasma which is associated with high morbidity.</p> <p>We have also shown that lung gene transfer leads to production of secreted protein in the lung. This data opens the possibility to apply our gene transfer vectors to a wide range of other lung disease including pulmonary alveolar proteinosis (PAP). PAP patients suffer from lipid accumulation in the lung and currently no good treatment options exist.</p> <p>In addition to applying the gene transfer agents directly to the lung we will also assess if cells that have been gene corrected outside the body can be reimplanted into the lung and lead to efficient production of therapeutically relevant proteins.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The development of new therapies may significantly improve survival and quality of life in patients with cystic fibrosis, haemophilia, TTP and pulmonary alveolar proteinosis</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mice and rats. We predict to use approximately 2500 mice or rats per year, but actual numbers will vary depending on the phase and the success of the individual projects.</p>

<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The vast majority of our procedures are mild (>90%) and will not cause severe adverse effects in the animal. The cystic fibrosis knockout mouse can suffer from intestinal problems, although we will use "gut-corrected" CF mice that do not suffer from intestinal disease. Some mice may be treated with pathogens known to cause lung diseases (eg <i>Pseudomonas aeruginosa</i> which is a common pathogen in cystic fibrosis patients). Some of our infection models may suffer from lung inflammation which may lead to adverse events such as weight loss and reduced mobility which will be carefully monitored and minimised wherever possible by using the lowest suitable bacteria dose. To assess the efficiency of new treatments for haemophilia it is important to quantify whether blood clotting time is reduced after gene therapy. The assay can only be done in living mice, because it requires complex interaction of many proteins involved in blood clotting which cannot be mimicked in other models. Haemophilia mice suffer from prolonged bleeding time. Adverse effects may include prolonged blood loss. This will be mitigated by quantifying blood loss and terminating the experiment if blood loss reaches a defined threshold. The mouse model for pulmonary alveolar proteinosis (PAP) has near normal life expectancy, but shows lipid accumulation in lungs, but not in other organs. We do not expect any significant side effects in this model. Treatment of PAP with a gene transfer vector may exacerbate the symptoms, but as described above this will be carefully monitored and acted upon appropriately. Procedures will be carried out using non-recover anaesthesia or allow animals to recover from anaesthesia as appropriate. We have no direct experience yet using a tail tip assay to monitor blood clotting time in haemophilia mice, but have and will further seek advice from collaborators. Based on available information we expect that an effective blood clot will form and bleeding will recede within 10 min. However, until we have gained more experience we will perform these procedures under general anaesthesia without recovery. At the end of the experiments</p>
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	<p>animals will be humanely killed. To assess the efficacy of gene therapy for TTP, a living animal must be used to understand the complex interplay of proteins and cell types contributing to haemostasis and thrombus formation. ADAMTS13 knockout mice and acquired TTP mice are healthy and have normal clinical symptoms, except for when challenged with ultra-large VWF which induces TTP-like symptoms in the mice. These mice display changes in behaviour and a range of haematological symptoms which can be detected through blood sampling, histological analysis and observations. Successful treatment will prevent onset of these symptoms and restore ADAMTS13 expression. According to literature, mice will revert to normal behaviour and clinical symptoms within 3-14 days following TTP induction. Following TTP induction, mice may exhibit severe symptoms, despite not being reported previously, thus animals will be monitored twice daily for 14 days or until mice return to normal behaviour and humanely euthanized in the event of severe symptom presentation.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Over the years we have learnt that the efficacy of gene transfer to the lungs can only be assessed in an intact organism because extra- and intracellular barriers and importantly inflammatory and immune responses cannot be reliably mimicked in cell culture systems despite our extensive attempts. However, we have access to several human lung ex vivo models including freshly extracted airway cells and cells grown at an air liquid interface which we utilise in parallel to working with mice. We are also aware of a more recent model, which involves growing airway cells and blood vessel cells on smallchips which we may be able to utilise when more widely available.</p> <p>In ADAMTS13 knockout mice, there are no clinical symptoms unless the disease is induced through administration of ultra-large VWF protein. This stimulus is required to trigger TTP pathogenesis which closely</p>

	<p>recapitulates how TTP individuals need a second vascular insult to trigger an acute TTP episode. These acute clinical symptoms cannot be reproduced in an <i>in vitro</i> model, however prior to using mice, gene transfer will be established in <i>in vitro</i> and <i>ex vivo</i> ALI models.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We use inbred strains wherever possible as inbred animals are effectively identical which reduces experimental variability and minimises numbers of animals used. We seek statistical advice to ensure experiments are appropriately designed to achieve research objectives with the minimum number of animals. We will breed “on demand” to avoid over-breeding. To reduce bias and confounding factors animals will be randomly assigned to treatment and control groups at the start of an experiment. In addition, wherever possible, the person conducting the measurements will be “blinded” i.e will not know what treatment the animals has received.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>There is no natural animal model for CF, but a mouse model has been generated using standard gene knockout technologies. The cystic fibrosis knockout mouse can suffer from intestinal problems. However, we will use a “gut-corrected” CF knockout mouse which has the CF ion transport abnormalities in the nose, but is otherwise healthy and normal. The mice do not have the characteristic CF gut disease, are not runt and do not suffer from increased mortality.</p> <p>There is a naturally occurring haemophilia A dog model, but this model is unsuitable for early phase research. Haemophilia knockout mice are available and will be used for part of this research.</p> <p>ADAMTS13 knockout mice are available with a range of genetic backgrounds. For this investigation the mildest genetic background and method for TTP induction has been selected in order to reduce suffering whilst still being able to gain meaningful and clinically relevant data. Mice will be humanely killed if, or as soon as treatment is seen to be ineffective</p>

	<p>in order to reduce unnecessary suffering.</p> <p>Wherever possible we will use non-genetically modified mice.</p> <p>Animals will be closely monitored following procedures and killed immediately using a humane method if undue suffering is likely and cannot be prevented by veterinary intervention. Procedures will be carried out under anaesthesia wherever feasible.</p> <p>Wherever possible, we will be using in vivo bioluminescent imaging, a technique that allows repeated assessment on gene transfer in the same animal over time. This will reduce the number of mice required.</p>
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Project	Viral vaccines (research)	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective is to make the materials and perform tests to be able to assess the quality and effectiveness of new and existing biological products, such as vaccines. These tests are essential to ensure the vaccines are safe and effective before being administered to humans.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	One part of the work will be to develop tests that can replace the use of animals and in one case a test in rats that could replace a test currently performed in primates. Other tests could lead to improvements in vaccines so that are better at preventing disease and help to make vaccines for new diseases or for existing diseases that do not currently have effective vaccines. Safety tests of viruses used in	

	<p>biological products could improve their safety or potentially reduce the time taken to make urgently needed vaccines. The consequences of using a vaccine of low potency or inappropriate strain are that it will fail to protect recipients and disease burden in the human population could increase. Serological surveillance of the population is dependent upon robust assays, those used in Rubella diagnostics have evolved faster than the qualifying standards. A better understanding of the interpretation of the assays available will ensure a safe balance preventing unnecessary vaccination or the loss of immunity against this pathogen in the human population.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>Mouse 4700 Rat 7000 Rabbit 100 Ferret 550 Chicken 20 over 5 years. Mice are used because they make good immune responses to many test materials and there are a large range of commercially available materials to analyse the responses. Rats, chickens and for some tests ferrets are used because it is a regulatory requirement to use a particular animal for that test. Rabbits are used if a large quantity of serum is needed to make material for an in vitro test. Ferrets are used for influenza tests because the immune response developed and the illness they experience both closely resemble those seen in humans.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Animals may be injected with substances by the intraperitoneal, intramuscular, subcutaneous, intradermal or intravenous routes, or dosed with substances by the intranasal route. Newly born rats will be injected intracranially with mumps viruses. Some animals will have a microchip implanted under the skin for the purpose of identification and to monitor temperature. Samples may be collected e.g. blood, nasal washings, or mouth or eye swabs. Injection and dosing procedures, microchip implantation, and sample collection procedures are expected to cause no more than mild and transient discomfort. Where appropriate anaesthesia is provided to limit distress. Repeated anaesthesia may be given to immobilise animals for non-invasive procedures e.g.</p>

	<p>imaging. For animals being immunised there may be some local irritation at the site of inoculations particularly where adjuvants are used. Any animal showing signs of adverse effects as a result of the regulated procedures will be humanely killed unless there is a rapid return to normal using no more than minor medical treatment. Some animals will be infected with influenza viruses and will experience influenza like illness. If possible, animals that become ill will be treated with medicines to alleviate symptoms according to a regime recommended by the vet. Ferrets and mice infected with virulent influenza may become seriously ill, experiencing weight loss and impaired movement and may be at risk of death from the disease unless there is prompt intervention. Where possible animals will be treated with anti-viral medicines to prevent development of serious illness. The outcome of these infections can be unpredictable and so animals will be monitored very closely by experienced staff with knowledge of humane end points. Rubella is not thought to generate adverse effects in mice. Mice infected with related viruses have been known to develop arthritis 6-8 days post infection. Injections at the sites chosen for this work will not lead to arthritis; however, mice will be monitored for signs of distress suggesting these complications. Any animal that has any significant adverse effect will be humanely killed using an overdose of anaesthetic. All animals used under this licence will be humanely killed at the end of the study, or before if it is necessary for the welfare of the animal.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The data concern the immune response and, in some cases, the protective immune response to viruses and vaccines and the pathogenesis of disease, which cannot be generated without the use of protected animals.</p> <p>In some cases, data will be generated to validate <i>in vitro</i> assays with a view to</p>

	eliminating the use of protected species.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>The types of experiment proposed have been conducted for many years and the numbers of animals required in each approach to give a successful outcome are well established by experience. Where appropriate, statistical input is sought on animal experiments so that the numbers of animals used are the minimum needed to produce statistically reliable results. Sometimes the numbers used are based on regulatory requirements, for example to test a vaccines strength or for safety tests</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Ferrets are among the few animals other than primates whose response to infection with influenza reflects that of humans; both the immune response and the clinical signs closely resemble that seen in humans. Methods for observation of clinical signs have been developed for recognition of onset of disease allowing earlier intervention with the use of anti-viral drugs or medication to relieve symptoms or to identify and humanely kill animals before progression to severe disease states.</p> <p>It is recognised that group housing is preferable for optimum well-being of ferrets and wherever possible they will be group housed. There are situations where single housing is required due to husbandry needs or for safety reasons. In these situations wherever possible animals will be housed in cages in rooms with other ferrets.</p> <p>Best husbandry practices will be employed to reduce the possibility of rejection of the rat pups by their mothers. Mothers and pups will be closely observed following injections and any rejected pup will be humanely killed immediately</p> <p>Anaesthetics will be used for procedures where there is potential to cause pain or distress to an animal</p> <p>Immunisation with adjuvants suitable for use in humans will be used. Freund's adjuvants will no longer be included for use under this</p>

	licence. Studies under previous versions of this licence have established that alternative adjuvants are at least as effective in achieving the required outcomes.
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Project	VIRUS HOST INTERACTION STUDIES FOR CONTROL OF AVIAN TUMOUR DISEASES	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	The broad purpose of this project license application is to gain better understanding of the mechanisms of diseases characterized predominantly by cancer of different cell types, caused by a group of pathogens, commonly referred to as cancer-causing or oncogenic viruses, which include Marek's disease virus, avian leukosis virus and reticuloendotheliosis virus.	
Why is it important to undertake this work?	These cancers are complex diseases involving multiple steps and factors, and there are many unknown causes and steps through which cancer develops. Because of the complexity and multisystem involvement of these diseases, there are no in vitro models. Hence these studies can only be conducted in experimentally-infected birds and are very important to	

	develop better intervention strategies to control and prevent cancer in chickens.
What outputs do you think you will see at the end of this project?	This application is part of the overall research aimed at understanding the molecular mechanisms of cancer induced by these important group of viruses, particularly on supporting our laboratory based research on specific virus-host interactions associated with pathogenicity. The major output from this project will be advancement of scientific knowledge disseminated through scientific publications, research output in the form of new diagnostics and intervention strategies including new vaccines and eradication procedures for more effective control.
Who or what will benefit from these outputs, and how?	<p>Avian oncogenic viruses are associated with huge economic losses and major welfare problems in the poultry industry. Diseases such as MD have a worldwide distribution and are reported to cause economic losses of US\$ 2.0 billion annually. MD vaccination today is almost a routine practice of the poultry industry in most parts of the world, without which it is impossible to maintain healthy poultry production. Although vaccines are still valuable in preventing losses from MD, increasing virulence of MDV isolates remains a major threat. We demonstrated that HVT vaccines helped the spread of more virulent strains thereby providing opportunities for evolution of virulence (2). Our recent success with the CRISPR/Cas9-based gene editing of the MDV-transformed cell lines has given immense opportunities to investigate the role of virus-host interactions in situ in these cell lines and eventually in vivo. In addition, gene editing approaches that we have developed could help innovations in multivalent vaccine development to offer simultaneous protection to multiple avian diseases. Some of our current collaborative projects with the leading poultry vaccine industry could also help in faster translation of our research findings to the field. Similarly, recombinant viral vector-based immunoprophylaxis will be a novel approach against major avian diseases.</p> <p>Similarly, other tumour diseases such as avian leukosis and reticuloendotheliosis also remain major threats to the poultry production in many countries. The sudden emergence and spread of the new subgroup J associated with myeloid leukosis, and the continuing re-emergence of antigenic variants associated with syndromes such as haemangioma in both broiler and layer flocks are examples of great concern. Hence continuing research is essential to understand the molecular virus-host interactions of these viruses, as it will help to maintain expertise and develop novel control</p>

	<p>strategies. In addition, our recent success with <i>in vitro</i> induction of genetic resistance in DF-1 cells (12, 13), an innovative approach for <i>de novo</i> generation of chickens with induced genetic resistance to different ALV subgroups. The new Licence will give us the opportunity to test such birds for genetic resistance to infection.</p> <p>Summary of benefits</p> <p>1. In the short-term, the licence will give the opportunity to understand the complex virus-host interactions involved in these virus-induced cancers. Advances in molecular tools for global analysis of gene expression will allow us to gain significant insights into the molecular cancer pathways, pathogenic determinants and mechanisms of diseases caused by oncogenic viruses. This will benefit researchers from biosciences by increasing understanding of tumour pathogenesis and developing innovative approaches in disease control.</p> <p>2. In the medium-term, the licence is essential to the development of next generation MD vaccines to curb the continuing increase in virulence and emergence of hypervirulent pathotypes, which are threatening the sustainability of the control strategies. Similarly, development of novel multivalent recombinant vaccines that can simultaneously protect against a number of avian viral pathogens will be a very valuable benefit to a number of stakeholders of the poultry industry as well as the vaccine manufacturers. Improved control of diseases by avian oncogenic viruses will help improving animal welfare and important for global food security.</p> <p>3. In the long-term, research towards developing <i>de novo</i> genetic resistance to diseases such as avian leukosis, where vaccine-based control methods do not exist, will have immense benefit to the industry in the fight against some of these devastating diseases, particularly in ODA countries where the economic losses from such diseases are very high.</p>
<p>Will this work be offered as a service to others?</p>	<p>No</p>
<p>How will you look to maximise the outputs of this work?</p>	<p>Our research group has a long standing close interaction with different stakeholders of the poultry industry including breeding companies and vaccine manufacturers. For</p>

	<p>example, we work with major poultry breeding companies and have helped in controlling these group of diseases. We also work closely with all the major poultry vaccine manufacturers. This would allow us to translate the research findings for commercial applications. REDACTED</p>
<p>Explain why you are using these types of animals and your choice of life stages.</p>	<p>Poultry species such as chickens, turkeys and quails are the natural hosts of avian oncogenic viruses. Natural infections by these viruses occurs to embryos and neonates. In order to get the detailed output of virus-host interactions and vaccine responses, it is important that we use these poultry species and the embryos or neonates. Also these viruses do not infect any other animal species.</p>
<p>Typically, what will be done to an animal used in your project?</p>	<p>At the end of the experiment (usually 8 weeks after infection), birds will be killed by a Schedule 1 method or alternatively, birds may have their necks dislocated, and will then be exsanguinated via decapitation (regulated procedure)</p>
<p>What are the expected impacts and/or adverse effects for the animals during your project?</p>	<p>Animals included in this project will be subjected to experimental infections by oncogenic viruses. As naturally occurring endemic diseases in many countries including the UK, clinical diseases in these experimentally-infected birds are similar to those occurring in natural infections. Although small proportions of birds may suffer from clinical disease with moderate severity, most of the animals suffer from a mild chronic disease with weight loss, reduction in appetite and tumours. With most experiments of an 8-week duration, some of the animals may not have developed any symptoms at all.</p>
<p>What are the expected severities and the proportion of animals in each category (per animal type)?</p>	<p>Maximum severity of the protocols in this applications is moderate. This level of severity is reached only in birds infected with acutely transforming retroviruses or very virulent MDV pathotypes. In experiments with these viruses, most of the animals will reach moderate levels of severity. However, the robust clinical scoring methods and frequent inspections will help in majority of these birds not going to the maximum severity but humanely killed by a schedule 1 method.</p> <p>Infection with less virulent MDV and non-acute retroviruses, most birds will experience mild chronic disease.</p>
<p>What will happen to animals at the end of this project?</p>	<p>killed</p>

<p>Why do you need to use animals to achieve the aim of your project?</p>	<p>Diseases caused by these group of oncogenic viruses are exclusively seen in poultry. The oncogenic process and tumour formation are very complex events with the involvement of network of multiple genes that are associated with tumour suppressor functions, activation, signal transduction, immune checkpoint modulation etc. Because of the complex nature of oncogenesis, it is difficult to have suitable <i>in vitro</i> models that are comparable to <i>in vivo</i> disease models. Hence experiments in the natural avian hosts are essential.</p>
<p>Which non-animal alternatives did you consider for use in this project?</p>	<p>For some acutely-transforming viruses, cells from animals can be transformed <i>in vitro</i> and some aspects of the molecular mechanisms of induction of cancer can be studied. Similarly, for Marek's disease, some aspects of the virus-host interactions can be studied using transformed cell lines derived from lymphomas induced in the infected birds.</p>
<p>Why were they not suitable?</p>	<p>These <i>in vitro</i> models of transformation only gives a part of the story involved in virus-host interactions and mechanisms of transformation. As the lymphoma and other tumours induced by oncogenic viruses are complex involving multiple systems, none of the <i>in vitro</i> systems can reproduce the authentic multisystem involving lymphomas and other tumours induced <i>in vivo</i> from experimental infections. Moreover, there are no <i>in vitro</i> transformation models for Marek's disease virus. Studies on MDV-transformed cancer cells only gives the virus-host interactions in an already transformed cell, and not the dynamic changes in the neoplastic transformation process.</p>
<p>Enter the estimated number of animals of each type used in this project.</p>	<p>fowl: 4600 quails: 200 other-birds: -</p>
<p>How have you estimated the numbers of animals you will use?</p>	<p>The total number of animals requested is based on the estimated numbers used in the previous years. Experiments using the infectious agents would require comparisons to be made with those of uninfected control birds. Quantitative data will be compared using analyses of variance, t tests and/or chi-squared analysis. Final size of the experimental groups will be determined on the basis of procedures described for statistical methods using advice from statisticians. The number of birds in each group will be determined using 'power of experiment' calculations based on q-PCR data (means & standard deviations) from previous experiments.</p>

	<p>Typically, the group sizes of animals for experimental studies will be between 6 and 10 birds, based on calculations from previous studies. For example, using q-PCR measurement of the viral genome copies per 10⁴ cells (expecting a 5-fold difference required to be detected as the criteria), we have observed that a group size of 8 gave statistical difference at a 'p' value of 0.05 and a power of 80%.</p>
<p>What steps did you take during the experimental design phase to reduce the number of animals being used in this project?</p>	<p>Further reduction will also be achieved by using the same 'control' group for more than one experiment. Birds allocated to different groups with randomised wing-band numbers. With regard to the genetic variability of the host and the measures to control it, the proposed group sizes are considered appropriate, because the modern commercial broiler/layer birds have comparatively less heterogeneity based on the recent studies on MHC variability in birds from a number of commercial breeding companies (Kaufman, personal communication). Moreover, we will aim to use inbred lines of chickens where possible which will reduce the variability significantly. Gene-edited birds proposed to be used in a few experiments also have limited genetic variability as they are usually generated from single founder birds. Advice on experimental design and number of animals required will be sought from Statisticians REDACTED and will also make use of the N3CR's Experimental Design Assistant (EDA) https://www.nc3rs.org.uk/experimental-design-assistant-eda.</p>
<p>What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?</p>	<p>Experiments will be designed to keep the animal usage to the minimum, but without compromising the validity of the research findings. Most of our experiments are carried out using well characterised viruses using chicken lines with limited heterogeneity, allowing us to use minimum number of birds in different groups. Our long experience in these disease models will help to decide on the numbers needed. Moreover, we have access to specialist mathematical biologists at the Institute who advise us on the minimum numbers per group to achieve statistical significance of our data. It is a routine part of experiment planning to have approval from such experts on our animal experiments. Where control groups are required we will perform as many concurrent experiments as is practically and scientifically possible so that the same control groups can be utilised for achieving reduction in animal usage. We have not proposed to use rabbits or mice for the production of antibodies in this project (compared to the previous licence), partly from the availability of alternatives, such as the Adhiron technology. Thus, there has been an overall reduction of 10% in poultry numbers. 100% reduction in the use of rabbits and mice for</p>

	antibody production.
Which animal models and methods will you use during this project?	<p>Most of our experiments are carried out using well characterised viruses using chicken lines with limited heterogeneity, allowing us to use minimum number of birds in different groups. Our long experience in these disease models will help to decide on the numbers needed. Moreover, we have access to specialist mathematical biologists at the Institute who advise us on the minimum numbers per group to achieve statistical significance of our data. It is a routine part of experiment planning to have approval from such experts on our animal experiments. Where control groups are required we will perform as many concurrent experiments as is practically and scientifically possible so that the same control groups can be utilised for achieving reduction in animal usage. We have not proposed to use rabbits or mice for the production of antibodies in this project (compared to the previous licence), partly from the availability of alternatives, such as the Adhiron technology. Thus, there has been an overall reduction of 10% in poultry numbers. 100% reduction in the use of rabbits and mice for antibody production.</p>
Why can't you use animals that are less sentient?	<p>Chickens are the natural and only susceptible species to Cancer is a highly complex, multifactorial, multistep dynamic process involving several cell types and events. There are no complete <i>in vitro</i> models that can simulate this complexity. Hence there are no non-animal alternatives that can completely replace the use of birds. Similarly, the immune responses to these diseases can also be effectively studied only in an infected bird, again due to complex nature of the responses. However, we have tried to use alternatives wherever possible. For example, we have generated a number of cell lines from the cancer tissues derived from the infected birds. These have been used for a number of studies to examine the molecular changes that occur in the cancer cell, which are very similar to that seen in the primary cancers induced by these viruses <i>in vivo</i> in natural infection models. We are also using these <i>in vitro</i> systems for most of the recent gene editing work, to help identifying the genes that are important for inducing and maintaining the cancer cells.</p>
How will you stay informed about advances in the 3Rs, and implement these advances effectively,	<p>As a member of AWERB we constantly discuss 3R. We also follow up the guidelines fro NC3R. Studies in the past several years by many laboratories have confirmed that there is no alternative to <i>in vivo</i> animal models to study virus-host interactions in the pathogenesis of and immune responses to oncogenic viruses. Retroviruses are RNA viruses that</p>

<p>during the project?</p>	<p>reverse transcribe into DNA provirus which integrates into the host genome. Most of the pathogenic (oncogenic) effects of retroviruses are induced by the proviral DNA form that causes the induction of host oncogenes adjacent to their integration sites. Furthermore, the oncogenic process and tumour formation are very complex events with the involvement of network of multiple genes that are associated with tumour suppressor functions, activation, signal transduction, immune checkpoint modulation etc. Because of the complex nature of oncogenesis, it is difficult to have suitable in vitro models that are comparable to in vivo disease models. Because of this, it is imperative that we will have to conduct animal experiments for our research on these important pathogens. Marek's disease virus (MDV) also has a unique tropism for lymphoid cells for the induction of tumours. MDV can be grown in cultured chicken fibroblast cells in cell culture dishes and we use this system to grow up large stocks of the viruses and to make mutations in the viruses, without needing to use chickens. However, in the chicken, the natural target cell for the virus is the lymphocyte and these lymphocytes do not grow well in culture (unless they are derived from MD tumours), so we cannot study either the natural primary infection or the formation of tumours in vitro. Similarly, the different facets of MDV infection dynamics involving multiple cell types with distinct interactions and outcomes also cannot be studied in any in vitro models. Examination of the protective effects of vaccination has to be also performed in chickens in the absence of other in vitro models. Chickens, turkeys and quails are the natural hosts of these oncogenic viruses. Use of these avian species proposed in the project is also justified as there are differences between these species in virus-host interactions.</p> <p>Although there are no alternatives to the study of pathogenesis and vaccine responses to these viruses, we have considered the options of using cell lines derived from the tumours as part of principle of Replacement of animal usage for studying some of the aspects of virus-host interactions. For example, we are using gene editing approaches on avian oncogenic virus-transformed cell lines to examine the effects of knockout of oncogenes such as the Meq, c-myc and v-rel as well as miRNAs such as miR-155 and miR-17-92 cluster. However, these alone could not provide all the information as these may not be similar to the interactions in the primary tumours, and also may not be sufficient to know the pathways that trigger neoplastic transformation. Similarly, there are no in vitro alternatives for studying the immune responses to these viruses and vaccines, although we are exploring possibilities of using</p>
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	organoids (organ on a chip) for certain studies.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	<p>This project benefits from the long-standing expertise of scientists who have spent many years working with oncogenic viruses and animal models. We have developed robust clinical scoring systems to accurately identify the stages of infection and appropriate humane end points. Chickens are monitored twice daily (or more frequently as required by the clinical scoring sheet) and humanely killed by a trained personnel in an ante-room so as not to distress the other chickens in the pens.</p> <p>Wherever possible we will aim to carry out maximal observations of welfare of the infected birds. At our establishment, we have the expertise of a number of groups working on other avian diseases. Their expertise and experience with the clinical scoring systems will be used when the efficacy of recombinant vaccines are evaluated.</p> <p>The birds used in this research will be housed either in floor pens or in isolators depending on the experiments and types of samples (such as the infected dust) to be collected. We have refined the Marek's disease transmission experiments by changing from the isolators to the floor pens based on the data from the pilot experiments which showed comparable to those from isolator experiments.</p> <p>Provision of enrichment is a priority at the Institute including for those birds housed in the isolators. Foraging, scratching and pecking are all important behaviours to chickens and so we provide our birds with substrate on the floor to allow foraging and dustbathing and toys to enable them to express their species specific behaviour. We also in most cases provide more space than that is legally required within the Home Office Code of Practice. Animal facilities are managed by our Animal Technicians who are experienced specialists in the care of animals. They are all trained in daily animal handling, husbandry.</p> <p>Wherever possible, we have also carried out further refinement steps by using chicks derived from vaccinated parent flocks, and the maternal antibodies usually give better protection from early clinical disease.</p>
What published best practice guidance will you follow to ensure experiments are conducted in the most	<p>We have designed very accurate clinical scoring systems and humane end points for each of the animal experiments. These robust systems have refined the experiments significantly to reduce suffering and improve welfare.</p>

refined way?	
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Project	Vitamin A and retinoids in the central nervous system.	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>The essential nutrient vitamin A is converted to the compound retinoic acid and it is this molecule that controls the functions of cells. Relatively little though is known how retinoic acid regulates the function of the brain, and this project will explore this question. The project has three central objectives:</p> <p>1. Examination of how vitamin A and retinoic acid, control the rhythms of the brain. These brain rhythms are essential to control the body and its ability to anticipate the rhythmic changes in the environment whether these be seasonal (6 month cycles) or change in day (24 hour cycles).</p>	

	<p>2. Investigate how vitamin A synthesized by glia controls the brain. We have discovered that the glia cells, the main cell type of the brain in addition to neurons, convert vitamin A to retinoic acid under some circumstances and we will explore under what conditions this may be important and whether it controls the function of both glia and nearby neurons.</p> <p>3. Study of the mechanisms by which vitamin A and retinoic acid control the development of the embryonic brain. In contrast to the adult brain, the control by retinoic acid of the development of the fetal brain has been extensively studied. This is not the case though for the processes that convert vitamin A to retinoic acid which is a key to comprehend how the actions of retinoic acid may be limited for normal regulation of the fetal brain but also to understand why excess levels of retinoic acid severely disrupt brain development.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The research performed in this project investigates fundamental changes in cell function and gene expression regulated by retinoic acid. Understanding this, in the long term, will be essential to comprehend several disorders. For instance: 1. The brain (specifically the hypothalamus) controls the desire to eat in the summer and conserve food during winter. We have strong evidence that retinoic acid in the hypothalamus controls this mechanism and may be used to control our desire to eat, a beneficial behavioural change given the current “epidemic” in obesity. 2. Our study of the actions of retinoic acid in the brain point to parallels with actions of a number of hormones and through such pathways retinoic acid may influence body metabolism and, in turn, play a role in diabetes. A number of studies point to such an interaction. 3. Our study of retinoic acid synthesis by glial cells may point to a mechanism for their known role in Alzheimer’s Disease. 4. Retinoic acid (isotretinoin) is a successful treatment of acne but is known to result in a high incidence of birth defects in pregnant women. Our studies will help point to the cause of this to help develop drugs</p>

	which lessen this tragic side-effect.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats, 13,500 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The adverse effects will predominantly be minor for instance ear notching, reduction of retinoic acid signalling and injection of cell labelling compounds. A small number of experiments will be more severe and will include studying the effects on the rodent of vitamin A deficiency which leads to eye irritation, surgery on the rodent brain to expose it directly to retinoic acid like drugs and exposing rodent embryos to retinoic acid which severely disrupts their development. In all cases suitable analgesia, anaesthesia (including terminal anaesthesia for some procedures), close monitoring including regular weighing, will be used to reduce all adverse effects. The effects will be mild for all but a small minority of animals for which severity will only be moderate. One of the main approaches of this project will be to give mice and rats substances which alter retinoic acid signalling. These may be given in the diet, by various routes or directly into the brain. In all cases, at the end of experiments, the animals will be humanely killed and tissues analysed. We anticipate that 5% of animals will be of unclassified severity, 93% of animals will be of mild severity and 2% of animals will be of moderate severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The principle aim of the proposed program of work is to understand how retinoic acid regulates the function and rhythms of the developing and adult brain. Simple <i>in-vitro</i> systems (e.g. cell lines) cannot substitute for this highly complex biological system that involves interaction between multiple organ systems and the use of animals in the proposed project is therefore unavoidable.

<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>One of the underlying principles of the research will be to use a minimum number of animals to still obtain valid result. Careful planning of experiments together with statistical determination of number of animals required will allow this. The most sensitive methods for detection of molecules are used, with low background noise and variability, greatly reducing the amount of tissue required.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rodents are the species to be examined which provide a relatively “primitive” mammalian species on which there is a vast amount on information into which we can tap regarding brain function in order to understand what may happen in the human brain. Our research also includes studies on the human brain which allows us to determine in what way our research on rodents is applicable to the human brain. All adverse effects will be reduced to the minimum with suitable analgesia anaesthesia (including terminal anaesthesia for some procedures) , close monitoring including regular weighing. We constantly monitor the literature and other resources closely to refine our methods to enable the most effective possible and most likely to achieve the goals we have set. Such refinement includes the use of brain slices to study the effect of retinoic acid-like drugs directly on the brain which reduces the number of rodents required for such studies and removes any possibility of effect of the drugs on the living animal. A further example is our use of approaches to minimize any detrimental effect vitamin A deficiency may have on an animal by supplementing them with protective retinoic acid which maintains their health for most of the experiment.</p>

Project	Vitamin A, retinoids and other lipid signalling molecules in the central nervous system	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Fats (lipids) are molecules in the body used not just to store energy but for complex tasks of sending messages from cell to cell. In the brain and spinal cord (central nervous system) the retinoids and cannabinoids are two types of molecules with these types of functions. This project will study these and other types of lipids with complex functions in the central nervous system. The project has three aims, to 1) investigate how these lipids in the brain are necessary for control, by the brain, of many body functions, 2) understand how the different	

	lipid molecules interact with each other in the brain and finally 3) determine whether drugs based on these molecules can be used to treat diseases such as Alzheimer's disease and amyotrophic lateral sclerosis a disease causing the death of neurons controlling voluntary muscles and often leading to rapid death. Also explored will be treatments for Tourette's syndrome a disease causing a person to make involuntary sounds and movements called tics.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The research performed in this project investigates fundamental changes in the function of cells making up the brain regulated by lipids. Understanding this, in the long term, will be essential to comprehend several disorders. For instance: 1. We will identify pathways whereby retinoic acid may influence body metabolism and, in turn, play a role in diabetes and obesity. 2. We will determine whether different lipid molecules in the brain interact with each to either enhance or suppress the others actions and 3. Our research on lipid based drugs will identify possible treatments for neurodegenerative disease such as Alzheimer's disease and amyotrophic lateral sclerosis as well as diseases such as Tourette's syndrome.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats, 5950 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals will undergo procedures that change how lipids effect the brain. This will either be through applications of substances that influence these lipids or through use of genetically altered animals that alter the way these lipids work in the brain. The adverse effects will be minor for by far the majority of animals because the planned subtle alteration of lipid molecule function in the brain are not expected to have major effects on the body. A small number of experiments will be more severe and will include more major changes in levels of lipid molecules, administering substances directly into the brain and around the spinal cord or exposing rodent embryos to lipid molecules that can severely disrupt their

	<p>development. Recovery though from treatments is expected to be rapid and uneventful. In all cases suitable painkillers will be used and the animals will be closely monitored and so post-operative care will be just like people recovering in hospital. In some cases we will examine changes in animal behaviour and such experiments will have only minor disruption to the animals lives with nothing more done that might occur in normal human lives. In all cases, at the end of experiments, the animals will be humanely killed and tissues analysed.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>The principle aim of the proposed program of work is to understand how lipid molecules regulates the function of the developing and adult brain and possible ways they may be used to create drugs for brain disease. In many experiments we study how this happens with cells grown in a dish which can provide a lot of useful information on how these lipids function in single cells. In some cases though we need to know how lipids work in the brain itself. This is essential to be able to determine for instance whether certain drugs may have future use in the treatment of disorders such as Alzheimer’s disease. Simple cells in a dish cannot substitute for this highly complex biological system that involves interaction between multiple groups of cells. Trying to model this with, for instance, sophisticated computer systems, is impossible because how the brain works is not understood anywhere near well enough to write the appropriate software for a “machine brain”. Therefore the use of animals in the proposed project is unavoidable.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>One of the underlying principles of the research will be to use a minimum number of animals to still obtain valid result. Careful planning of experiments together with statistical determination of number of animals required will allow this. The most sensitive methods for detection of molecules are used, with low variability, greatly reducing the amount of tissue</p>

	<p>required. A further example is the use of brain slices to study the effect of lipid based drugs directly on the brain which reduces the number of rodents required for such studies.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Rodents are the species to be examined which provide a relatively “primitive” mammalian species on which there is a vast amount of information into which we can tap regarding brain function in order to understand what may happen in the human brain. Our research also includes studies on the human brain which allows us to determine in what way our research on rodents is applicable to the human brain. All adverse effects will be reduced to the minimum with suitable pain killing drugs, close monitoring including regular weighing. Aseptic techniques will be used when administering substances directly into the brain or around the spinal cord. We constantly monitor the literature and other resources closely to refine our methods to enable the most effective possible and most likely to achieve the goals we have set. Such refinement includes the use of certain animal models of disease which have a non-harmful effect on the animals but allow us to study the molecules we know to take part in disease.</p>

Project	Welfare of poultry under different housing and management practices	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Despite large changes in housing methods and legislation that governs the keeping of farmed animals, much is still unknown on how best to keep poultry in ways that both meet their behavioural and biological needs, while still making farming sustainable. For example, laying hens are still not commonly housed without beak trimming; broiler breeders are chronically food deprived (but physically healthy), without definitive evidence as to their overall welfare state; and different housing designs throw up benefits in some aspects of welfare but drawbacks in others, thus objective	

	<p>ways of measuring chronic stress from housing methods could be beneficial. This project licence would enable us to investigate ways in which to house and manage poultry that best meets their welfare needs while still working within viable methods of food production.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>This work may, in the long-term, enable us to house birds with less mutilations (e.g. beak trimming of laying hens), find better ways to feed broiler breeders (if current methods indicate chronic hunger and other methods do not) and identify what types of housing systems are the least chronically stressful for poultry (e.g. indoor versus free range), and what strains of growing birds are suited best to some food assurance schemes.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>In 5 years, we would use up to 10,000 birds (domestic chickens and/or turkeys). This is because we sometimes conduct our work under commercial conditions.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Some birds will experience mild to moderate discomfort due to the housing environment, but this will often be short term, or they will be allowed to make a choice of where to be. Short-term food or water deprivation will cause short-term hunger and thirst. Food and/or water composition are designed to restrict growth to reflect commercial levels of restriction in place for parent stock of meat birds, and not more. Mild aversive stimuli are designed to stimulate chronic stress or to disrupt sleep, but these will be designed to reflect stress/disruption seen in commercial systems and no more. Ironically, not beak trimming birds is regulated, because it can lead to greater feather pecking, cannibalism, and mortality, but this will be closely monitored and strict control measures will be in place. Where birds are beak trimmed for a study, only commercially-applicable methods will be tested. Transported birds will experience journeys no longer than is allowed commercially, but with greater attention to their welfare with frequent checks. Physiological manipulations (e.g. hormones) will only be done within the natural range for the species. A small proportion of birds will undergo brain surgery so</p>

	<p>that we can study sleep behaviour objectively by recording the brain's electrical activity (EEGs), but this will be performed under general anaesthesia and with post-operative care. Birds are expected to behaviour normally within a few minutes of recovering from surgery (e.g. eating, drinking, preening).</p> <p>The maximum severity level is moderate, however in reality most birds will experience mild severity. Some birds will be humanely killed at the end of a procedure for tissue collection, or because brain surgery means that they cannot be kept beyond the period of study. Where possible, birds will be rehomed (e.g. laying hens), or they will be released from the Act and culled as per normal farming practice.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	Behaviour and welfare studies need to study the whole animal and their responses to their environment.
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	We will base our animal numbers on prior experience and after discussion with a statistician, to ensure that the number of animals used is neither too high nor too low. We use optimal experimental designs and analyses that take account of all sources of variation, to get the maximum information from our studies. The use of commercial conditions impacts on the number of animals that are used (e.g. they are typically higher than laboratory-based studies), however this means that the relevance of our work to agricultural industry is likely to be greater.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to</p>	Poultry are used because their housing and management systems (and how they respond) are the areas of interest. With every experiment performed, we carry out a harms/benefits analysis, and review these retrospectively to see how measures can be refined for future similar trials. These are requirements of our AWERB. This assists us in planning future trials to minimise welfare costs to animals. Using EEG

the animals.	to objectively study sleep has been used previously in wild birds and the method is highly refined (e.g. wireless loggers, small implants) to give the desired results.
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Project	Zebrafish models for inherited neurological diseases	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Many inherited neurological diseases are very severe diseases starting already in childhood. In these diseases the brain, the nerve cells and the muscles don't function properly, and patients have difficulties with movement and coordination. The affected patients are disabled and often die prematurely. The cause of the diseases lies in the DNA of the patients, which is the material in the cells of all organisms that contains all the information for their characteristics and appearance. DNA defects are passed down from parents to children making the diseases inherited of familial. The	

	<p>diseases are currently incurable and have a devastating impact on patients and their families as well as requiring substantial resources from the national health service. Our overall aim is to examine in animal models how changes identified in the DNA of patients lead to the development of a disease and to evaluate existing and novel drug treatment options. The organs affected by the diseases studied by us are the brain, skeletal muscle, the heart and the eye. We will generate genetically modified zebrafish carrying the same changes in the DNA as identified in patients and investigate how these changes impact the development and function of the different organs. In these very basic studies into the underlying basis of disease we have elected to use the simplest vertebrate (animal group distinguished by the possession of a backbone) model available, the zebrafish. Zebrafish have a number of significant advantages for these studies including a short time to reach maturity, transparent embryos (which can be viewed under a microscope) and organ structures sufficiently related to humans.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The major benefit of this research is to the scientific community by progressing our understanding of how changes in specific parts of the DNA lead to a neurological disease. In the first instance, our research will generate new information for clinicians treating patients and other scientists in the field and improve diagnosis for the patients. By providing this basic understanding we aim to contribute to the development of novel and better treatments for patients in the long term. We also aim to test drugs for their ability to ameliorate defects in fish cells and their mechanism of action to inform ongoing drug development programs and contribute to the advancement of treatment options</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We estimate that we will need to use 25500 zebrafish over the 5-year course of the project. The majority of adult fish will be used for breeding purposes only. Approximately 10000 animals will be used for testing drug treatments. We minimise the number of zebrafish used by</p>

	<p>keeping breeding pairs to a minimum and by testing the success of introducing changes into the DNA a few days after the injection with the reagents that modify the DNA. This way we avoid raising fish to adulthood unnecessarily if the DNA modification turns out to be unsuccessful.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The experiments that we undertake involve the use of animals as models for human neurological diseases and so the effects on the animals in part reflect those diseases. However, we will limit the effects on the animals to the first 120 hours of life (embryonic period) wherever possible. During these first 120 hours, zebrafish are believed to have less capacity to experience suffering and are not considered protected animals by the Home Office legislation; thus the expected level of severity of the experiments is mild. When drug tests are done on zebrafish, the substances are administered by dissolving them in the tank water. No invasive methods are required minimising the stress for the animals during the experiment. This is one of the big advantages of using zebrafish to test potential drug treatments. Most animals will be humanely killed at the end of the experiments except those required for breeding who will be expected to be only mildly affected, if at all.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Muscle, brain, heart and eye are the organs affected by the diseases we are studying; all of these are very complex organs made up of multiple cell types. This means that cell culture models, which generally consist of a single cell type, have limited applicability to patients. Therefore, many pre-clinical studies can realistically only be achieved in whole animals. The detailed structures of interest for us are too different in invertebrate species such as worms and flies, especially the contact sites between nerve and muscles (called neuromuscular junctions) which are of particular interest for the diseases studied by our group. However, where possible, we will also use primary cells derived from patients in parallel to investigate aspects which can be addressed in this way. In case</p>

	<p>muscle samples become available from patients we will use them instead of animals to study the consequences of the disease on this tissue. Tissue samples from patients will also be archived for future use to replace animal experiments.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Adult fish are required mostly for the purposes of breeding and production of embryos (which form the basis of our experimental protocols). There is no aim to produce adults with a neurological disease. Adult carriers have only one copy of the modified gene and will be healthy. We reduce animal numbers wherever possible by reviewing our experimental data rapidly following an experiment and planning follow up experiments to resolve outstanding experimental questions. In this way the information generated by our research is maximised while experimental animal use is minimised. We will use the PREPARE and ARRIVE guidelines recommended by the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs) for designing our experiments and publishing our results.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The protocols on this licence are designed to minimise any effects on zebrafish after 5 days of development, where the animals have more substantial capacity for suffering. We aim to restrict any harmful effects where at all possible to the early stages before 5 days where we can, closely monitor the health of embryos and humanely kill severely affected individuals before they develop into hatchlings.</p> <p>In our zebrafish facility, the environmental conditions such as water temperature, food and lighting are strictly monitored according to Home Office guidelines to ensure the health of the animals. All fish are inspected daily and obtain daily live prey (brine shrimp) feeding to allow them to express natural feeding behaviours. Together with the animal technicians in our facility we will also test new environmental enrichment options for the fish tanks and use them if they are compatible with routine husbandry and tank cleaning</p>

	<p>procedures. As zebrafish are social animals, single housing of individual fish during experiments will be kept to an absolute minimum. When the fish are moved to new tanks or put together with other unfamiliar individuals, we will allow sufficient time for them to adapt to the new environment before starting the experiments. Handling of the animals by using a net is kept to a minimum to avoid unnecessary stress and damage to the animals.</p>
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Project	Zebrafish models for investigating cancer formation and progression, immune responses and immunotherapy	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	1) <i>To learn more about melanoma, including how it escapes destruction by the immune system.</i> Melanoma is a cancer of pigment producing cells known as melanocytes that are mainly found in the skin. Approximately 15,000 new diagnoses are made each year in the UK. Around 20% of melanoma patients currently die from their disease. Exposure to strong sunlight results in mutations in melanocytes that consequently grow out of control. Ordinarily, the immune system is equipped to detect and remove abnormal cells but for a number of reasons this process is not always	

	<p>100% effective and cancer can progress. Modern (immuno)therapies seek to restore immune responses that once more destroy cancer cells. While very promising (a significant fraction of patients have been cured), they do not always succeed: some tumours fail to respond while others stop responding.</p> <p>2) <i>To evaluate new melanoma treatments including chimeric antigen receptor T cell (CART) therapy.</i> CART therapy is a new form of immunotherapy that has proved effective in treating blood cancer but not yet solid cancers like melanoma. In CART therapy, immune cells known as T cells are taken out of the patient and genetically modified to enhance their ability to detect cancer cells. They are then reintroduced into the patient. Current obstacles with CART therapy is lack of potency and equally problematic the possibility of serious autoimmune-style toxicity.</p> <p>3) <i>To learn more about the immune system of zebrafish.</i> Our knowledge of immunity in fish trails far behind that in rodents and humans. Among fish species, zebrafish has not been intensively studied as it is of no commercial value. But owing to the potential of zebrafish models for generating insight into human disease mechanisms, and also embracing technological advances that can rapidly generate data, it is now worth addressing that gap.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>1) The research will reveal the disease mechanisms which result in the maintenance and progression of melanoma. 2) The research will identify and validate novel biological targets for drugs that can treat melanoma. 3) The research will expand our understanding of the function of the immune system in zebrafish, and indicate whether it is a suitable model for research into human disease. It could also benefit the aquaculture industry that is trying to improve disease management in fish stocks through developing vaccines, which requires knowledge of immune system function. 4) If we are successful in advancing basic understanding of the function of the immune system in zebrafish, subsequent research could uncover what limits host immune responses to cancer and how to improve</p>

	immunotherapy of cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	22,500 zebrafish over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Genetic modification of these animals could result in genetic disease or cancer, causing moderate suffering. The implantation of cancer cells and exposure to experimental treatments with unanticipated toxicity could also cause moderate suffering. Zebrafish will also be treated with factors that stimulate immune cells which is assumed to cause only mild irritation. During investigations, they may be rendered temporarily unconscious using anaesthetic or in limited circumstances by inducing hypothermia in order to image animals, for which they need to be still. At the end of study, the animals will be humanely killed.
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Exploratory studies, where possible, are first performed in a test tube or in zebrafish embryos. Data is also generated from human tumours. However, the involvement of multiple cell types in the process of cancer formation and progression and treatment response is currently impossible to fully represent other than in a mature organism. For the same reason, the complexity of the immune system is impossible to represent outside an organism.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs</p>	

(harms) to the animals.	
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Project	Zebrafish Models for Neuromuscular Diseases	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	<p>Inherited neuromuscular diseases such as muscular dystrophy are currently incurable and have a devastating impact on patients and their families as well as requiring substantial resources from the health service. Our overall aim is to examine in animal models how changes identified in the DNA of patients lead to the development of a neuromuscular disease and to evaluate existing and novel pharmacological treatment options. Muscle, brain and heart are the organs affected by the diseases we are studying; all of these are very complex organs made up of multiple cell types. This means that cell culture models, which generally consist of a single cell type, have limited</p>	

	<p>applicability to patients. Therefore, many pre-clinical studies can realistically only be achieved in whole animals. In these very basic studies into the underlying basis and mechanisms of disease we have elected to use the simplest vertebrate model available, the zebrafish. Zebrafish have a number of significant advantages for these studies including a short time to reach maturity, transparent embryos (which can be viewed under a microscope) and a muscle structure closely related to mammalian muscle (unlike invertebrate models).</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>The major benefit of this research is to the scientific community by progressing our understanding of how deficiencies in specific genes lead to a neuromuscular disease. By providing this basic understanding we aim to enable the development of novel and better treatments for patients with neuromuscular disease, although much of this more directed research lies outside the scope of this project. We also aim to test drugs for their efficacy and mechanism of action to inform ongoing drug development programs. In the future, we hope that the continuation of our research will also benefit patients and their families.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We estimate that we will need to use approximately 20000 zebrafish over the 5 year course of the project. The majority of adult fish will be used for breeding purposes only. A small number of zebrafish (250) between the age of 0-3 months will be used for pilot studies to assess dosage toxicity and effectiveness of pharmacological compounds. Approximately 4750 animals, mainly young embryos and larvae, will be used for testing pharmacological treatments. We minimise the number of zebrafish used by keeping breeding pairs to a minimum and using efficient genotyping strategies.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>The experiments that we undertake involve the use of animals as models for human neuromuscular diseases and so the effects on the animals in part reflect those diseases (muscle weakness and wasting). However, we will limit the effects on the animals to the embryonic period wherever possible and so the expected level of</p>

	<p>severity is moderate. The zebrafish embryos will be used for the creation of new strains and to test pharmacological compounds and so may exhibit muscle weakness and wasting as a consequence of the genetic alterations. A small number of zebrafish (250) will be exposed to therapeutic compounds as part of dosage pilot studies, however as the optimal dose of these compounds will be unknown, there is the potential for adverse side effects. Adverse effects of specific therapeutic compounds are difficult to predict in advance even when in-vitro laboratory testing has been undertaken as this is unlikely to be relevant to zebrafish. Therefore, we cannot rule out acute toxicity and lethality in the case of novel experimental therapies. Consequently, the adverse effects have the potential to be severe. All animals will be monitored closely during and after the pilot to look for any signs of distress and will be humanely killed if adverse effects present. Adult zebrafish will be used solely for the purpose of breeding and are not expected to exhibit adverse effects. Most animals will be humanely killed at the end of the experiments except those required for breeding who will be expected to be only mildly affected, if at all.</p>
Application of the 3Rs	
<p>1. Replacement</p> <p>State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Muscle and brain are complex organs consisting of multiple cell types which interact both during development and as a mature tissue. Unfortunately, currently it is not possible to model this structure in cell culture (which generally consists of single cell type) with sufficient fidelity to make the conclusions drawn applicable to patients. Where possible, we use primary cells derived from patients in parallel to investigate those aspects which can be addressed in this way, but these studies are of limited scope in terms of understanding the impact of changes found in patients on muscle and brain function as a whole. To date, the only way of studying these aspects is by using animal models.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of</p>	<p>Adult fish between the ages of 3 months to 2 years are required solely for the purposes of breeding and production of embryos (which form the basis of our experimental protocols). There is</p>

animals	no aim to produce adults with a neuromuscular disease. We reduce animal numbers wherever possible by reviewing our experimental data rapidly following an experiment and planning follow up experiments to resolve outstanding experimental questions. In this way the information generated by our research is maximised while experimental animal use is minimised.
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>The protocols on this license are designed to minimise any effects on zebrafish after 5 days of development, where the animals have more substantial capacity for suffering. We aim to restrict any harmful effects where at all possible to the embryonic stages where we can closely monitor the health of embryos and humanely euthanize severely affected individuals before they develop into hatchlings.</p>

Project	Zebrafish models for understanding human genetic disease affecting ciliary function	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
	<input checked="" type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input checked="" type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	For many years the primary cilium, a hair-like cell surface structure, was believed to be functionless . Over the last 15 years, an expanding group of diseases with shared clinical features have been found to have defects in genes whose encoded proteins are found localised to the cilium. Consequently, these patients have abnormal cilia . These groups of disorders are collectively known as the ciliopathies. Cilia are found on almost all cells in the body. Unsurprisingly, loss of cilia affects multiple tissue types. Ciliopathy patients become obese, develop kidney	

	<p>disorders, have heart developmental defects, and become blind before they reach 30 years of age . There is no cure for ciliopathy related disease.</p> <p>As yet, many ciliopathy patients do not have a gene associated with their disease. We are now able to sequence the whole DNA of patients to identify genes that are defective. This project studies those genes identified in patients and evaluates the likelihood that they cause the disease. By blocking the function of these specific genes in zebrafish, we can analyse if the resulting changes are similar to the patient's symptoms. Thus, this project aims to identify mutated genes in ciliopathy patients and prove that they cause the disease by using zebrafish as a tool.</p> <p>Mutated genes that cause a developmental affect in zebrafish, similar to those seen in humans, will then be used to investigate which cell molecular pathways are disrupted . Here, we will research the function of the genes during development and cilia function within the whole animal and by looking at the behaviour of cells growing in a petri dish.</p> <p>Zebrafish embryos will be used to screen drugs to identify potential therapies to prevent kidney disease, prolong vision, and manipulate perturbed signalling pathways.</p>
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<ul style="list-style-type: none"> - discover new genes involved in cilia development and disease. - increased our understanding of embryonic development, both normal and abnormal leading to disease. - identification of new pathways that might be amenable to drug treatment - Identify potential therapies to prolong vision or prevent chronic kidney disease.
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<ul style="list-style-type: none"> - Zebrafish (Danio rerio) approximately 2500 fish per year
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity?</p>	<p>Adverse effects to experimental protocols are most likely to manifest in a set of mild behavioural perturbations such as disinterest to food, bullying by other fish, rapid respiration</p>

<p>What will happen to the animals at the end?</p>	<p>(seen by gill movement), glancing off the side of the tank, lethargy or abnormal swimming. After procedures, zebrafish will be closely monitored for abnormal swimming or feeding behaviours. Individuals displaying persistent signs of distress (indicating moderate suffering) will be humanely culled using an overdose of anaesthetic. As the creation of new mutations might produce unexpected results, we will seek advice from the home office inspector whenever this occurs. Our animal house staff are very observant and will contact a team member if any animal appears to be suffering.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Embryonic development is a four-dimensional process (i.e. varying in space and time), and studying it therefore requires the analysis of whole developing embryos. Direct genetic studies of embryonic humans are difficult practically, and only descriptive analysis is possible, with experiments ruled out on ethical grounds. Tissue culture systems, although they can provide useful information on certain molecular or cellular phenomena, cannot mimic the complexity of functioning organs, let alone the developing embryo. Computer simulations can be valuable in extending theoretical approaches to embryonic development, but cannot tell us about real biological situations, such as those occurring in the embryo.</p> <p>The zebrafish offers a fantastic whole organism system to study vertebrate development. Their embryos develop outside of the mother and are transparent allowing cell movements to be visualised down the microscope. This also means that the mother does not have to be culled to collect the embryos. The produce lots of eggs, allowing many experimental individual to be analysed. Furthermore, they share over 80% of disease causing genes in common with humans. Thus, a suitable replacement for higher vertebrate organisms.</p>
<p>2. Reduction Explain how you will assure the</p>	<p>We will actively participate in the zebrafish community by sharing resources to reduce the</p>

<p>use of minimum numbers of animals</p>	<p>number of newly bred genetically modified fish.</p> <p>We will design animal experiments efficiently using the most appropriate breeding schemes in order to produce as many embryos with the required genotype as possible, and therefore reducing the needless generation of non affected animals.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Zebrafish produce hundreds of eggs per adult female, providing large datasets for genetic and statistical analyses. The equivalent of birth in zebrafish is the point they become independent feeders, at approximately 5 days post fertilization (dpf). At this point the zebrafish becomes protected by the Animals (Scientific Procedures) Act 1986. This project uses many protocols where the zebrafish eggs are not permitted to develop beyond 5 dpf, requiring fewer animals to fulfill the project aims compared to a similar mouse project. Thus, we have minimized the potential for actual stress/pain by refining the majority of our experiments to embryonic stages of development. Indeed, the zebrafish are small and transparent making them optimal organisms to conduct non-invasive techniques that would otherwise be used on mammals. For the purpose of drug screening, pharmacological agents can be administered in the embryo medium opposed to injection procedures performed in adult mice.</p> <p>Where we anticipate animals might suffer pain, appropriate anaesthesia will be given pre-operatively. Animals are also monitored (e.g. post-operatively) to assess the need for (further) pain relief, or antibiotic treatment where infection may be an issue</p> <p>As the creation of new mutations might produce unexpected results, we will ask advice from the home office inspector whenever this occurs monitor the breeding very closely and will euthanase any animals that appear to be suffering</p>

Project	Zebrafish models of cardiac development and disease	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input type="checkbox"/>	Translational and applied research
	<input type="checkbox"/>	Regulatory use and routine production
	<input type="checkbox"/>	Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/>	Preservation of species
	<input type="checkbox"/>	Higher education or training
	<input type="checkbox"/>	Forensic enquiries
	<input type="checkbox"/>	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Heart defects are the most common human birth defect, occurring in around 1% of live births. These arise due to abnormal formation of the heart during development of the embryo in the womb, and often require surgery after birth as well as lifelong care for the patient. This project aims to understand which genes are important for ensuring the normal formation of the heart in an embryo, and to generate zebrafish models to better understand how specific gene mutations cause specific heart defects.	
What are the potential benefits likely to derive from this project	1) To improve our understanding of which genes are important for normal heart	

<p>(how science could be advanced or humans or animals could benefit from the project)?</p>	<p>development, and how they help our hearts develop normal-ly. 2) To provide information for clinicians and pa-tients with structural heart defects on why mu-tations in specific genes may cause heart de-fects, and improve the genetic counselling of-fered to such individuals. 3) The generation of specific animal models of heart defects caused by abnormal embryo de-velopment, based upon mutations found in patients, can provide information on disease progression, any additional health problems associated with the heart defects, and as a tool for screening potential drug treatments. 4) By improving our knowledge of how a heart is built, we may also gain ideas around how to help a heart to regenerate, or grow new tissue, after damaging events such as a heart attack.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>We use zebrafish (Danio rerio), and will use up to 13,000 fish over 5 years</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</p>	<p>Since we study embryonic development, most of our experimental work is on zebrafish embryos at a very young stage when the embryos are not fully developed and are not protected by law. Most of the adult zebrafish we will use are maintained for breeding and embryo production, a mild procedure for the fish. Fish kept under breeding protocols are routinely handled and occasionally anaesthetised, which do not usually cause detectable adverse effects on the fish. The majority of the adult fish we use carry fluorescent transgenes, or one copy of a mutated gene, however these fish do not appear to have any adverse effects from carrying these modifications. We will on occasion alter specific genes in a zebrafish embryo in order to grow them to adulthood and generate new models, however we carefully monitor these growing zebrafish larvae daily to ensure the mutations do not cause any health issues, and generating new models in this way is also a mild procedure for the fish. Occasionally fish grown in this way may display behaviour that indicates the fish is distressed, for example unusual swimming or feeding behaviour, or weight loss. Fish that behave abnormally like this are euthanised to</p>

	<p>prevent any ongoing discomfort. A small number of fish that we raise completely lack function of specific genes – and we raise them to see what effect loss of that gene has on development of the heart. We can then for example perform exercise tests on these fish to assess how well their hearts function without, or special imaging to see how well the heart forms without these genes. In these cases, some fish may experience low levels of discomfort for example exhaustion after exercising, which similar to above can manifest itself in abnormal swimming behaviour, resting in the tank, and reduced feeding. We will only perform these experiments a small number of times on each fish to ensure that we gain enough data to be useful, but that we do not continually expose the fish to a distressing experience. All fish are humanely killed at around 18 months old, similar to lifespan in the wild.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Heart development is a very complex process by which a 3-dimensional heart tube has to undergo reorganisation in order to form a functioning organ. This 3D development of the heart occurs within the environment of an embryo, from which the heart receives many genetic and physical cues. In order to understand this process as well as possible, we need to study it in an environment as close to the normal situation as possible – i.e. in a developing embryo. Throughout the duration of the project we will continue to monitor the possible use of replacements where suitable.</p>
<p>2. Reduction Explain how you will assure the use of minimum numbers of animals</p>	<p>We mainly use adult zebrafish as breeding stocks to generate the embryos which we use to assess heart development. Since one female zebrafish can give many hundreds of eggs per mating, we can work to minimise the numbers of fish we need while ensuring that we keep our lines safely. Our estimates of numbers of animals are the outcome of careful experimental design together with our dedicated aquarium team, ensuring good density of fish to promote normal socialisation and behaviours, and good sex ratios for breeding purposes. We will also</p>

	<p>use where appropriate a method for ensuring we only raise the animals that carry the genetic mutations we need to adulthood, allowing us to reduce further the amount of animals required. Finally, we will share wherever possible the animals with other users in our facility.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Although zebrafish are a lower vertebrate compared to humans and animals, their hearts develop in a very similar manner to humans, and form many similar structures. Over 80% of disease-causing genes are conserved in zebrafish making them a suitable model to understand the genetic basis of human disease in a non-mammalian system. Furthermore, since zebrafish embryos are fertilised externally, it allows embryo collection without sacrificing the mother, reducing the overall number of animals required to perform a desired experiment when compared with mammalian models.</p> <p>Animals welfare will be maximised by ensuring fish are housed at appropriate density to promote normal social behaviours, with environmental enrichment where appropriate (for example use of snails, and objects for the fish to interact with). Limits on handling frequency will be put into place to minimise handling and reproductive stress. Finally, our aquarium is managed and maintained by a dedicated team of technicians with extensive experience in fish husbandry and welfare.</p>

Project	Zebrafish models of cellular immunity
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/> Basic research <input checked="" type="checkbox"/> Translational and applied research <input type="checkbox"/> Regulatory use and routine production <input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals <input type="checkbox"/> Preservation of species <input type="checkbox"/> Higher education or training <input type="checkbox"/> Forensic enquiries <input type="checkbox"/> Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	White blood cells called neutrophils are necessary for fighting infections, but can also contribute to tissue damage in inflammatory diseases when they are not cleared from inflammatory sites in a timely manner. We aim to find ways to remove neutrophils where they have the potential to do damage, while preserving their ability to fight infection. Our previous work has generated a number of interesting leads that we wish to pursue with the overall aim of identifying new treatments for conditions such as chronic obstructive pulmonary disease (COPD) and rheumatoid arthritis.

	We will use drug treatments and changes in the genes of zebrafish in order to advance our understanding of how these genes contribute to neutrophil activation, the inflammatory process and how we may target these for the treatment of inflammatory disease.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project seeks to better understand how neutrophils, white blood cells, respond to and are cleared from sites of inflammation. We hope that by increasing our understanding of this process we will identify targets for new treatments for inflammatory disease.
What species and approximate numbers of animals do you expect to use over what period of time?	A maximum of 82,700 zebrafish (5 days or older) will be used over 5 years. The majority of experiments will be conducted on immature zebrafish forms (less than 5 days old) generated from these adults.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most fish are expected to live out their lives in the same levels of comfort as any aquarium fish. A very small number may experience adverse effects such as oedema (mild swelling) e.g. around the heart and eyes. The majority of adults will be used for breeding only. Some adult fish will undergo the removal of a small portion of their tail fin for genetic analysis (fin clipping). Fish recover and regenerate their tail fin quickly. In a minority of cases (2500 over 5 years) fish will undergo tail fin transection when they are less than 5 days old and will be allowed to live until older larval stages or adulthood. Some gene changes may result in unexpected adverse effects e.g. increased incidence of infection. In these cases, fish will be sacrificed without delay. Animals will be sacrificed before they suffer disease at the end of their natural lifespan, or earlier if indicated.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Inflammation is a complex process requiring interaction of multiple cell types and cannot meaningfully be modelled in a cell-based system. The majority of our experiments are

	<p>carried out on larval zebrafish before the age of protection (5 days old). Although human neutrophils cannot be genetically altered, we have shown that many of our zebrafish drug studies can be confirmed in human neutrophil experiments. Therefore, our work limits the use of mammalian models.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>Most experiments are performed on larval zebrafish. We have extensive experience of the assays used, and are confident of our calculations of the minimum number of fish required. Where experiments have been conducted previously, and the data is available, these studies will not be repeated in our model.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>Zebrafish are the model with the lowest neurophysiological sensitivity suitable for such studies (a vertebrate immune system is sufficiently similar to humans to be useful, but insect or worm immune systems are not). This model has minimal impact on animal welfare. We are continually striving to refine our procedures and within the lifetime of this Project licence will move, where feasible, to swabbing for genotyping rather than fin clipping.</p>

Project	Zebrafish models of haemorrhagic stroke	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input checked="" type="checkbox"/>	Basic research
	<input checked="" type="checkbox"/>	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Intracerebral haemorrhage (ICH) is a type of stroke that is caused by bleeding in the brain and can cause disability and death. We currently know very little about how this disease works and because of this, we don't have any medicines for patients. We do know that bleeding in the brain causes a lot of brain damage and also an inflammatory response which worsens the injury. However, we do not fully understand which molecules are controlling this inflammation and injury. If we did, we would stand a better chance of being able to treat patients with specific drugs. We are using zebrafish models of ICH as a new way of trying to understand this disease better. Zebrafish	

	<p>embryos are see-through meaning we can very easily observe cells and molecules in the brains of living animals. This makes it easier for us to understand the biological processes that happen after bleeding in the brain. Furthermore, these fish recover very quickly from ICH, so we think that understanding the underlying biology of this might possibly provide clues into how we might be able to one day recover the human ICH brain.</p> <p>Therefore, the aims of this project are to:</p> <ol style="list-style-type: none"> 1. Improve our understanding of the molecules that control inflammation and brain injury after ICH. 2. Determine how long it takes for zebrafish to fully recover from brain injury after ICH and which molecules control this recovery. 3. Identify new drugs which can reduce brain injury or speed up the recovery process. 4. To compare the injury, inflammation and recovery processes in different types of ICH model, to see if there are similarities (and whether they can all be treated with the same types of drug).
<p>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</p>	<p>We do not fully understand the disease processes that happen after ICH, and we don't have any medicines for ICH patients. The expected benefits of this project will be an improved biological understanding of the injury, inflammation and recovery processes that happen after ICH. This will allow us to understand which drugs could be used to treat patients in the future. We believe that findings from this project will lead to the identification of new treatments for ICH patients.</p>
<p>What species and approximate numbers of animals do you expect to use over what period of time?</p>	<p>47,000 zebrafish over 5 years.</p>
<p>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at</p>	<p>During this project we will make and maintain a number of different genetically-altered zebrafish which will be necessary for our research into ICH. These fish are not expected to show any adverse effects. However, if any fish shows any</p>

<p>the end?</p>	<p>unexpected sign of stress or illness, they will be humanely killed using a schedule 1 method. Some fish will be anaesthetised for identification purposes. The type and dose of anaesthesia will be discussed with the named veterinary surgeon. Occasionally, a fish may not recover well from anaesthesia. Under these circumstances, these fish will be killed using a schedule 1 method. For identification purposes, a small piece of tail fin will be removed from some anaesthetised fish. Following this operation, fish will be treated with analgesics to control pain. Fish will be monitored closely following the operation and any fish showing abnormal behaviour will be killed using a schedule 1 method. Some animals will experience brain haemorrhage during the embryonic stages, but these fish recover from brain injury very quickly and grow up to be healthy adult fish. If we could understand these recovery processes then it might provide clues into how we may improve recovery in ICH patients. We will monitor live, anaesthetised animals under a microscope to study the responses of brain cells in real-time. We will also monitor their swimming behaviour over time. As these animals are undergoing a recovery process, we do not expect to observe many, if any, adverse effects when the animals are in their tanks. All animals will be killed at the end of the experiments using humane methods.</p>
<p>Application of the 3Rs</p>	
<p>1. Replacement State why you need to use animals and why you cannot use non-animal alternatives</p>	<p>Although experiments using cells or in test-tubes can be informative, these systems cannot mimic the complex interactions that occur between blood vessels, brain cells and the immune system in ICH. The only way we can perform the necessary experiments to understand the biological processes that occur in the brain after ICH is to use animal models– where the natural environment within the brain remains intact.</p> <p>Historically rodents have been used to study ICH biology. Zebrafish are animals with lower neurological complexity than mice and rats. As such, the use of zebrafish embryos and early-stage larvae can be considered as a form of replacement of the existing rodent models of</p>

	<p>ICH. Furthermore, many of the experiments performed in this proposal will be performed on fish embryos and larvae during the pre-regulated stages, which we know experience minimal distress and recover quickly after ICH.</p> <p>To compliment our research further, we will also perform some experiments in tissues obtained from zebrafish and in brain slices obtained from human ICH patients.</p>
<p>2. Reduction</p> <p>Explain how you will assure the use of minimum numbers of animals</p>	<p>We know that zebrafish embryos/larvae recover quickly after ICH. Wherever possible, experiments will be performed on animals during the pre-regulated stages. Efficient experimental design and statistical techniques such as power analysis will allow us to generate meaningful results which take into account any sources of variation between experiments, but that keep the number of protected animals used down to a minimum.</p>
<p>3. Refinement</p> <p>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.</p>	<p>In terms of Home Office regulations, zebrafish are animals with the lowest neurological complexity that can be genetically modified to study human disease. Any animal that has undergone ICH in this study that behaves unexpectedly will be killed using a schedule 1 method.</p> <p>We will use anaesthesia and analgesics for identification purposes where appropriate and under the guidance of the named veterinary surgeon. We will also continue to optimise the use of skin swabbing as an efficient alternative to fin-clipping, and will use this technique preferentially in the future as it is less invasive and does not necessarily require anaesthesia.</p>

Project	Zebrafish models of neurological disease
Key Words (max. 5 words)	
Expected duration of the project (yrs)	5 Years 0 Months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	<input type="checkbox"/> Basic research
	<input checked="" type="checkbox"/> Translational and applied research
	<input type="checkbox"/> Regulatory use and routine production
	<input type="checkbox"/> Protection of the natural environment in the interests of the health or welfare of humans or animals
	<input type="checkbox"/> Preservation of species
	<input type="checkbox"/> Higher education or training
	<input type="checkbox"/> Forensic enquiries
	<input type="checkbox"/> Maintenance of colonies of genetically altered animals
What's the aim of this project?	The aim of the project is to address the lack of treatments for neurological diseases. We hope to address this by generating zebrafish disease models, characterising them, and using them to investigate new therapies.
Why is it important to undertake this work?	The work is important because there is currently a lack of effective treatments for the vast majority of neurological diseases.
What outputs do you think you will see at the end of this project?	We expect to publish new findings in relation to the underlying biology of neurological diseases, and the identification of novel therapeutic approaches for these diseases. In addition work conducted during this project will

	provide important pilot data to enable us to secure future funding.
Who or what will benefit from these outputs, and how?	We hope that our findings will be of relevance to a number of stakeholders. For example, other researchers in the field of neurological disease, patients and families afflicted by the diseases we study, and pharmaceutical companies interested in utilising our models and therapeutic approaches in order to formally progress our ideas into a clinical setting. In addition we hope the findings will enable us to secure future funding for research.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	We disseminate our findings through scientific and lay presentations. We collaborate closely with other investigators where it is mutually beneficial, for example working with clinical geneticists to generate disease models.
Explain why you are using these types of animals and your choice of life stages.	<p>We use zebrafish because they offer several technical advantages compared to alternative species such as mice for our experiments.</p> <p>Fish are vertebrates (i.e. they have a spinal cord) so represent a simple yet appropriate model for studying human neurological diseases.</p> <p>The majority of our work involves the use of non-regulated embryonic life stages.</p>
Typically, what will be done to an animal used in your project?	Zebrafish will be used to generate genetic models of human neurological disease. Mutations will be introduced into the genome to mimic mutations found in human patients (100 procedures over 3 months). We will then characterise these zebrafish to determine whether they develop a version of the human disease at the molecular, physiological and pathological level (500 procedures over 1-2 years). At the molecular level we will look for biochemical changes associated with the mutation in man (e.g. changes in mitochondrial form and function). At the physiological level we will look for alterations in swimming behaviour. At the pathological level we will look for pathology reported in patients, e.g.

	<p>death of specific neurons and protein aggregation.</p> <p>If we are able to show that these zebrafish model the human disease then we will use them to help to find new treatments for the human disease. This will usually involve treating the fish with drugs, typically delivered in their diet, and then seeing if drug-treated fish show any improvement compared to fish receiving placebo (200 procedures over 1-2 years).</p> <p>Further analysis of these drug trials will typically involve pathological and biochemical confirmation of the effects of the drug (no additional procedures, over 1 year).</p>
What are the expected impacts and/or adverse effects for the animals during your project?	The most likely adverse events will be the development of symptoms of neurological disease, such as impaired swimming. These are likely to be moderate severity, and may last several months. Animals will be sacrificed humanely to provide tissue samples for our research.
What are the expected severities and the proportion of animals in each category (per animal type)?	<p>All experiments will use zebrafish.</p> <p>mild: 80%</p> <p>moderate: 20%</p> <p>severe: 0%</p>
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	To characterise molecular mechanisms of neurological diseases we must perform some experiments at the level of the whole organism. We use cells and tissue samples where it is possible to do so, but ultimately we need to understand how neurons die in their natural context.
Which non-animal alternatives did you consider for use in this project?	<p>Human cells expressing disease-associated mutations.</p> <p>Primary cultures of rodent-derived embryonic neurons.</p>
Why were they not suitable?	Neurons are highly specialised cells, which interact with a wide variety of other cell types both inside and outside the brain and spinal cord. For example a motor neuron in the lower spinal cord (small of the back) can send processes, over a metre long, out to muscles in the foot

	<p>and in so doing makes unique and intimate interactions with at least four different cell types. Each interaction has its own complicated chemical and physical signals. Such complexity is impossible to replicate in culture systems</p>
<p>Enter the estimated number of animals of each type used in this project.</p>	<p>zebra-fish: 39,500</p>
<p>How have you estimated the numbers of animals you will use?</p>	<p>We estimate that this is the maximum number of animals we will need to use in order to meet our objectives over a 5 year period.</p> <p>Power calculations are used to determine the number of zebrafish required for each experiment.</p>
<p>What steps did you take during the experimental design phase to reduce the number of animals being used in this project?</p>	<p>It is important to use an appropriate number of animals for each experiment, that is not too large, as this would be wasteful, or too small as this may not provide statistically significant results.</p> <p>We base the number of animals to be used for each experiment on our previous experience. This may be from similar studies using the same GA zebrafish model, or on pilot studies used to look for the variability in the data we will obtain, and the size of the difference observed between control and GA zebrafish.</p>
<p>What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?</p>	<p>Zebrafish are highly efficient breeders, so we are able to obtain large numbers of embryos <5.2dpf from a small number of parents. We will use pilot studies to optimise the number of animals used in this project. We will share tissue where it is feasible to do so.</p>
<p>Which animal models and methods will you use during this project?</p>	<p>We will use zebrafish models of human neurological disease because they offer several technical advantages compared to alternative species such as mice for our experiments.</p> <p>Fish are vertebrates (i.e. they have a spinal cord) so represent a simple yet appropriate model for studying human neurological diseases.</p> <p>The models themselves are generally mild, but</p>

	occasionally moderate severity. On balance, this outweighs the unmet need for scientific advance in order to identify therapeutics.
Why can't you use animals that are less sentient?	The majority of animals used under this license will be embryos <5.2 days old. These are not considered protected animals for the purposes of ASPA, thus we already use the least sentient model. Where we do use life stages >5.2 days old we believe that zebrafish are less sentient than mice, which would be the most suitable alternative model system.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	I will follow the relevant literature for experimental design, use twitter to find new information, attend relevant meetings (eg FELASA). To implement changes I will work with my research group to pilot new approaches where we believe these may improve 3Rs aspects of our work.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	Sometimes we require single housing of zebrafish, and where we don't think this will jeopardise the welfare of the fish we will use companion animals. We use close monitoring of adult zebrafish disease models using score sheets to monitor levels of distress. In the event that genetically altered zebrafish shows any distress, for example caused by abnormal swimming, this allows us to implement a humane endpoint.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	To the best of my knowledge there are no published best practice guidelines for the zebrafish work in this project license. However we use philosophies of experimental design advocated by the likes of Festing and Wurbel in order to refine out experiments.