Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2019 Volume 2 (N to Z)

Contents

Project	**1. 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)		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 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	

think you will see at the end of this project?	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 paratmers 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: 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 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.
Who or what will benefit from these outputs, and how?	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 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 teat cancer.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	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".
Explain why you are using these types of animals and your choice of life stages.	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.

Typically, what will be done to an animal used in your project?	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.
What are the expected impacts and/or adverse effects for the animals during your project?	Animals may experience local irritation to injections. This is usually mild and passes within 24 hours. Some of the 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. We anticipate that by operating within the guidelines for blood drawing and tumour challenge, and with careful monitoring then animals are not expected to to experience significant adverse effects related to these procedures.
What are the expected severities and the proportion of animals in each category (per animal type)?	We anticipate the 90% of animals will have severity scores of mild, and that up to 10% will have a severity score 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?	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.

Which non-animal alternatives did you consider for use in this project?	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.
Why were they not suitable?	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 systemsdo 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?	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. 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.
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 of animals you plan to use in your project?	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 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.
Which animal models and methods will you use during this project?	We will use genetically altered immunocompromised mice as our animal model. We will use the following methods: 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

Why can't you use animals that are less sentient?	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.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	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 Norecopa web pages. Any changes will be implemented directly through the experimental design, and if necessary through a project license amendment.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	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 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. 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. 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'.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	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.

Project	**2. 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)	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)	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. Repairing nervous system damage in a rodent model involves inducing cut nerve fibres to regenerate across the injury and to make connections below it.	

	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. 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. Our treatments for nervous system damage aim: (1) to block the degeneration process around the lesion and/or to inhibit the gradual loss of cellular function in chronic neurodegeneration, (2) to repair the lesion, by inducing nerve fibre regeneration or reactivating plasticity/sprouting in the brain and/or spinal cord. 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).
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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 dam-aged 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

	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to 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 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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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

	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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. 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

	of animals used, in addition to seeking statistical advice from experts within the applicant's research establishment.
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.	Project Goals and choice of animal models 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. 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. 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. Surgery, post-operative care and humane endpoints 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

	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 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

	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 end points may be reached.

Project	**3. 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)	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)	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	

	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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the	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

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 form anaesthetic. The tissues will be collected an 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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. Appropriate experimental design will help us plan ahead and avoid unnecessary use 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	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

measures you will take to minimise welfare costs (harms) to the animals.	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.

Project	**4. 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)	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)	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?	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year period we will need: 1650 rats 1050 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?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	1. By refining behavioural tasks to improve performance/reduce variance in control animals so impairments can become apparent with fewer animals. 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.

	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. 4. By using anatomical tracers with different wavelengths so parallel tracing experiments can be carried out. 5. The in vivo imaging studies naturally reduce the number of animals needed as extensive information can be acquired from even a single animal.
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.	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. 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. 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. 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

	or with gross sensory-motor impairments would be uninformative for these types of studies.

Project	**5. 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)	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)	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

	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.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. Finally, we will share our data with other researchers to reduce the risk that experiments are repeated.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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. 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

	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	**6. 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)	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
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	

	health and disease. 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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

	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.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 12000
In the context of what you propose to do to 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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of	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

animals	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.
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.	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. 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.

Project	**7. 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)	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)	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.	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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
	experiments for this research.

2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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. 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.
Project	**8. 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)	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)	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 humans or animals could benefit from the project)?	Improving our understanding of the neural basis for perception and action at the level of electrical impulses in individual neurons is in the first 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of	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.

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.
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.	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. 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. 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. 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. 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 animals needed.

Project	**9. 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)	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)	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	

	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.
What species and approximate numbers of animals do you expect to use over what period of time?	A rat model will be used, and the number of animals required under this project is estimated at 700 (maximum) 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?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.

2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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.

Project	**10. 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)	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 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. 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	

	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. 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.
What 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-reliving 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	
1. Replacement State why you need to use animals and why you cannot use non-animal	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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.

Project	**11. 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)	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)	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. 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. 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	

	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. Wherever possible, we do experiments on cell lines or brain sections in dishes to reduce the number of experiments to be undertaken on 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 the animals.	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

	be seen and modified. Most experimental animals receive minor procedures such as monitoring of reproductive cycles and blood sampling before being anaesthetised and humanely killed. 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.

Project	**12. 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)	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)	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. We are investigating the molecular regulation of brain stem cell proliferation and differentiation into specialised brain cells. We are also	

	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. The brain injury and disease models we plan to use include: neurological injury (traumatic brain injury), neurodegenerative disease (Alzheimer's), and neuropsychiatric disease (depression & schizophrenia). 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	During the next 5 year period we have predicted use of up to 17,750 mice and 1,275 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?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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

	sections. These data are directly compared with our animal work, to help validate the latter. 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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. 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. 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 amount of 5 periods of general anaesthesia.

Project	**13. 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)	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)	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. 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	

	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. 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 2000 rats will be 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?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 *in vitro* methods. The use of computational modelling to understand brain function underlying behaviour is feasible and we collaborate with local colleagues using such *in silico* 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 *ex vivo* methods to measure the levels of certain brain chemicals in relation to learned fear and its inhibition in this work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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 electrophysiology methods of assessing activity in more than one brain area. Both of these aspects will

	further reduce 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.	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.

Project	**14. 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)	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)	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. The problem of FASD cannot be controlled by health education alone. At least half of all pregnancies are	

	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. 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. The aim of this project is to use a mouse model of FASD to look at the biochemical changes caused by alcohol.
What 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 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.
What species and approximate numbers of animals do you expect to use over what	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
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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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

	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. 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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 behviour 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.
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 used cause no distress. The training of our researchers ensure that the animals have the highest possible standards of welfare.

Project	**15. 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)	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)	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	

	development; and whether these insights identify novel therapeutic opportunities The key elements of this project licence are to: 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, 10,000 (largely through complex breeding programmes) over the 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?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever possible we have developed *in vitro* 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 *in vitro*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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-

	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 symptons 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.

Project	**16. 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)	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)	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	

	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). There are three main aims of this project: 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the	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

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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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).
2. Reduction Explain how you will assure the	We have considerable experience in developing zebrafish models of human disease. We do this by making animals which have an extra gene and this

use of minimum numbers of animals	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%.
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.	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. Zebrafish are a social species - we have refined our protocols to reduce the number of fish which are

	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.

Project	**17. 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)	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)	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	

	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.
What species and approximate numbers of animals do you expect to use over what period of time?	20,000 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?	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).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 systems, which cannot possibly be recreated in vitro: therefore, these processes must be investigated in a living mammal to ensure our results are physiologically relevant.

	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 *in vivo* experiments with *in vitro* 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 *in vivo* testing which will establish physiological relevance.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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: 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.
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 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. 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; (Iii) have the least impact on the animal's welfare, compatible with our scientific objectives. 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 independent conduction of experiments.

Project	**18. 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)	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)	Objective 1: A brain hormone called kisspeptin (Kiss) 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, Kiss 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 Kiss cells is connected throughout the brain and is regulated to control reproduction and fertility. Objective 2: More recently it has been suggested that gene mutations in hypothalamic proteins (e.g. Makorin Ring Finger Protein) can cause pubertal disorders by misregulating the functions of Kiss cells in children. Importantly, altered Kiss 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 Kiss cells to control puberty onset. 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 Kiss 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 Kiss cells influence sexual, social and affective behaviours differently between the sexes and if these neurons protect males from anxiety-related behaviours.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to 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 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 stables. 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 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.
Application of the 3Rs	
1. Replacement	The brain signals governing the control of

State why you need to use animals and why you cannot use non-animal alternatives	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 Kiss 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reduction of animal use is built into the design of this project at several levels. ● 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 maximise the amount of information extracted from test samples. Experiments will be designed that where possible an animal can act as its own control.
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 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.

	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. 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. Non-schedule 1 methods of killing are required in order to obtain tissue of sufficient quality to obtain sceintific outputs.

Project	**19. 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)	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)	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	

	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. 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. 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.
What 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 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

	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.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, I will use approximately 3200 mice over 5 years (including about 1500 for 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?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. Mice are the most appropriate animals for these experiments because: - 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.

2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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. 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. 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

	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.

Project	**20. 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)	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)	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. The objective of our project is to increase the scientific understanding of what nerve cells and	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use roughly 10000 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?	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,

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 *in vitro* 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.

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	**21. Neuron-glial-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)	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)	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.	

	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.

What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice: about 8000, Rats: about 2500
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	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

	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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 experiments we propose.

Project	**22. 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)	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)	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	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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).
In the context of what you propose to do to 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 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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 *in vivo* testing compounds will be screened using a range of *in silico* (computer based) and *in vitro* testing to ensure efficacy. *In vitro* and *in silico* 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).
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to use the lowest number of animals we will carry out an *a priori* 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. 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
	these types of experiment.

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.	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.

Project	**23. 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)	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)	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.	

What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-	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

animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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.

Project	**24. 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)	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. 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	

	of the lens. No surgery will be required. 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to 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 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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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

	resolved. The study results will be reviewed as they proceed. This means that studies can stop as soon as their objectives are achieved. Variation between animals will occur. However, the other eye will be used as a control. This means that fewer animals will be required.
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 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. 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. 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.

Project	**25. 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)	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. 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.	

	The main aims of this project are to: 1) understand how the sealant behaves. How long does it take to degrade? Does it cause any response from the body? Is it safe? 2) show that needle biopsy with the new device causes less lung collapse than the standard technique.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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.

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 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. 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. 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.

Project	**26. 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)	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)	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: 1. Assess pesticide resistance in UK mite populations and develop a test to detect	

	pesticide resistance in mites which will determine the effectiveness of current treatments for sheep scab mites. 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. 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	Sheep, approximately 36 over 3 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?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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

	to provide good quality animals which responded well to the mite infestation, thereby reducing the need for further 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 the animals.	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. 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. 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.

Project	**27. 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)	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)	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 billon 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	

	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 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. 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. ii. Use of environmental enrichment in housing to relieve stress of isolation. iii. iii. Acclimation to restraint before blood pressure measurement. 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 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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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. 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. 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 the future.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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). 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. We have access to in house statisticians with whom we consult as necessary when planning in vivo studies.
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.	Mice and rats are small and easily handled species with a highly characterised immune system and well-defined biology. Mouse and rat models of metabolic and kidney disease have been established by other groups and reported in the literature. 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. 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). Best practice, for example the use of analgesics after surgical implantation of continuous delivery devices, will be employed to minimise suffering.

Project	**28. 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)	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)	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	

	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.
What 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 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

	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.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 500 rats over the five 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?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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'

	own baselines in our calculations of surface temperature changes to maximise accuracy.
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 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.

Project	**29. 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)	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)	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	

	mechanisms ('targets') that could cause seizure. This project is based on identifying such novel targets, for example a protein termed Pumilio. 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 312 mice over the course 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?	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

	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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Epilepsy is a complex disease that cannot, at present, be modelled by cell culture or *in silico*. Moreover, whilst lower organisms (e.g. *Drosophila*, 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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. 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

	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 *Drosophila*, which will reduce the number of mice used.

Project	**30. 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)	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)	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	

	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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	12,000 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?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals	To understand ageing in humans it is necessary to study a mammal and we will perform these studies in mice. We have

and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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. We will follow the NC3Rs ARRIVE guidelines in all of our research.

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.	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. 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.

Project	**31. 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)	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)	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	

	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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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

	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.
In the context of what you propose to do to 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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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

	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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. 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

	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.

Project	**32. 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)	X	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)	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 • 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. The objectives of this project are:	

	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. *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. *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.
What 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 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
What species and approximate numbers of animals do you expect to use over what period of time?	Cattle, age 1 day to adult, up to 2,200 over 5 years. Sheep, adult, up to 20 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 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.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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).
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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. Non-lactating cattle will be used for some studies of rumen function and digestibility because they are easier to maintain. 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. 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

	stress on animals. In all cases where there are alternatives, we will utilise the procedure that imposes the least harm to an individual animal.

Project	**33. 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	

	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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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 to12 weeks of age) will be up to 1000 per year (i.e. 5000 pigs over the course of this license).
In the context of what you propose	Nutritional supplements are intended to

to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
3. Refinement	Pigs, housed in a standard production environment, are used because the purpose of

Explain the choice of species and why the animal model(s) you will use 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 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. 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.

Project	**34. 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	

	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.
What outputs do you think you will see at the end of this project?	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. 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.

Who or what will benefit from these outputs, and how?	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. 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).
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	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
	impact on a variety of scientists. It is expected that this work will result in scientific publications in peer reviewed journals.

Explain why you are using these types of animals and your choice of life stages.	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 exists 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. 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.
Typically, what will be done to an animal used in your project?	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
	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-

	clinical disease. 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. 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.
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?	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. 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.
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

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	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
Which animal models and methods will you use during this project?	For our rat and mouse studies, we focus on the N. brasiliensis and H. bakeri 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. 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 H. bakeri 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 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	**35. 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)	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)	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. To address this question, we will first develop	

	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. 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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

	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 *propose* how the memory is formed; they cannot answer the question *directly*. 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.

Project	**36. 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)	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
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	

	memory' - but it is also known that some chromatin states can be modified by factors such as nutrition or the environment. 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.
What outputs do you think you will see at the end of this project?	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.
Who or what will benefit from these outputs, and how?	(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

	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. (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. (iii) REDACTED Our research underpins the delivery of strategic priorities for these funders. (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. (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.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	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.
Explain why you are using	The aims of the project are to understand epigenetic

these types of animals and your choice of life stages.	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 *in vitro* cell systems, thus reducing the number of animals used and refining the experimental approaches before applying them to mouse models.
Typically, what will be done to an animal used in your project?	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. We shall also be generating new genetically altered mouse strains using highly refined genetic modifications that will selectively affect genes in the placenta. 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.
What are the expected impacts and/or adverse effects for the animals during your project?	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. 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. 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.
What are the expected severities and the	Overall, the expected severity of this project licence is Mild, with fewer than 5% of animals expected to

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 *in vitro* 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 *in vivo* 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 *in vivo* 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 *ex vivo* 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,

	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.
Why can't you use animals that are less sentient?	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 (*ex vivo* analysis of placenta), or will be done under terminal anaesthesia (e.g., placenta function assays).
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	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 in *vivo* situation.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	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. 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. 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.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	NC3Rs Arrive Guidelines.

Project	**37. 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)	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)	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	

from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to 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 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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-	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

animal alternatives	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 *in vivo* 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 *in vivo* 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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 levelsand biomarker responses to give statistically robust data in proof of concept trials.
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	Mice are the lowest sentient species that are appropriate for in vivo 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

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.

Project	**38. 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)	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)	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.

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.	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.

Project	**39. 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)	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)	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	

	switched on and off during the embryo formation and can have lasting impact by changing the traits of the offspring. The objectives of this project are: 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
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	Zebrafish, 300 adults, 3 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 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.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 Drosophila and *C. elegans* are other popular model systems for studying inter- and trans-inheritance. However, invertebrates show differences in epigenetic mechanisms. For example *C. elegans* presents an evolutionary loss of DNA methylation. However throughout the course of the project we will look for non-animal alternatives to parts of our work by constantly reviewing the literature
2. Reduction Explain how you will assure the use of minimum numbers of animals	General considerations: 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. . 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. 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 . Environmental stressors: exposure to ethanol and nicotine. 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

	number of fish. The amounts of ethanol and nicotine here chosen are the lowest between those previously reported by other groups working with zebrafish. During the exposures, we will control variability and bias by using the experimental designer provided by the 3Rs website. The sample sizes determined for the exposures to environmental stressors are based on our previous experiments in collaboration with collaborators Larvae will be randomly and blindly allocated in the experimental well plates. Sample size has been determined based on literature and advice of statistician
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.	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. 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. 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. 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)

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

Project	**40. 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)	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)	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. 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 *M.*	

	tuberculosis infection who are at a high risk of relapsing with active, contagious disease. Many of the fundamental aspects of the host-pathogen relationship between *M. tuberculosis* and humans are poorly understood and there is an urgent need to define clear correlates of protective immunity and immunological biomarkers of 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)?	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. 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	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 aneasthesia 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

	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. All studies will have clear strict indicators of disease progression ensuring the lowest level of distress possible. Following aerosol challenge, *M. tuberculosis* 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 *in vitro* due to complex infection processes with many unknown mechanisms of evading the immune system.
2. Reduction Explain how you will assure the	This strategy will ensure that only the most promising candidates reach clinical/field trials thus reducing the numbers of animals used in

use of minimum numbers of animals	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.
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.	Vaccine and drug targets will be generated from a series of non-animal studies using defined culture conditions that mimic environments that *M. tuberculosis* may experience in humans during TB infection. The candidate vaccines will then be evaluated in a step wise progression of *in vivo* 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 *in vivo* 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.

Project	**41. 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)	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)	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 be 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	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 '*in silico*' and '*big data*' 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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

	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.

Project	**42. 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)	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)	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 (PDTX), and the corresponding short-term cultures of PDTX cells, retain most of the original cancer's variability and capture the diversity of inter-patient responses to	

	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. 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. 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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

	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.
In the context of what you propose to do to 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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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

	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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
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 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. 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

	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.

Project	**43. 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.

animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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.

Project	**44. 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)	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)	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. 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.	

	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

	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. • 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice and over the five years we will use a maximum of 1000 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?	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 as 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 scaned, we can overcome this by appropriately maintaining the body temperature by using heated air blowers. Similarly after animals are

	imaged they will be kept in a warming cabinet and will be kept under continuous observation until they have recovered from the procedure. In conjuction 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 studies. 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use	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,

of minimum numbers of animals	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.
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.	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 *a priori* - 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.

Project	**45. 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)	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)	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	

	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. 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect	Over the 5 year duration of the licence the work is expected to use: Rats 2000 Rats (GAA) 4000
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?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction	We will ensure that only the minimum number of animals are used by careful experimental design

Explain how you will assure the use of minimum numbers of animals	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.
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.	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.

Project	**46. 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
	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?	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.	

	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.
Why is it important to undertake this work?	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.
What outputs do you think you will see at the end of this project?	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

	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.
Who or what will benefit from these outputs, and how?	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.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	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

	interests and the contribution to the publication of research articles in high quality biomedical journals will maximise the outputs from this work.
Explain why you are using these types of animals and your choice of life stages.	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.
Typically, what will be done to an animal used in your project?	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 administerd by injection, inhalation, or oral administration. Outcome measures will include physiological assessments for example non invasive temaperature 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

	the anaesthetised state, and invasive physiological montioring undertaken with the use of surgically implanted telemetry devices. 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 therpeutically target a dysfunctional immune response to improve outcomes for inflammatory disease states for which no effective treatments currently exist.
What are the expected impacts and/or adverse effects for the animals during your project?	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 effetcs 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 exagerrated 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.
What are the expected severities and the proportion of animals in each category (per animal type)?	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.

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?	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.
Which non-animal alternatives did you consider for use in this project?	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. We have also considered the use of none-mammalian animal models.
Why were they not suitable?	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. 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.
Enter the estimated number of animals of each type used in this project.	mice: 33,000

How have you estimated the numbers of animals you will use?	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.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	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.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	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.
Which animal models and methods will you use during this project?	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

	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.
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 groupto 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/laban.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.

Project	**47. 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)	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)	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). 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	

	amount of protein goes up or down. 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".
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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
In the context of what you propose to do to 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 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

	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 handed 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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.

Project	**48. 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)	X	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
	X	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 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. 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.	

	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. The majority of diagnostic manufacturers require normal animal sera for the dilution of antibodies and control components. It is a legal requirement to test donated blood for 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)?	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
What species and approximate numbers of animals do you expect to use over what period of time?	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
In the context of what you propose to do to 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 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. Animals used for the production of syphilis pathogens are dosed with antigen once and

	monitored closely for 2 weeks. Animals are weighed prior to the procedure; adverse effects are controlled by pain relief and husbandry refinements. On completion of the schedule, tissue is harvested under terminal anesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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. 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.

Project	**49. 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)	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)	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. 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.	
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

	carry out this procedure for more than 2-3 animals per season. 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. We will monitor the benefit each animal brings to the study and stop adding new individuals once we have sufficient data.
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.	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.

Project	**50. 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
	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 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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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. 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. A review of the most appropriate approach for generating our medicines will be carried out at the beginning of each programme. We will also

	apply the learnings from successful projects carried out on this licence to help build our knowledge of how to make in vitro antibody generation technologies more reliable in future. Furthermore, we aim to introduce new technologies to allow us to deep mine in vivo 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 *in silico* to ultimately replace both *in vivo* and *in vitro* antibody discovery technologies. The datasets generated from in vivo studies are especially valuable as these antibodies have been generated naturally to the target antigen, and then affinity matured by natural in vivo sequence diversification processes, whereas in vitro 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 *in vitro* system that can be used to model the complex antibody generation process that the *in vivo* antibody generation and affinity maturation provides.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the experience gained from the previous licences to guide the design of our studies. 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

	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 *in vivo* studies. We will use randomisation, blinding etc. where appropriate to avoid biases.
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.	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. 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. 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. 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.

Project	**51. 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)	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)	The objectives of this programme of work are: 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	

	either alone or simultaneously in chickens 4. To strengthen the virulence of respiratory viruses by passaging in poultry. 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. 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: To improve understanding on the disease and immunity in poultry infected with respiratory viruses of infectious bronchitis and metapneumoviruses To examine the impact of vaccination in improving respiratory protection against virulent viruses 1. To understand the underlying mechanism

	which confers the protection when on or more respiratory vaccine viruses are given either alone or simultaneously in chickens 2. To strengthen the virulence of respiratory viruses by passaging in poultry. 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, 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:- 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 dead of millions of birds due to diseases and production losses (e.g. poor body weight gain or eggs).

	2. *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 dead of millions of birds due to diseases and production losses (e.g. poor body weight gain or eggs). 3. *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 dead of millions of birds due to diseases and production losses (e.g. poor body weight gain or eggs). 4. *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. 5. *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. 6. *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.
	7.	*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.
	8.	*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 *E coli* and avian mycoplasmas. Thus, antibiotics use can be reduced, which will contribute in avoiding antimicrobial resistance.
	9.	*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 *E coli* and avian mycoplasmas. Thus, antibiotics use can be reduced, which will contribute in avoiding antimicrobial resistance.
	10.	*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 *E coli* and avian mycoplasmas. Thus, antibiotics use can be reduced, which will contribute in avoiding antimicrobial

	resistance. 11. *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 *E coli* and avian mycoplasmas. Thus, antibiotics use can be reduced, which will contribute in avoiding antimicrobial resistance. 12. *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. 13. *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. 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.
What 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 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:- 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 dead 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 diseases 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.
What species and approximate numbers of animals do you expect	The proposed work in this project will use specific-pathogen-free and commercial chicken and turkey breeds that are available in the UK.

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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.

2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	Chickens and turkeys will be used because they are the most susceptible host to the diseases under study. 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. 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.

Project	**52. 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)	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)	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.	

What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to 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 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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 *in vitro* 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having	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

regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	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. 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.

Project	**53. 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)	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)	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	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity?	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

What will happen to the animals at the end?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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). 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.

Project	**54. 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)		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 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	

	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.
What outputs do you think you will see at the end of this project?	Our work is expected to have the following key benefits: − - 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. 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.
Who or what will benefit from these outputs, and how?	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).

	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). 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). 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). 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).
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	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.

Explain why you are using these types of animals and your choice of life stages.	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 Trypanosoma infection, since it is a natural host for these parasites, and can be infected through physiologically relevant routes, recapitulating livestock disease phenotypes.
Typically, what will be done to an animal used in your project?	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.
What are the expected impacts and/or adverse effects for the animals during your project?	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.

What are the expected severities and the proportion of animals in each category (per animal type)?	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.
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 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.
Which non-animal alternatives did you consider for use in this project?	In reaching this point, we have adopted sophisticated in silico models to replace as much in vivo 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 in vitro 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 in vivo models to confirm the most significant findings arising from our *in silico* analyses and in vitro 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.
Why were they not suitable?	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 *in vitro* model. Furthermore, one of our

	chosen parasites, *Trypanosoma vivax*, 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.
Enter the estimated number of animals of each type used in this project.	mice: 700
How have you estimated the numbers of animals you will use?	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. 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 T. congolense and T. vivax fly-bite infections of livestock, and T. congolense and T. brucei 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.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	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.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	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

	bead array, histology, and transcriptomic analysis of both host and pathogen gene expression. Where possible, tissue will also be archived for future investigations. 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.
Which animal models and methods will you use during this project?	We will use a mouse model of trypanosome infection. Mice will be immunised with candidate peptide antigens, and then challenged with *T. congolense* or *T. vivax* via Tsetse fly bite. This mimics natural infection.
Why can't you use animals that are less sentient?	Ultimately, we hope to develop a vaccination that protects against *T. congolense* and *T. vivax* 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 Trypanosoma 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.
How will you stay informed about advances in the 3Rs, and implement these advances effectively,	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

during the project?	field to keep informed of any developments in our particular model. We will continuously seek to refine our experimental procedures to reflect new advice.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	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. 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 *T. vivax*, at which point antibodies can be detected in the lymph nodes. Much of our analysis to determine correlates of protection could therefore 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.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	We will refer the 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.

Project	**55. 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)	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)	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. 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	

	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. 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. 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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

	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

	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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. 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

	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.

Project	**56. 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)	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)	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 dysentry. 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	

	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. 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. Furthermore there are no vaccines to prevent diarrhoeal disease caused by *Shigella*. 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 *Shigella* based on the natural route of 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)?	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 Neisseria gonorrhoeae and Shigella, 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. Neisseria gonorrhoeae and Shigella spp.. have developed resistance to multiple antibiotics, and we are now faced with

	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.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year course of the licence, we will use up to 9,000 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 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 N. meningitidis (Protocol 2), mice might developed 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

	than the development of signs and symptoms. For infection with Shigella (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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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. 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. For the protocol that involves mice receiving live *N. meningitidis* (a cause of meningitis, Protocol 2), we

	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 takes 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	**57. 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)	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. 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. 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	

	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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	400 mice, 400 rats, 120 rabbits, 180 sheep and 180 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 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.
Application of the 3Rs	
1. Replacement	The formation of adhesions is a complex process involving many different components within the

State why you need to use animals and why you cannot use non-animal alternatives	body (blood, lymph, enzymes, etc) all interacting and as such a complete live animal is needed to form adehsions 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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. 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. 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.
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	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 intial 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

(harms) to the animals.	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. 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. 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.

Project	**58. 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)	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)	To produce unique strains of genetically altered Laboratory mice as required by various research programmes within the establishment. To remove potentially harmful diseases and organisms through a process called rederivation thus improving their health status To freeze tissues from genetically altered mice for future use. 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.	

What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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
In the context of what you propose to do to 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 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

	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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) the can produce in a short space of time, a large number of genetically identical animals. nevertheless, we are constantly reviewing procedures and current literature to look for alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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. Impact on the animals' welfare will be minimised

	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. 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. We are in regular communication with similar animal units to discuss better ways of doing things and pass on what we have learned

Project	**59. 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?	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. 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. 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. Much of the research is aimed at developing techniques to reduce the future need for animals.
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?	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. 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.
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,

	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.
Typically, what will be done to an animal used in your project?	Animals are immunised by injection with the material to which a response is sought and subsequently blood samples are withdrawn. IgY Polyclonal Antibodies are produced by immunising chickens and collecting the eggs to obtain and purify the IgY.
What are the expected impacts and/or adverse effects for the animals during your project?	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.
What are the expected severities and the proportion of animals in each category (per animal type)?	The severity limit for this work is mild and no adverse effects are expected. 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. Cattle – 200 Sheep – 75 Pigs – 50 Poultry – 100 Goats – 30 Camelids – 50
What will happen to animals at the end of this project?	kept-alive, rehomed, used-in-other-projects
Why do you need to use	Alternative methods for antibody production are used

animals to achieve the aim of your project?	where available however, there are currently no methods available for the production of specific polyclonal and monoclonal antibodies using non animal alternatives. Much of the research is aimed at developing techniques to reduce the future need for animals.
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.	fowl: 100 pigs: 50 goats: 30 sheep: 75 cattle: 200 camelids: 50
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.

Which animal models and methods will you use during this project?	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.
Why can't you use animals that are less sentient?	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. The animals that are to be used in this project need to have For blood collection the products needed are species specific.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	Through regular contact with advisory bodies and effective staff training.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	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.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	Work will follow published monographs, LASA Guidelines on administration of substances and NC3Rs Guidelines on blood sampling.

Project	**60. 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)	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)	(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 *in vitro*. 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

	mechanisms can be identified in the laboratory through simplified cell culture and fruit fly approaches.
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.	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.

Project	**61. 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)	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)	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. 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 diesases. We will test this exciting possibility in diverse models of degenerative diseases. The 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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have discovered novel, powerful and straightforward approaches to rescue form failure of protein quality control. We have a group of pharmacological modifiers with high potential to ameliorate protein misfolding diseases. 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. 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. The work in mice we propose to carry out is essential to validate our discoveries and may have a big impact on human health.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Mouse breeding will be carefully monitored to ensure that surplus animals are not generated. 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. To reduce the sources of variability and bias mice will be randomly assigned to experimental groups. Experiments will be run in a blind fashion. Cryopreservation will be used to preserve important lines and remove the necessity to hold

	stock for extended periods.
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.	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. 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. 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.

Project	**62. 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)	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)	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.

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.	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. 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.

Project	**63. 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)	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: 1. identify drugs which combined with radiotherapy are more effective against tumours located just	

	under the skin, 2. see if a high fibre diet might be as effective as adding a drug, 3. finding drugs and dietary effects which cause minimal effects to the normal tissues surrounding the bladder when combined with radiotherapy, 4. to test the most promising treatment combinations in mice which have the tumour growing in the bladder, 5. to test strategies to prevent tumours spreading into the bladder wall muscle 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. 7. to test if feeding normal mice and germ-free mice with the gut bacteria from mice and humans alters their responses to radiotherapy. If successful, such treatments could be tested in humans in clinical trials.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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

	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. 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.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice and expect to use approximately 5,600 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 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. 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.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. We will keep the numbers of animals used in the early test experiments to an absolute minimum, usually 2 or 3 per group. 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. 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.
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 mice as they are the animal species where most is known about how tumours respond to drugs and radiation. 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.

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	**64. 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?	The objective of this project is to produce reagents for either direct use in diagnostic tests or the improvement of them. The Unit producing these reagents is responsible for producing approximately 300 different polyclonal antibodies and 50 antigens for diagnostic work.	
Why is it important to undertake this work?	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. The Unit producing these reagents is responsible for	

	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.
What outputs do you think you will see at the end of this project?	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. For example the antibodies against different salmonella serotypes will allow epidemiological investigation of disease outbreak, preventative action and indirectly support publications and other communications. Reagents are also sold commercially for similar reasons.
Who or what will benefit from these outputs, and how?	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. These reagents are also supplied to several research groups therefore minimizing the numbers of animals required.
Will this work be offered as a service to others?	Yes
How will you look to maximise the outputs of this work?	This is a service licence with production of these biological reagents is carefully linked to demand.
Explain why you are using these types of animals and	The animals selected have been proven to be the best model to produce the material of the best specificity

your choice of life stages.	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. 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.
Typically, what will be done to an animal used in your project?	**Protocol 1 Polyclonal antiserum production in rabbits** 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. For short (somatic) programme which lasts 19 days, 5 inoculations followed by exsanguination under terminal anaesthesia. For long (flagella) programme which lasts 35 days, 6 intravenous inoculations followed by exsanguination under terminal anaesthesia. For the Adjuvant (somatic) programme which lasts 43 days, 4 inoculations either subcutaneous or intramuscular followed by exsanguination under terminal anaesthesia. **Protocol 2 Production of Marek's Disease Virus Antisera** 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. **Protocol 3 Production of Marek's antigen** 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

	suitable antigen harvest.
What are the expected impacts and/or adverse effects for the animals during your project?	Protocol 1. As the inocula have undergone inactivation procedures, no adverse effects are anticipated. With the protocol using adjuvants there may some localised swelling or inflammation around the injection site. 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. 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.
What are the expected severities and the proportion of animals in each category (per animal type)?	Rabbit - all mild severity Chicken - mild severity for antisera raising, 50% moderate for the MDV antigen raising (rest mild).
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?	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. 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.

Which non-animal alternatives did you consider for use in this project?	Use of recombinant antibodies from genetically modified cell lines. In-vitro (cell culture) production for Marek's Disease Virus.
Why were they not suitable?	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. 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.
Enter the estimated number of animals of each type used in this project.	rabbits: 1000 other-birds: -
How have you estimated the numbers of animals you will use?	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. 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.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	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.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	These reagents are also supplied to several research groups therefore minimising the numbers of animals required.
Which animal models and methods will you use during this project?	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.
Why can't you use animals that are less sentient?	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. 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.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	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.
How will you refine the procedures you're using to minimise the welfare costs	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,

(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	**65. 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)	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)	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our laboratory is combining experimental analysis in mouse with mammalian cells grown in cutlure as well as using the fruit fly *Drosophila melanogaster*. All the molecules and molecular pathways that we are studying in mice are also present in *Drosophila*. In our research pipeline, we are starting our analysis of the YAP protein and other proteins in the Hippo pathway in *Drosophila* before confirming our results in mammalian cell culture and later in mice, *in vivo*. 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 *Drosophila* has just a single one, Yorkie. Ultimately, we must perform these experiments in mice to establish the relevance of these genes to human development and disease. Our work in *Drosophila* 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. 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 *in situ* may be used for.

2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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. Once we will acquire significative 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.
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.	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. 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. 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

	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. 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.

Project	**66. 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)	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)	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	

	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. Our main objectives are to: 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.
What 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 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.
What species and approximate	Over a 5 year period: 2720 mice (2400 for

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.

	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.
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 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.

Project	**67. 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)	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)	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. 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	

	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 develolpment, 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 Xenopus laevis and Xenopus tropicalis. 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 *Xenopus* 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.

Project	**68. 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)	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)	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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. We will also use theoretical modelling approaches with the help of computers to replace animal experiments where possible and

	to help us formulate better supported scientific questions for our animal experiments (see refinement).
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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. 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. 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.

Project	**69. 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)	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)	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	

	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. 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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)
In the context of what you propose to do to 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 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

	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.

Project	**70. 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)	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)	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 suffers 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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 suffers and healthy human volunteers. These samples will be obtaining 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. In addition to this, we will use experimental

	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.
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 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. 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. 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.

Project	**71. 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)	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)	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 *in vitro* models as investigative tools whenever possible, and a large part of this work uses *in vitro* tissue culture techniques. However, at this point it is necessary to use animals to answer questions

alternatives	about VGSC function during metastasis *in vivo*. 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use *in vitro* 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.
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.	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.

Project	**72. 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)		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?	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	

	blood called the platelet. 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. 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. 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.
What outputs do you think you will see at the end of this project?	We expect the following outputs by the end of the programme of work in this project: - publication of new insights into platelet receptor function in thrombosis and haemostasis in scientific journals - identification of potential new drug targets for thrombotic disease
Who or what will benefit from these outputs, and how?	**Immediate 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. **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. **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. **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.
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

	thrombosis experiment before being culled by a Schedule 1 method. 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.
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)?	The major severity category is mild. Less than one in twenty of the animals will experience a moderate level of severity due to: - 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?	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. 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.
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

take during the experimental design phase to reduce the number of animals being used in this project?	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. 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.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	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. 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.
Which animal models and methods will you use during this project?	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.
Why can’t you use	We cannot use non-mammalian species for this work, as

animals that are less sentient?	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. The majority of procedures outlined in this project will be under terminal anaesthesia, and therefore harm to the animals will be low.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	We will stay informed by advances in the 3Rs through attendance of seminars and conferences, as well as discussions with the NVS and NACWOs. We will review each experiment on completion to determine any refinements that can be applied to future experiments. 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. 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 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.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	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.
What published best practice guidance will	Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be

you follow to ensure experiments are conducted in the most refined way?	generated in the experiment. Experiments will be conducted in accordance with the guidelines published by the Laboratory Animal Science Association (LASA). The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE guidelines.

Project	**73. 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)	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)	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	

	health. 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 1450 rats and 1060 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?	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

	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).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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

	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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.
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 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. 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.

Project	**74. 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)	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)	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. 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	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use a maximum of 15,000 mice over 5 years, the majority of which will be genetically altered.
In the context of what you propose to do to 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 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 anesthetised 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
	harm.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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. Our experiments also involve cell lines, to inform us about the function of altered glutamate receptors before testing them in neuronal settings.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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.
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 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

minimise welfare costs (harms) to the animals.	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. 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' 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.

Project	**75. 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)	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)	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,	

	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 *BDNF* 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
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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
	(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.

to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	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 aneasthetised throughout and do not recover.

Project	**76. 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)	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)	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	

	factors that can lead to faulty regulation of angiogenesis. The aim of this study is to develop a better understanding of how angiogenesis effects 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.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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

	techniques can potentially be used in detection and assessment of disease in patients.
In the context of what you propose to do to 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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Alternatives to animals cannot be used to meet the aims and objectives of this work at present, because *in vitro* models of angiogenesis differ fundamentally from the *in vivo* 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.

	However, we are also continuing to refine our *in vitro* methods and, if successful, these will lead to a degree of replacement of animals in future research. Notably, data collected from experiments outlined in this protocol will be used to develop both a novel 3D in vitro model (placenta-on-a-chip) and an in silico 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. By using appropriate in-vitro systems throughout we will reduce animal usage significantly. We share tissues and data with others where 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.	

Project	**77. 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)		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 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	

	infarction (heart attack) and brain infarction (stroke). 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.
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 imposssibility 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 plateklet 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 estab-lished 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?	2. Non-sentient animals 4. Productioin of genetically modified platelets using gene silencing in vitro from bone marrow culture or stem cells.
Why were they not suitable?	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. 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. 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. 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.
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 animnal material. Surplus animals and tissue that is not nof interest for our ptojects is routinarily shared with other
plan to use in your project?	investigators

Which animal models and methods will you use during this project?	Mice are the lowest vertebrate group amenable to genetic modifications on which well characterised models for the study of platelet function are established. 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. Power calculations have been performed to enable the minimal use of animals to obtain statistically meaningful data.
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	**78. 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)	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)	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). Despite high prevalence and acute nature of	

	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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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 in 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.
In the context of what you propose to do to 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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 aviod 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

	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. The mice (and not non-protected animal alternatives) have been chosen because: a) the system of blood clot development in mice in 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
2. Reduction Explain how you will assure the use of minimum numbers of animals	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

	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.
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 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. 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 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

	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.

Project	**79. 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)		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
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	

	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. 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.
What are the potential benefits likely	The overall aim of the programme of work is to

to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	Cattle 2000 Pigs 1900 Sheep / goats 2000 Chicken 2600 Turkey 700 Rabbit 700
In the context of what you propose to do to 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 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

	animals. Where this cannot occur (an unregistered product for example), animals will be humanely euthanised and the carcases will be incinerated. 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
3. Refinement Explain the choice of species and why the animal model(s) you will use	The animal species we propose to use are as dictated by European guidance documents. In most cases the adverse effects are likely to be

are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	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. 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. 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. 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. Animal husbandry is to a very high standard, by experienced staff following up to date guidelines and regulations. Prompt veterinary

	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.

80. Salmonella Virulence

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Bacterial, pathogen, virulence, immunity, antibiotics, Salmonella

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

In this project we will study mechanisms by which *Salmonella* causes disease and how our immune system works to resist this. Infection of humans with *Salmonella* can lead to gastroenteritis and typhoid fever, depending on the strain type. It is estimated that over 90 million cases of *Salmonella* gastroenteritis and 13 million cases of typhoid fever (with approximately 130,000 deaths) occur globally each year. There is no vaccine against *Salmonella* that cause gastroenteritis and current typhoid vaccines do not work well for everybody.

Infection of humans with *Salmonella* Typhimurium usually results in self-limiting gastroenteritis but this strain causes a systemic 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.

A large part of *Salmonella* 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 *Salmonella* 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 *Salmonella*, 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.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

Through this research we are likely to discover new processes of pathogen and host cell biology, which could have implications for other 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.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Approximately 6000 mice will be used over the 5 year period.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of 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?

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.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

Our aim is to understand how *Salmonella* (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 *Salmonella* 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 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.

Reduction

Explain how you will assure the use of minimum numbers of animals.

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.

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.

Mice are the most appropriate species in which to model systemic infection by *Salmonella*. 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.

81. Salmonella Virulence

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Bacterial, pathogen, virulence, immunity, antibiotics, Salmonella

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

In this project we will study mechanisms by which *Salmonella* causes disease and how our immune system works to resist this. Infection of humans with *Salmonella* can lead to gastroenteritis and typhoid fever, depending on the strain type. It is estimated that over 90 million cases of *Salmonella* gastroenteritis and 13 million cases of typhoid fever (with approximately 130,000 deaths) occur globally each year. There is no vaccine against *Salmonella* that cause gastroenteritis and current typhoid vaccines do not work well for everybody.

Infection of humans with *Salmonella* Typhimurium usually results in self-limiting gastroenteritis but this strain causes a systemic 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.

A large part of *Salmonella* 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 *Salmonella* 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 *Salmonella*, 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.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

Through this research we are likely to discover new processes of pathogen and host cell biology, which could have implications for other 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.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Approximately 6000 mice will be used over the 5 year period.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of 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?

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.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

Our aim is to understand how *Salmonella* (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 *Salmonella* 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 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.

Reduction

Explain how you will assure the use of minimum numbers of animals.

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.

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.

Mice are the most appropriate species in which to model systemic infection by *Salmonella*. 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.

Project	**82. 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)	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)	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	

	but the mechanisms behind this process are still unclear. 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to 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 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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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

	they show any signs of suffering. All procedures have been refined to use the most humane techniques possible to reduce distress to the mice.

Project	**83. 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)	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)	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	

	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. Peripheral nerve damage is seen in approximately 3-4% of all trauma cases and another part of Schwnan 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.
What 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 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 schwanomas 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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
	50% will have a mild or moderate phenotype.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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
	function and tumour development. This is why we require the use of mice and rats for this work.

2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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.
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 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

	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 .

Project	**84. 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)	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)	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	

(how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	This project uses zebrafish, a small tropical freshwater fish species. Adult fish are maintained for breeding purposes to generate embryos and larvae which are them 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.
In the context of what you propose to do to 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 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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. (3) Using statistical tests, where possible, to ascertain the minimum number of animals required to produce statistically robust datasets.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having	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

regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	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.

Project	**85. 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)	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)	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	

	patients. 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. 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. 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. 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could	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

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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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

	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 rats and mice because these species have told us quite accurately about many aspects of pain processing in humans. 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. We will use great care in ensuring that rats and mice are well maintained and suffer minimal

	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.

Project	**86. 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)	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)	**Our project aims to begin to tackle the following current unknowns:** 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	

	to it. **Overall Aims:** 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. **Specific Objectives:** 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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: 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-dependent differences in obesity and health problems linked to it. 9. Increase our understanding of new ways to prevent or treat obesity and/or the health problems linked to it.

What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to 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 METHODS USED AND HARMFUL EFFECTS** 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 to 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. By changing specific genes we can learn how

	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

	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. The results and new knowledge gained from our work will be made publicly available.
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; 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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

	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. 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. 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. 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.
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.	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. 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

	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. 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. 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.

Project	**87. 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)	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)	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	

	(*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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to the animals, what are the expected adverse effects and the	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

likely/expected level of severity? What will happen to the animals at the end?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of	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

animals	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.
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.	**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. **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. **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 obtained meaningful outcomes that stand up to statistical testing and scrutiny by other scientists. Doses of drugs and routes of administration will be chosen so as not to have adverse effects.

Project	**88. 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)	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)	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

	canine osteoarthritis. In each of the protocols we aim to emulate some aspect of the human pathbiology 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. Our program of work will principally investigate: 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.
What 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 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.
What species and approximate numbers of animals do you expect	We will use a maximum of 9,000 rats and mice, mainly mice over a period of five years. The

to use over what period of time?	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.
In the context of what you propose to do to 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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. *In vitro* 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 *in vitro* 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

	integrated, organ-level, physiologically intact environment in which such responses are normally coordinated. These *in vitro* 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).
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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. 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).

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 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 *in vivo* approach to address questions in bone and joint mechanobiology. 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).

Project	**89. 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)	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)	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.	

	Therefore there is a lot of interest in *i)* understanding more of the basic biology of cancer and metastasis; and *ii)* use this basic information to find novel therapeutic targets and avenues. 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. 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. 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. 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.
What 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 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

	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
What species and approximate numbers of animals do you expect to use over what period of time?	3000 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 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

	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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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
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.	Two animal models will be used: 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

	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	**90. 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)	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)	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 of 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

	the simultaneous activity in their brain is recorded. In some studies, the function of specific neurons may 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.
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 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,

	because other approaches such a 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.
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 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.
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.	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.

Project	**91. 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)	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)	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	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to 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 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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	It is essential when animals are used in experiments to ensure that too many animals are not used unnexcessarily, but equally that a sufficient number of animals is used to allow clearly experimental conclusions to be drawn

	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. 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. 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
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 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. 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

	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 eachlung, 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 bysuitable 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	**92. 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)	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)	The objectives of this licence are to: Determine the response of skeletal tissues to mechanical and biological stimulation. Enhance the integration of prosthetic implants with tissues. Develop strategies to regenerate skeletal tissues.	
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	

humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years: Mice 200 Rats 300 Sheep 700
In the context of what you propose to do to 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 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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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 animals will be used in order to achieve statistical differences.

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.	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.

Project	**93. 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)	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)	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	

	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 (*Poecilia reticulata*) 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).
What 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 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

	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.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years we expect to use approximately 5100 fish. Most will be guppies (n=3300, *Poecilia reticulata* and/or congenerics *P. wingei*, *P. obscura*). We will also use their close relatives in the family Poeciliidae, including fish from the swordtail/platie species complex (Xiphophorus spp, n=900) and black-barred limia (*Limia nigrafasciata*, n=450). We will also use some small freshwater fish from the minnow (cyprinidae) family (specifically zebrafish n=450).
In the context of what you propose to do to 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 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

	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,

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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

	improvement to improve welfare in captive populations of this widely used scientific 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.	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). 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). 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.

Project	**94. 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)	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)	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	

	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. 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	This project proposes to use mice. Approximately 5900 mice will be used over five years (1180 mice 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?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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

	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.
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.	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. 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.

Project	**95. 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)	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)	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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
3. Refinement	The choice of species is mice, because they are the smallest mammal that is routinely

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

Project	**96. 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)	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)	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 from the project)?	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 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 is 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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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). 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

	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	**97. 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)	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)	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). Additionaly, 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 minimum numbers of animals	We assure the use of minimal animal numbers by use of calculations based on statistical significance and use of power calculations as valid.

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.	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.

Project	**98. Studying neuron-glial 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)		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 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	

	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.
What outputs do you think you will see at the end of this project?	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

	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.
Who or what will benefit from these outputs, and how?	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.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	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.
Explain why you are using these types of animals and your choice of life stages.	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

	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.
Typically, what will be done to an animal used in your project?	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
	regeneration of brain cells in disease-like states.

What are the expected impacts and/or adverse effects for the animals during your project?	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.
What are the expected severities and the proportion of animals in each category (per animal type)?	We do not study animals that exhibit severe adverse effects. 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. 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. 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

	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 cnstruct 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?	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. 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

	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 indvidual 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

	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.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	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.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	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.
Which animal models and methods will you use during	We use zebrafish to study neuron-glial interactions during nervous system formation, function, disruption

this project?	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 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. 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. 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.
Why can’t you use animals that are less sentient?	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.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	REDACTED. We will implement appropriate advances through discussions with our local vets and named animal care and welfare officer.
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	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.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	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.

Project	**99. 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: ● 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.	• 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”. In work done in previous projects, refinements to the precise location of the immunisation site and the technique used have minimised inflammation. Monitoring sheep for general welfare and for any adverse reactions is regularly conducted.wer

Project	**100. 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)	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)	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.	

What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at	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-

the end?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 *in vivo* 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.
2. Reduction	Calculations are carried out to determine the necessary number of animals for each

Explain how you will assure the use of minimum numbers of animals	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.
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 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. 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.

Project	**101. 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)	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)	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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. 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.

Project	**102. 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)	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)	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	

humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	It is estimated that approximately 7000 mice will be used during the 5-year course of this proposal.
In the context of what you propose to do to 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 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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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

	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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

	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.

Project	**103. 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)	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 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	

	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	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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. 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,

	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. 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.

Project	**104. 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)	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)	**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	

	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. **Our solution :** We wish to apply our knowledge of the molecular pathways that cause liver 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 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** 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.
What 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 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

	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 complexitys and test the use of new therapies without using animal models. 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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

	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. 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.
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.	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

	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. 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.

Project	**105. 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)	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)	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	

	our understanding of these processes. 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, ~30,000 over 5 years Xenopus, 150 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 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

	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 Xenopus (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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 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. 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 *in vitro* 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

	example, mice carrying a mutation that causes neonatal diabetes in humans should help us understand precisely what causes the human phenotype.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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 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. 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 appropriate.

Project	**106. 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)	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)	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	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	Rodents (predominantly mice, some rats), approximately 5,000 over the 5 year 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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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
	animals.

2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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. 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.
Project	**107. 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)	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)	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	

	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. 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. 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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

	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.
What species and approximate numbers of animals do you expect to use over what period of time?	This application will support the work of 5 scientists over 5 years. We propose to use up to 17000 mice including genetically modified 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?	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.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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

	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	**108. 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)	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)	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. 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	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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 is we use them again.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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. There is no *in vitro* model available. Culturing of nematodes *in vitro* 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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

	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. 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.
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 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.

Project	**109. 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
	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 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	

	suitable for scientific research. 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. As a result of naturally occurring genetic mutations, certain animal strains will display similar diseased states to that of humans e.g: ● 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. 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.
What 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 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

	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 bought 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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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.
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	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

general measures you will take to minimise welfare costs (harms) to the animals.	requirements. 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. 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. 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. Animals will be housed in optimal social groups, allowing for a reduction in potential aggression or overt dominance behaviours, thus reducing any associated stress. 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.

Project	**110. 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)	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)	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	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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
	from each 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?	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 cages 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

	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 temporally 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

	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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have adopted modern methods of measuring blood pressure, renal sympathetic activity, renal or carotid blood flow using radio-transmitters. This are viable for 3-4 months. This increased longevity and the development to measure multiple parameters within the same rat (e.g.

	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.
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.	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.

Project	**111. 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)	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)	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 structrues 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	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will involve up to ~ 20 homing pigeons (Columba livia), which will be flown over the duration 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?	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
	establishment after the end of the project.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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: ● 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

	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. ● Final protocols will involve the simultaneous collection of multiple data types. This will reduce the number of overall trials. 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.
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 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. 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

	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. Protocols will be refined to keep handling of animals to a minimum. 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. 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.

Project	**112. 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)	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)	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.	

	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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	All our in vivo experiments will be performed in mice. We expect to use approximately 7,500 mice over 5 years.
In the context of what you propose	The majority of the experiments will involve

to do to 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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 *in vitro* modelling of candidate human disease variants in immortalised human podocytes, to generate preliminary data and select the most promising candidates for in vivo study.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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

	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.
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 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. 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

	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.

Project	**113. The genetic control of development in the life cycle of the parasitic nematode Strongyloides 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)	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)	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 discoverer 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) to the animals.	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 which do not cause noticeable harm to them.

Project	**114. 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)	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)	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	

	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to 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 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

	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 a 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.

	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.

Project	**115. 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)	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. The data will be used to review substances under development or satisfy governmental requirements necessary for approval of clinical trials (dosing in	

	humans or animals) or bringing products to market. 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. 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).
What 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 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 protections 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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 future needs.

In the context of what you propose to do to 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 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. 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). 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

	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. If an animal is suitable for rehoming as a companion animal, after being considered suitable by a Vet, this may be allowed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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 alone.

2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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. 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.
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.	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. 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. 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. All animals are monitored for signs of any adverse effects on their health or wellbeing, and to prevent

	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). 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. 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. 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. 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.

Project	**116. 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)	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)	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 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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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

	to gain more information from the data, so potentially increasing statistical power and enabling a reduction in numbers.
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.	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. 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. 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.

Project	**117. 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)	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)	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	

humans or animals could benefit from the project)?	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).
What species and approximate numbers of animals do you expect to use over what period of time?	The estimated numbers of mice to be used are 10,000 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?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-	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

animal alternatives	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
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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.

Project	**118. 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)	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)	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	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to 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 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

	gene modifiers to test their ability to ether 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), intra-venously (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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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)	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

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 effecst 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	**119. 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)	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)	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. Over the past two decades we have identified pathways operative during an inflammatory response that help inflammation to resolve and	

	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. The aims and objectives of this programme of work are therefore to: 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.

In the context of what you propose to do to 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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-	Where possible we perform experiments using human samples from both healthy people and patients with inflammatory conditions and aim to

animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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.
3. Refinement Explain the choice of species and	The mouse is the most appropriate species to conduct these studies as it is the lowest

why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	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. 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. 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. 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.
Project	**120. 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)	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)	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. 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	

	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

	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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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,

	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 then 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.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, 60,000 over 5yrs.
In the context of what you propose to do to 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 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

	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 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

	progress and the mice show no clinical sings 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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

	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.

Project	**121. 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)	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)	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. Current standard therapies (e.g. non-steroidal anti-inflammatory drugs, NSAIDs) are often inadequate	

	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. 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 750 rats will be 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?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 *in vitro* 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 *in vitro* therefore need to be studied in the whole animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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

	additional pharmacokinetic studies or anatomical studies will also be incorporated.
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.	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.

Project	**122. 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)	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)	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?	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.
Application of the 3Rs	
1. Replacement	To understand the effects of parasites, pollutants and other factors on seabirds, the

State why you need to use animals and why you cannot use non-animal alternatives	only option is to work on wild birds living in their natural environment
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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.

Project	**123. 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)	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 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: 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	

	tadpole body and subsequent escape behaviour? 3. How do ionic pump proteins in the nervous system regulate tadpole swimming?
What 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 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 Xenopus 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.
What species and approximate numbers of animals do you expect to use over what period of time?	A colony of about 50 males and 50 female Xenopus laevis 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.
In the context of what you propose to do to 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 regulated procedure is needed to inject hormone into adult Xenopus 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

	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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 *in situ* where the connectivity is intact and animal behavioural outputs can be monitored: - 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have the following ways to reduce the number of animals and protocols required: ● 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
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 use of *Xenopus laevis* for neurobiological studies has a long history. *Xenopus laevis* 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. We use tried and tested measures to minimise

	animal welfare costs: - 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.

Project	**124. 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)	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)	**Purpose of the project.** 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;	

	(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. **Clinical needs.** 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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”.
What species and approximate numbers of animals do you expect to use over what period of time?	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

	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 foetuses for the study. Mated mice will be killed to remove embryos and foetuses for the studies or recycled into the breeding colony.
In the context of what you propose to do to 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 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

	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

	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.
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 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.

Project	**125. 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)	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)	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	

	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

	mouse colony will be managed efficiently in order to avoid breeding excessive mice.
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 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.

126. The role of centrosome-dependent cell cycle regulation in mammalian development

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Development, Stem cells, Cell cycle

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

All living things are composed of cells that multiply through cell divisions. Centrosomes are small cellular structures that produce and organise microtubules, protein polymers with vital roles in cell division, cell-to-cell communication and in cellular architecture. The intricate design of the centrosome requires the coordinated assembly of over one hundred different proteins, each in multiple copies. Inheritance of faulty centrosomal proteins is known to cause a range of human disorders, including proportional dwarfism and abnormally small brain (i.e. microcephaly). In addition to small body and brain size, mice with faulty centrosomal proteins, also show impaired blood production with too few red blood cells. We do not understand how and why defective centrosomal proteins result in these abnormalities.

The data gathered by our team so far point to the existence of important crosstalk between centrosomes and the cell division cycle; the latter is a highly ordered series of events that collectively ensure the correct division of one cell into two daughter cells. In particular, faulty centrosomes seem to prevent cells from undergoing normal number of division cycles not only in the early embryo, but also during blood development. The aim of this project is to uncover the mechanisms by which abnormal centrosomes influence the cell division cycle in different tissues during embryo development. Because centrosome defects are common in many types of human cancers, our results will improve our understanding of how these defects might impact the growth of cancer cells.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these

could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

Certain mutations in our genes lead to developmental disorders such as dwarfism or microcephaly. We propose to use animals to model these disorders in order to improve our understanding of the link between centrosomes and organismal growth. In particular, it is important to reveal how defects in centrosomes cause major physiological perturbances. Our study will also improve our understanding of key cellular mechanisms responsible for determining normal body and brain size. In addition, since abnormal centrosomes can also be found in the majority of human tumours, this study could identify cellular pathways that sense these abnormalities. Modulating such pathways in cancer cells may open up new therapeutic strategies.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Number: 5420 mice; Period: 5 years

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of 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?

Mouse strains with centrosomal gene mutations exhibit small body and brain size but otherwise develop and age normally; and show no sign of discomfort. The Cdk5rap2 mutant strain also has fewer but abnormally large red blood cells, a condition called macrocytic anaemia. Although biologically significant, the anaemia is subclinical in this strain and has no adverse effect on the animals. Both Cdk5rap2 and Cenpj mutants have a normal, healthy lifespan, indicating that the impact of these mutations is greatest during development. The new cell cycle reporter strains that will be created to enable visualisation of the cell cycle are expected to be healthy; several combinations of these reporters exist already and none of these mouse strains have been reported to show adverse phenotypes. We have been using animals lacking the p53 protein, which controls the cell cycle and is involved in preventing tumour growth. These animals develop tumours after 3 months of age, but in our proposed work the majority of animals will be used for breeding prior to this age. If animals develop tumours despite their young age, these tissues will be collected for analysis. Informed by our results obtained with the cell cycle reporters, we may acquire additional cell cycle mutant strains and amend our licence accordingly. Such strains are likely to be well characterised, so we would be able to plan for any adverse effects that might arise. The substances administered are not expected to cause any lasting adverse effects. Of itself, injection of substances will cause no more than transient discomfort and no lasting harm. In all cases, the general health and condition of an animal will remain the overriding determinant. Mice will be killed if they show signs of ill health, such as piloerection, hunched posture, inactivity or inappetence,

which cannot be alleviated by minor veterinary intervention.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

Prior to embarking on animal experiments, we will collect as much evidence as possible from studying cultured cells and cell-derived organoids. There are two reasons why we cannot exclusively use these in vitro models to attain our goal. First and foremost, our study aims to understand mutations that interfere with embryonic development and stem cell function. These are complex physiological processes that cannot be effectively modelled in culture. Therefore, this work necessitates animal models. Second, for in vitro experiments cells must be removed from their natural environment. Interaction with this environment may affect their capacity to divide, survive or die, and thus isolated cells may not reflect the process that takes place in intact tissues/organs. The genetically altered mice selected for this work are valid models for human developmental diseases, as indicated by their shared characteristics.

Reduction

Explain how you will assure the use of minimum numbers of animals.

Over the past 4 years, we established that centrosomal gene mutants exhibit abnormalities during development but otherwise they have a normal and healthy lifespan. This has prompted us to focus on embryonic development, and as a result this current application will primarily use early embryos, thereby reducing the number of runty adults produced. When designing experiments, we will perform statistical analyses to ensure that we use the minimum number of mice per group that will be informative, including carrying out pilot experiments.

To reduce the number of mice in our colony, when a strain is not required for a certain period of time, stocks of frozen sperm and embryos will be made. This will help to avoid unnecessary breeding, reducing our animal usage.

We will minimise use of animals by teaming up with other research groups interested in surplus tissues from same animals that are not used for this study. To maximise the information from a single animal, we will aim to collect samples from most organs. These samples may be shared with other scientists to minimise the breeding of further animals

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 already demonstrated that genetically altered mice strains can mimic a range of clinical features present in patients with primordial dwarfism. We therefore believe that despite their small size, mice are a good model system to study growth retardation. When similar genes are mutated in lower vertebrates such as zebrafish, the phenotypes are very crude (i.e. kinky tail) and as such are less informative.

We aim to understand a human developmental disease caused by homozygous mutations in a single gene, meaning that all tissues of patients carry only the faulty copy of this gene. To best mimic this scenario, we need to generate mice that produce only the faulty gene (called homozygotes). If homozygotes exhibit a harmful phenotype or are infertile, we will breed heterozygotes (animals that have one working and one faulty copy of the gene; but appear completely normal) to maintain the colony and use these to generate homozygous embryos for experiments. In the case of centrosomal gene mutations, homozygotes show no suffering with small body size and low weight being their only distinguishing features. Moreover, given that homozygous embryos can be obtained by breeding heterozygous adults the use of early embryos substantially reduces the need to produce homozygous adults.

Project	**127. 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)	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)	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.	

	our work in mice.**REDACTED.** 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	We should use no more than 1000 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?	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.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.**
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.	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.

Project	**128. 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)	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)	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	

	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. 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. 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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
	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.

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 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.

Project	**129. 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)	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)	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	

	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. The objectives include 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?
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats (n=250) Mice (n=5060) Over 5 years
In the context of what you propose to	Animals are likely to lose weight after

do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 *Clostridioides difficile* (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.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We have consulted the departmental statisticians on experimental design and group size to minimize the number of animals used and maximise the information gained. 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.
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.	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. 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'.

Project	**130. 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)	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)	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 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. 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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

	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.
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.	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. 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.

Project	**131. 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)	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)	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	

	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. 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity?	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

What will happen to the animals at the end?	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.
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 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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

	giving us confidence that mice are an appropriate species to use in our studies. 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.

Project	**132. 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)	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)	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.

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.	REDACTED. Additionally, the dosage of any intervention required will be more similar to that for a human. 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.

Project	**133. 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)	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)	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.	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	It is estimated that up to 430 wild type and genetically altered mice will be used for this project over a 3-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?	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 intracolonically at low volumes to anaesthetised mice, at the lowest possible therapeutic concentration, in order minimise

	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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 *in vitro* or *in silico* assays, to recapitulate the human IBD are completely missing. For the identification of the substances to be tested in mice, we have employed cell-based assays. By employing *in vitro* assays, we replace a large number of mice that would be required to test the whole range of compounds.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will analyse the effects of the tested substances on functional endpoints relevant to IBD, the inflammation and mucosal healing *in vitro*. 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. 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. The variation between individual mice and the variable development of the disease has been taken into consideration in order to ensure the

	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.
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 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. 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. 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. In the DSS model, upon each cycle of DSS administration animals experience gradual weight loss and reduced stool consistency. Progressively,

	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 relief 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	**134. 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)	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)	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 procedues. 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

	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.
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.	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. The health of animals throughout all procedures will be monitored daily.

Project	**135. 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)	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)	1: To investigate how tolerance develops to opioid drugs. 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). 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	

	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. 2: To investigate the role of learning and memory in drug addiction. 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. 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. By gaining greater understanding of these memory processes and devising strategies to inhibit these memories novel anti-addiction therapies can be designed.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	'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 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?	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
	death.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals is determined using statistical methods. We have extensive experience in these techniques to ensure the robustness of statistical analysis. 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.
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.	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. 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. When animals are given drugs that can cause addiction, this can be used to model aspects of human drug addiction but exploits normal

	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. 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. 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.

Project	**136. 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)	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)	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	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose	This project uses animals to test new ways of

to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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

	molecules that we will then use in our animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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. 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.

137. Transgenerational consequences of preconceptional and in utero exposure to real-life chemical mixtures on fertility and metabolic health

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

endocrine disrupters, environmental chemicals, metabolism, reproduction, cardiovascular function

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We are all exposed to a cocktail of environmental chemicals, which, if they enter our bodies can affect body function and health. Importantly, our chemical exposure may also affect the health of our children and even subsequent generations. This project, uses a unique animal model and expertise from the UK and USA to investigate the mechanisms by which a mother's exposure to a real life cocktail of chemicals, during pregnancy, can affect the long-term metabolic cardiovascular and reproductive health of her offspring and that of subsequent generations. Studies will characterise the effects of maternal exposure to a real-life mixture of environmental chemicals on the metabolic cardiovascular and reproductive health of three generations of male and female offspring, investigate what physiological systems are affected by exposure to environmental chemicals and how these changes are passed between 3 subsequent generations.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

We are currently facing a global epidemic of obesity and its associated metabolic disorders and rising infertility. While these health problems are undoubtedly associated with lifestyle factors including diet, and lifestyle choices for example a delay in the age at which childbirth occurs, there is evidence that exposure to environmental chemicals may also be having effects on both our metabolic cardiovascular and reproductive health. Importantly, such effects of environmental chemical exposure, may be persistent and passed between generations so the effect we see now could have been influenced by the exposure of previous generations and our current chemical exposure may affect the health of subsequent generations. A greater understanding of both the effects and the mechanisms through which environmental chemicals may affect our bodies, provides benefits at multiple levels. Policy making bodies will benefit from increased knowledge to safeguard the general public and wild/companion/domestic animal populations from harmful environmental chemical mixtures. Clinicians and researchers (human and veterinary) will benefit through understanding how chemical exposure may be predisposing certain people and animals to ill health. Students and researchers will benefit from data generated by this project, which may inform their areas of research. Students will also learn important skills in the area of environmental toxicology, chemical analysis and physiology and in vivo large animal experiments.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

The license will initially cover 350 adult ewes that will be grazed according to normal husbandry practice on either control pasture or pasture treated in line with European Sewage Sludge (biosolids) Directive 86/278/EEC with biosolids during gestation. These ewes will be able to be returned to stock, either when sufficient lambs are born for investigation or when their lambs are weaned. The license will then cover up to 350 of their offspring over the course of three generations. These animals will be studied from birth until up to 27 months of age.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of 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?

There are no major expected adverse outcomes for the animals included on this project license. Biosolids are used as an agricultural fertiliser throughout Europe and the USA and no major effects have been reported by farmers. The changes we are interested in are likely to be subtle and/or affect processes that only have a physical outcome when combined with other (lifestyle) factors. The experimental manipulations that will be conducted on the live animals are all classified as mild as they consist of blood sampling, ultrasound scanning, well defined and described tests that examine how the body responds to natural hormones and nutrients and semen collection. Some animals will be humanely killed and tissues recovered so that we can see what changes have been

induced by chemical exposure and how these changes are transmitted between generations.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

The proposed work aims to look at the interrelated effects of exposure to environmental chemical on the systems that regulate our metabolism and reproduction and to investigate how such effects can be passed between generations. As such these studies require theuse of an appropriate animal model. These types of studies cannot be accomplished using in vitro systems, computer simulations, or mathematical models as they are currently unable to allow assessment of the interactions and changes that occur between complex physiological systems over time and between generations of animals.

Reduction

Explain how you will assure the use of minimum numbers of animals.

The number of animals to be used will be kept to a minimum following mathematical analysis of the amount of variation that naturally occurs in the traits to be looked at and an estimate, based on previously published studies, of the size of any effects of treatment. In the proposed work the effects of treatment are compared to a control group, the mathematical analysis has recommended that each groups should ideally contain between 11 and 22 animals to ensure valid results are obtained. To ensure sufficient animals are born in the F3 generation, it is proposed that the F1 cohort will number control n=22 male and n=78 female and biosolid exposed n=22 male and n=78 female.Similarly the F2 cohort will number control n=22 male and n=25 female and biosolid exposed n =22 male and n=25.Therefore not all animals, will undergo all physiological tests. Allocation to optional steps will be randomised but controlled for such no animal is disproportionally represented in the tests. All experiments will be conducted so as to be able to publish the results according to the ARRIVE guidelines and NCCRswill be used for guidance with regard to best practice www.nc3rs.org.uk

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.

Sheep were chosen as the most appropriate animal model for use in this study for a variety of reasons. Sheep exhibit numerous features that make them ideal for comparative studies with humans. Sheep are ideal for reproductive/developmental studies, as they have a prolonged gestation and an extended pre and peripubertal period. Given the relatively large body size, it is possible to collect repeated blood samples from sheep and at post mortem; their large organ size facilitates collection of multiple samples for subsequent tissue and anatomical analyses. Relative to rodents, sheep brains are more comparable to human brains, with regards to size, blood and CSF flow and neuroanatomical organization. Due to the role of sheep in comparative studies and their agricultural importance, extensive information on their physiology and behaviour is also

available from the literature.The proposed procedures (mild) will include the collection of blood samples, well characterised physiological challenges, morphometric measurements, trans-rectal ultrasound (female only) and semen collection (male only), the project team have extensive experience with all procedures and therefore are able to minimise any suffering associated with the procedures.

Project	**138. 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 dia-betes 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 sensa-tion. 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 re-sult of the procedures. Animals will be closely moni-tored and any animals experiencing more than mod-erate 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 *in vitro. In vitro* experiments on cell lines and ex vivo 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.

2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used will be the minimum required to ensure meaningful data is acquired. 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 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 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. 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. 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.

Project	**139. 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
	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 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 treat-ment 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 pulmo-nary disease COPD and Asthma) can cause chang-es 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, pos-ture and lethargy. Adverse effects expected on the treatment and prevention of peritoneal inflammation project include body weight loss and changes to ap-pearance such as coat condition, posture and leth-argy. 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 appear-ance 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 mod-els is moderate. Measures are taken to limit harms such as

	frequent monitoring of disease-specific clin-ical 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 experi-ment, 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

	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. 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. 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.

Project	**140. 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)	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)	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	

	understanding of the disease mechanisms. 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. 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.
What 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 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
What species and approximate numbers of animals do you expect to use over what period of time?	We will only use rats and mice in this project. We expect to use around 6000 rats and 3000 wild type 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	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 neuroinflamation, a key driver of neurodegeneration (usually once but at times repeated dosed may be

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 locormotor 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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. The minimum number of animals will be used for each study in order to obtain information and data required. 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. 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

	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.
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 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. 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.

Project	**141. 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)	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)	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. 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	

	(helminth) infection and allergic responses. **Specific objectives**: 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 *Schistosoma mansoni* Our ultimate goal is identification of cellular and molecular targets for rational development of therapeutics.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at	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

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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 Drosophila 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. Our research also depends on generating the egg stage of *Schistosoma mansoni*for use in subsequent studies. There is no alternative means of generating this life cycle stage other than in a mammalian host.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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 *in vivo*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. 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.

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 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. Miceare the most established animal model for study of the parasitic worm that we work with (schistosomes). Further, *Schistosoma mansoni* in miceis 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 *Schistosoma mansoni* infected mice may show signs associated with hepatosplenic disease from approximately 6 weeks post infection. Doses are carefully adjusted to minimise adverse effects. 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. 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.

Project	**142. 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)	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)	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.	

What species and approximate numbers of animals do you expect to use over what period of time?	3,000 animals/year, 15,000 for 5 years total across all 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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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.

	Achieving it would mean that we have understood the cerebral cortex, which is the very goal of our research.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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: ● 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.

	• 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 a sweet water, we avoid dehydration and reduce weight loss, while maintaining appropriate levels of motivation.

Project	**143. 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)	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)	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,	

	but they can be grouped into two basic factors: the anatomical localization of this cancer, and the lack of understanding of its biology.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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 interven-tions (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.
In the context of what you propose to do to 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 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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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

	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.
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 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. 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 were 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

	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.

Project	**144. 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)	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)	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).	

	Healthy skin can be achieved by avoiding disease promotors, 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. 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. 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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

	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?	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. 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

	pain, it will be humanely culled. 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. 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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

	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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
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.	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. 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

	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.

Project	**145. 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)	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)	Key specific objectives: 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

State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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 be-fore 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.
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.	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

	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.

Project	**146. 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)		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 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	

	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.
What outputs do you think you will see at the end of this project?	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.
Who or what will benefit from these outputs, and how?	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. 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. 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. 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.

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

impacts and/or adverse effects for the animals during your project?	, 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.
What are the expected severities and the proportion of animals in each category (per animal type)?	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.
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?	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.
Which non-animal alternatives did you consider for use in this project?	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.
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 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. 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)

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?	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. 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.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	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 standards currently applied in terms of sample size determination. 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. 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.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	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. 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. mouse colonies will also be maintained using efficient breeding.
Which animal models and methods will you use during this project?	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 animals have a very low risk of infections. 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.

Why can't you use animals that are less sentient?	Mice are the least sentient , and most understood (in terms of gene expression and modifiying and cell labelling) animals that represent mammalian biology. They allow us to recaptulate 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 stratagies that are most suited to mice.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	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
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	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.

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	**147. 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)	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)	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. A key type of cell that infiltrates inflammatory sites is the neutrophil. It is believed that by	

	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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	12525 adult 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?	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

What will happen to the animals at the end?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of	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

animals	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.
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 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.

Project	**148. 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)	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)	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. However, how our brain selects sensory information is not well understood. The aim of our	

	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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to 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 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

	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).

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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

	the required level of detail to answer our research questions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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.

Project	**149. 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)	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)	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	

	test potential treatments to enhance neural repair. 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. 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. 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.
What are the potential benefits likely to derive from this project (how science could be advanced	Peripheral neuropathy and pain are common problems. Peripheral neuropathy affects 6% of the elderly population and chronic pain up to 20% of

or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 pleuripotent 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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 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.
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.	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 between drug treatments so that they do not interact and have combined effects.

Project	**150. 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)	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)	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	

	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.
What 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 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

	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 *ex vivo* alterative to assess complex behavioural functions of the CNS. *In vitro* slices and cell preparations may be used to examine

animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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.

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

	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.

Project	**151. Understanding The Pathogenesis Of Diabetes And Optimising Islet Transplantation In 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)	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)	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	

	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.
What 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 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.

What species and approximate numbers of animals do you expect to use over what period of time?	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
In the context of what you propose to do to 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 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

	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
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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.

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 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.

Project	**152. 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)	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)	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.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 6000 mice and 3000 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?	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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

	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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.

Project	**153. 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)	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)	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. Our project aims to investigate how molecular	

	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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	Less than 4000 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 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.
Application of the 3Rs	
1. Replacement State why you need to use	Cardiovascular disease is a complex condition which involves both the endothelium and a variety of other cell types, including many components of

animals and why you cannot use non-animal alternatives	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
2. Reduction Explain how you will assure the use of minimum numbers of animals	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
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 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.

Project	**154. 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)	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)	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	

	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. 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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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 inibit VM and could be used in combination with anti-angiogenics 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.
What species and approximate	We are planning to use up to 7200 mice over a

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

regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	used cell lines of mouse tumours. 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.

Project	**155. 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)	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)	Malaria is a major health problem that is especially prevalent in tropical/sub-tropical regions. The causative agent is *Plasmodium*, 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	

	of these unusual cell divisions (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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use 8000mice 200 rats during the course of five year period in this project 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?	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.

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 P falciparum 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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

	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.
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 rodent malaria model *P berghei* shares many feature close to human malaria *P falciparum* 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.

Project	**156. 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)	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. We have shown this can work for a range of cancer drugs and some example vaccines but	

	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.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 4975 mice over the next 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 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.

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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. 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
	of the mammalian situation.

2. Reduction Explain how you will assure the use of minimum numbers of animals	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. 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 miceWe 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.
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.	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 ($1000mm^3$). 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. Combining simple procedures during periods of anaesthesia, for example microchipping whilst

	anaesthetised for tumour cell implantation reduces the exposure to the anaesthetic and the number of times the mice experience unconsciousness. 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.

Project	**157. 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)	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)	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	

	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 pigs 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.
What 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 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.
What species and approximate numbers of animals do you expect to use over what period of time?	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.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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. 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.
Project	**158. 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)	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)	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 consequentlyand 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

non-animal alternatives	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. 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. 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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 infusedmice 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.

Project	**159. 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)	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)	Our Objectives are to use imaging of neural activity in the brain of larval zebrafish to: 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	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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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 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, larval zebrafish are less sentient than mammals.

Project	**160. 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)	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)	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. We published the first *rnaset2*-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 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

	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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.

Project	**161. 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)	X	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)	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	

	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
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	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 .
What species and approximate	Approximately 150 wild animals will be used in

numbers of animals do you expect to use over what period of time?	total, over the 5 years of this 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?	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 is to confirm the palatability of baits during the scaling up process for production. Subsequently animals will be used in work to needed 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 that 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the	Professional statistical advice to ensure that the minimum number of animals are used to generate sound and valid data. The protocol for each study

use of minimum numbers of animals	is scrutinised in the context of the whole R&D programme in order to focus on the most relevant questions to answer.
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.	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 M. bovis 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.

Project	**162. 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?	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. 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

	strategies. In addition, our recent success with *in vitro* induction of genetic resistance in DF-1 cells (12, 13), an innovative approach for *de novo* 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. **Summary of benefits** 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. 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. 3. In the long-term, research towards developing de novo 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.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	Our research group has a long standing close interaction with different stakeholders of the poultry industry including breeding companies and vaccine manufacturers. For

	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
Explain why you are using these types of animals and your choice of life stages.	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.
Typically, what will be done to an animal used in your project?	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)
What are the expected impacts and/or adverse effects for the animals during your project?	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.
What are the expected severities and the proportion of animals in each category (per animal type)?	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. Infection with less virulent MDV and non-acute retroviruses, most birds will experience mild chronic disease.
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?	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 *in vitro* models that are comparable to *in vivo* disease models. Hence experiments in the natural avian hosts are essential.
Which non-animal alternatives did you consider for use in this project?	For some acutely-transforming viruses, cells from animals can be transformed *in vitro* 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.
Why were they not suitable?	These *in vitro* 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 *in vitro* systems can reproduce the authentic multisystem involving lymphomas and other tumours induced *in vivo* from experimental infections. Moreover, there are no *in vitro* transformation models for Marek's disease virus. Studies on MDV-transformed cancer cells only gives the virus-host interactions in an already tranformed cell, and not the dynamic changes in the neoplastic transformation process.
Enter the estimated number of animals of each type used in this project.	fowl: 4600 quails: 200 other-birds: -
How have you estimated the numbers of animals you will use?	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.

	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%.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	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.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	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
	antibody production.

Which animal models and methods will you use during this project?	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.
Why can't you use animals that are less sentient?	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 in vitro 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 *in vivo* in natural infection models. We are also using these *in vitro* systems for most of the recent gene editing work, to help identifying the genes that are important for inducing and maintaining the cancer cells.
How will you stay informed about advances in the 3Rs, and implement these advances effectively,	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 *in vivo* animal models to study virus-host interactions in the pathogenesis of and immune responses to oncogenic viruses. Retroviruses are RNA viruses that

during the project?	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. 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

	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?	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. 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. 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. 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. 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.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	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.

Project	**163. 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)	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)	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

	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.
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 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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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

	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.
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.	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 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.

164. Welfare assessment of decompression killing in laboratory rodents

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Key words

Laboratory rodent, Welfare, Euthanasia, Decompression

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Rodents are the most widely used species for scientific research and therefore approximately 1.1million mice and 234,000 rats are used in scientific procedures in the UK, each year. The vast majority of these animals are killed either during or after the research, where the most common method is to expose them to an increasing concentration of carbon dioxide (CO2). However, studies have shown that CO2 exposure can be aversive to rodents, inducing anxiety, breathlessness and, if above certain concentrations while the animals remains conscious, pain. Achieving unconsciousness with lack of oxygen (hypoxia) may be more humane but achieving this by exposure to other specific gases has been explored and is technically problematic and too expensive for use. Decompression is a possible alternative, whereby animals lose consciousness and die by being placed in a chamber where gradually reducing air pressure reduces oxygen availability. This is called hypobaric hypoxia and is equivalent to rapidly ascending to high altitude, which is reported as not unpleasant or painful to humans within certain rate ranges. The aim of this project is to systematically evaluate, for the first time, the potential of hypobaric hypoxia (decompression killing) as a humane method of euthanasia for laboratory rodents. Decompression has already been validated as a humane method to slaughter chickens.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

There is a need to find alternative humane methods of euthanasia for laboratory rodents and hypobaric hypoxia is a promising option. This project will generate detailed data on the

behavioural, physiological and pathological responses of laboratory rodents to hypobaric hypoxia, including negative effects on welfare. We will also conduct a parallel detailed evaluation of CO2 and argon euthanasia to determine whether hypobaric hypoxia is as humane, or more humane than the current primary method. The results of the research will directly inform policy makers in laboratory animal sector both in the UK and elsewhere; if hypobaric hypoxia is found to be a welfare friendly approach to killing laboratory rodents then this will have significant implications for how laboratory rodents are killed. If successful, wide spread application of decompression killing has the potential to improve the welfare of approximately 1.1million mice and 234,000 rats used for biomedical research in the UK each year, and many millions (>35 million) more globally.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We expect to use 920 mice and 60 rats over the five-year course of the project.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of 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?

The project has moderate severity because (1) we will implant rodents with wireless transmitters to measure their cardiac responses and brain activity (essential to accurately assess time to loss of consciousness) and (2) we will expose rodents to carbon dioxide gas which may be aversive, cause breathlessness and/or pain and to hypobaric hypoxia, which though hypothesised to be more humane may also cause harm. Given that the purpose of the work is to assess the welfare impact of nonrecovery euthanasia methods, the animals will not survive the procedures. To understand the welfare impact of hypobaric hypoxia, rodents will be exposed to decompression and carbon dioxide and their behavioural and physiological responses compared. We will use measurements of brain activity (EEG) to determine when the rodents become unconscious, so that we can determine what welfare harms occur up to that point. Following surgery, some rodents will just be placed in the decompression chamber for the same amount of time as a decompression cycle then removed, after which they will be culled by one of the humane methods used for research animals. This is to determine the effect of being placed in a novel chamber. We will train rodents to indicate that they want to leave a situation, and then measure these responses during decompression or exposure to carbon dioxide.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

There are no alternatives to the use of rodents for this work because intact animals are required for the study of the specific welfare effects of each method of killing on this species. We have fully considered alternatives, but the nature of animal welfare assessment is that many body systems contribute to the animal's conscious experience which cannot be adequately reproduced by other methods.

Reduction

Explain how you will assure the use of minimum numbers of animals.

We have carefully calculated the minimum meaningful numbers of animals for each experiment, based on previous studies. We will employ a factorial design 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 where possible, the same animals will be used for behavioural, physiological and pathological assessments, which will further strengthen the data we gather in terms of scientific relevance.

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.

Our use of rodents is essential as we are investigating a welfare issue specific to this species. Measurement of brain signals requires the surgical placement of very small electrodes through the skull. We will refine this by using anaesthetic techniques that have proven efficacy and suitability in mice and we will provide post-operative pain relief. After surgery, rodents will be returned to their home cage and monitored closely. We will acclimatise all animals to handling as well as to all testing apparatus before each experiment. We will use non-aversive handling methods such as tunnel or cup handling for laboratory mice. Emergency methods to euthanise rodents will be in place in case of unexpected events. The work is staged from studies under anaesthesia to conscious studies; as the work proceeds we may find that decompression is not a suitable method for killing rodents because of unexpected and/or unavoidable welfare costs or from technical or application difficulties, and if this happens the work will not continue. However, the work will then allow this method to be ruled out.

Project	**165. 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)	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)	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	

	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.
What 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 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
What species and approximate numbers of animals do you expect to use over what period of time?	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

	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.
In the context of what you propose to do to 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 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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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

	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Adult fish are required mostly forthe purposes of breeding and production of embryos (which form the basis of ourexperimental 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.
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 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. 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

	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.

Project	**166. 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)	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)	1) *To learn more about melanoma, including how it escapes destruction by the immune system.* 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	

	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. 2) *To evaluate new melanoma treatments including chimeric antigen receptor T cell (CART) therapy.* 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. 3) *To learn more about the immune system of zebrafish.* 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.
What 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) 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
	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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers 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 the animals.	

Project	**167. 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)	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)	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 (how science could be advanced or	1) To improve our understanding of which genes are important for normal heart development, and how they help our hearts	

humans or animals could benefit from the project)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	We use zebrafish (Danio rerio), and will use up to 13,000 fish 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?	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

	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.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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 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.
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.	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. 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.

Project	**168. 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)	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)	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

	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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.

Project	**169. 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)		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 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.	We use zebrafish because they offer several technical advantages compared to alternative species such as mice for our experiments. Fish are vertebrates (i.e. they have a spinal cord) so represent a simple yet appropriate model for studying human neurological diseases. The majority of our work involves the use of non-regulated embryonic life stages.
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.

	death of specific neurons and protein aggregation. 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). 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).
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)?	All experiments will use zebrafish. mild: 80% moderate: 20% severe: 0%
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?	Human cells expressing disease-associated mutations. Primary cultures of rodent-derived embryonic neurons.
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

	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
Enter the estimated number of animals of each type used in this project.	zebra-fish: 39,500
How have you estimated the numbers of animals you will use?	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. Power calculations are used to determine the number of zebrafish required for each experiment.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	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. 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.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	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.
Which animal models and methods will you use during this project?	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. Fish are vertebrates (i.e. they have a spinal cord) so represent a simple yet appropriate model for studying human neurological diseases. The models themselves are generally mild, but

	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 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.