Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 44

Project Titles and key words

- The mechanisms of tissue formation and regeneration

  Development, regeneration, healing, neuronal differentiation

- Anti-cancer therapies and biomarkers

  Tumour, drugs, radiation, microenvironment, hypoxia

- Seasonal biology of sheep

  Seasonal, livestock, reproduction, hormone

- Visualising NF-kB pathway dynamics in IBD in mice

  Inflammatory bowel disease, NF-kB, oscillation, IL-10

- Control of mammary gland function

  Mammary, breast, integrin, adhesion, stem

- Type 2 Inflammation in Health and Disease

  Inflammation, immunity, parasitic infection, allergy

- Embryo Development and Implantation

  Embryo, implantation, chondrogenesis

- Prefrontal-hippocampal function

  Behaviour, electrophysiology, memory, neural networks

- The aetiology of diabetic neuropathy

  Diabetes, neuropathy, nerve, regeneration, sensory

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">The mechanisms of tissue formation and regeneration</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Development, regeneration, healing, neuronal differentiation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td>Yes</td><td>~~No~~</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The ultimate goal of our work is to identify means of promoting better healing and regeneration in humans following injury or disease. Before reaching this goal we need to understand better the molecular and cellular pathways responsible for tissue formation, growth, repair and regeneration, which is the overall aim of this project licence. The objective of our research is to better understand how tissues form in the embryo, in particular the nervous system, and how long-lasting neural stem cells are established during embryogenesis. Furthermore, we plan to investigate the mechanisms responsible for repair and regeneration of tissues following injury.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">We expect that identifying the mechanisms responsible for tissue formation and regeneration will provide us with critical information, which will help us identify novel therapeutic targets that will allow human patients to repair and regenerate tissues better following injury</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">We will use two species of frogs, Xenopus laevis and Xenopus tropicalis. Frog embryos are very useful to ascertain the molecular and cellular mechanisms responsible for tissue formation during embryogenesis, as frog embryos can be produced in very large numbers and they are relatively easy to manipulate. In addition, frog embryos develop externally, so that all stages of development are easily observed. This is in contrast to mammalian embryos, which are produced in small numbers, are</td></tr>
</table>

[1] Delete Yes or No as appropriate.
[2] At least one additional purpose must be selected with this option.

	very small and develop inside the mother. In addition, frogs are considered a lower vertebrate with lower neurophysiological sentience than mammals. Nevertheless, the molecular events responsible for early embryogenesis are very similar in all vertebrates, so what we learn in the frog is immediately applicable to our eventual aim of understanding the molecular control of mammalian embryogenesis, including man. Furthermore frog tadpoles have remarkable capacities to repair and regenerate their tissues following injury. For example, frog tadpoles can regenerate their tails, with its cohort of tissues, such as muscles, vasculature and spinal cord, within 5-10 days following injury. The molecular and cellular mechanisms responsible for these remarkable capacities remain largely unknown. We estimate that this five year project will involve 14,000 procedures in adult animals (injection of hormones and establishment of genetically altered organisms) and 33,000 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?	The main procedures performed on adult animals will be injection of hormones for the purpose of induction of mating or laying eggs, and the production of genetically altered animals. Injection of frogs with hormones is a very mild procedure, which does not cause an adverse affects on the animals. The generation of genetically altered tadpoles and frogs is also not expected to result in adverse affects on the animals. Given the remarkable capacities of tadpoles to repair and regenerate tissues following injury, the creation of wounds and extirpation of tissue in tadpoles does not lead to long-lasting effects on the animals.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The main objective of this project is to investigate the molecular and cellular bases of tissue formation, repair and regeneration. Most of these experiments are conducted in embryos, tissue explants and tissue culture cells, and thus are not licenced procedures. However, to study the formation of complex tissues it is necessary to perform some work in vivo, as it is not possible to recreate fully the complex environment of the developing and regenerating tissues in culture. However, we have chosen to pursue this work on a “lower” vertebrate (i.e. frogs) with lower neurophysiological sentience.
2. Reduction Explain how you will assure the use of minimum numbers	The primary means by which the number of animals will be minimised will be through the co-ordination of our experiments between four collaborative research groups. Frogs produce

of animals	thousands of eggs, which can be shared between multiple groups. By co-ordinating our use of frogs for our experiments we are able to significantly decrease the number of animals needed by nearly 30%. For the experiments conducted in the post-embryonic larval (tadpole) stages, we will initially perform a small pilot study on untreated tadpoles for which we will design a power analysis (t-test, alpha=0.05, power=80%, effect size as a minimum reduction of 60%) that will provide us with the required number of tadpoles needed to perform each experiment. During this pilot study we will seek the advice the named statistician, to confirm that we use the minimal number of tadpoles for each specific objective.
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.	Frog embryos have been used to investigate the mechanisms responsible for tissue development for over a century. Indeed, much of what we currently know about how the vertebrate embryo develops has come from experiments initiated in frog embryos. Using an established experimental species reduces the use of animals, as one does not have to replicate accrued knowledge in another species. Frog embryos and tadpoles are particularly well suited to this project because they have remarkable capacities to heal wounds quickly, without leaving scars, and to regenerate complex tissues within days following injury. This makes this model organism particularly useful in studying both the development of tissues and their repair following injury. Finally, frogs are of lower neurophysiological sentience, and thus it provides a powerful replacement model for similar studies performed in higher vertebrates, such as mammals.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Anti-cancer therapies and biomarkers</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Tumour, drugs, radiation, microenvironment, hypoxia</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Despite significant progress over recent years there is still a pressing need for better anti-cancer therapies. New approaches can come from understanding the changes that occur within the tumour cells themselves and targeting these processes. Another important aspect is to understand the contribution that “non-cancer” cells within the tumour microenvironment make towards tumour progression and whether these can also be targets for therapy. Furthermore cancers are very complex and even within the same disease type, not all tumours will respond to a specific therapy. We need to be able to identify patients most likely to respond and be able to monitor their response using “biomarker” approaches. Within this project we are aiming to generate preclinical evidence that aids progression of 5-10 therapeutic strategies and/or “biomarkers” in to the clinical setting. This will be achieved by determining how well therapies work using appropriate tumour models; investigating how the response to therapy is influenced by the interaction between “non-cancer” and tumour cell populations and developing non-invasive imaging-based “biomarkers” that will inform whether a particular tumour may respond to treatment, how well it responds and when changes have occurred that stop the tumour from responding.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>Although we already have a proven track record of translating preclinical work into clinical trials, there is still a need for further refinement of our preclinical</td></tr>
</table>

[3] Delete Yes or No as appropriate.
[4] At least one additional purpose must be selected with this option.

advanced or humans or animals could benefit from the project)?	models and studies to ensure the greatest chance of success in the clinic. We accept that not all tumours are the same, but we have to be sure that we treat appropriate populations in our clinical trials to have any chance of success. We have to design our preclinical studies in such a way that enables the most rigorous testing of the drug in question and that enables clinicians to make informed decisions within the trial process. The benefit of this work will be 1) a greater understanding of the tumour microenvironment and it's contribution to therapy 2) proof-of-concept studies that drive new targets towards clinical development 3) refinement of established models enabling more robust translation from preclinical to clinical research and 4) validation of imaging based biomarkers that can be applied in clinical studies to enhance trial design and decision making. The quicker we can initiate well informed clinical trials, the quicker we can drive towards benefit for patients and impact upon the 150000 lives that are lost per year to cancer in the UK.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 5000 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?	In a typical experiment, we will establish tumours in mice, treat them with various therapeutic approaches and monitor tumour development and response using direct calliper measurements or imaging depending on tumour site. Some of the tumour models are established following a surgical procedure. Adverse events as a consequence of tumour initiation are rare, but could include infection, pain or introduction of mouse pathogens which are countered by good aseptic technique, use of analgesics and screening of murine cell lines to ensure free from pathogens. Tumour growth is occasionally associated with a reddening of the skin in superficial tumours. These models are moderate in severity. With tumours implanted at orthotopic sites following surgery, tumour location dictates the adverse event that could occur. Studies using these complex models are labelled severe although we intervene wherever possible prior to the onset of significant detriment. Initial pilot studies are used to determine model progression and to identify early indicators of a decline in well-being that could be used as an end-point indicator in subsequent studies (for example general loss of condition or weight that precedes a severe adverse event). Imaging is incorporated, if possible, where tumours are not palpable to monitor burden. Burden is

	restricted to below 1.25cm^3 that equates to less than 5% body volume. Severe events that could occur are: brain implants- neurological symptoms; lung tumours- laboured breathing; abdominal tumours- abnormal defaecation or urination. There is a potential risk of death, but experience, refined monitoring procedures and clear intervention end-points limit this to a very low probability. Treatments used can cause weight-loss and in superficial tumours, skin reddening or scabbing at the tumour site. Ulceration is very rare and would indicate mouse cull. Overall wellbeing is monitored using a Health Score Sheet recording system that uses measures such as weight, appearance and behaviour in addition to evaluation of tumour burden/appearance to give an overall assessment of mouse welfare. This enables early interventions before substantial deterioration of mouse condition is observed and allows the accrual of information used to improve the overall process in subsequent studies (e.g. recognising the timeframe over which metastases occurs within specific models and increasing frequency of monitoring accordingly) and the identification of clear end-points. Through such recording and monitoring, even when using protocols and models for which we have given a severe banding based on what *could* happen, we endeavour to ensure the actual severity of adverse events experienced by the animals is moderate. In all cases the animals are culled at the end of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We cannot yet model fully the complexity of the interacting cell populations within a tumour, the dynamic state of the tumour microenvironment or the signalling processes that regulate tumour growth. Before initiating in vivo studies we undertake comprehensive in vitro studies, which include using 3D-cell systems and modified culturing conditions, such as manipulation of oxygen availability, to mimic as closely as possible tumour conditions in vivo. Coupled with in silico evaluations of drug/target interactions and pharmacokinetics, these enable us to make informed go/no-go decisions as to whether a study should progress in to the in vivo setting. We use genetic approaches to provide supporting data that a particular pathway may be a good therapeutic target before progressing to a drug treatment which has the potential to have more systemic effects. Ethically we cannot test in the clinical setting and therefore must use species with physiology that best represents what we would expect to find in the human diseased state.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We reduce inherent variability by using adult, same sex, age matched animals. We treat animals bearing tumours of equivalent size, which markedly improves the uniformity of response, thereby requiring fewer mice. We use a randomised design with group sizes determined by power analysis using freely available software (SISA, http://www.quantitativeskills.com/sisa/). Tumour growth data from either pilot or archived experiments is used in the power analysis. Archived control data sets are used as a cumulative resource to allow recalculation and consequent reduction in group sizes. If several related agents are assessed, initial experiments use 2 animals per dose level. If both fall beyond the 95% confidence interval for the control data, the agent is taken forward. Where studies assess a novel therapeutic combined with a standard therapy (eg radiotherapy), sample size calculations will be based on the radiation response (not untreated control data) determined in pilot studies. Where appropriate we adopt imaging in our studies to enable multiple assessments to be made in the same mouse, pre and post therapy. We often apply a sequential study design in this case as it may not be possible to image all mice required in a single imaging session. The initiation of tumours for imaging studies is staggered over time, with generally 4 mice imaged per day depending on tracer. This is repeated until the experimental group sizes are achieved. Archived imaging data is used to refine sample size calculations enabling a reduction in group sizes.
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 as these allow the growth of a range of tumour models which mimic the clinical situation. They are the species with the lowest neurophysiologic sensitivity in which such well-characterised models of cancer exist. Further correlative studies between mouse and man indicate the potential for results to translate between the species. We aim to use multiple, complementary models within these studies. We can use tumours implanted at superficial sites for routine and proof-of-concept studies, but follow up studies frequently require the use of more complex models that reflect more closely clinical disease (for example patient derived samples established as tumours). We commonly use models that metastasise and have refined processes enabling the monitoring of metastatic disease burden using imaging based systems. We use a Health Score Sheet recording system (described above) that enables robust monitoring of mouse wellbeing and identifies appropriate intervention points to minimise welfare costs. We have refined implant

	sites to reduce detrimental implications for the mice (for example replacing the hind limb intra muscle inoculation model that can result in impaired mobility with the peritoneal muscle wall inoculation model). Real-time optical imaging is undertaken using tumours established into surgically attached window chambers. This is a refinement to a standard histological study in that you can visualise changes over time and then determine when best to analyse tumours by histology/non-invasive imaging. We have refined surgical and treatment recovery procedures by housing mice in temperature controlled incubators for 24-48h and making feed more accessible.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Seasonal biology of sheep.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Seasonal, livestock, reproduction, hormone</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The goal of our research is to identify the genetic mechanisms whereby changes in photoperiod and melatonin drive seasonal changes in physiology in a livestock species, the sheep.

The vast majority of wild animals and most of man’s domesticated species are adapted to live in seasonal environments and experience significant annual changes in food supply and temperature. In order to time the onset of breeding and lay down and store fat at the appropriate time of year such animals operate a seasonal clock which controls timing of many hormone rhythms. A key hormone regulating this seasonal timer is called Melatonin, which is produced within the brain in the pineal gland and secreted at night. The pattern of secretion changes seasonally, with longer-duration profiles produced on the long winter nights. It is known that these changes in seasonal duration drive seasonal hormone rhythms and provide the brain with an internal representation of external photoperiod change, acting on physiology and behaviour. Melatonin is known to act on a specialised structure called the pars tuberalis (PT), located in the pituitary gland, in a region close to the hypothalamus in the base of the brain. This structure is now thought to regulate seasonal rhythms by responding to the melatonin signal and driving hormone pathways in the pituitary and on the hypothalamus.

The project is expected to yield for the first time detailed insight into the molecular pathways</td></tr>
</table>

[5] Delete Yes or No as appropriate.
[6] At least one additional purpose must be selected with this option.

	activated by melatonin.
What 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 are a number of important wider practical benefits. *Benefits to agriculture and animal breeding:* Most species used in agricultural systems retain a strong seasonal ancestry, and their physiology is driven by photoperiod (ie sheep, goats, horses, chickens). Knowledge of the genetic mechanisms regulated by melatonin will thus provide important prior knowledge in driving future animal breeding selection schemes. *Insight into mechanism controlling feeding and fat metabolism:* Key physiological processes such as feeding behaviour and seasonal fat deposition are known controlled by central biological clocks regulated by melatonin. Knowledge of how such metabolic cycles are regulated is likely to be of major importance in identifying brain pathways involved in feeding behaviour and energy balance, and will therefore be of relevance to the study of causal mechanisms underpinning human obesity and metabolic disorders. *Melatonin as a drug and chronopharmaceutical:* Melatonin is now widely used as a chronopharmaceutical compound, to shift body rhythms in man. Although not licenced for such use in the EU, in the USA and other countries it is extensively taken and sold openly. Within the EU, clinical trials are now underway of a novel drug with dual action on melatonin receptors and 5-HT receptors (Agomelatine; Servier, Paris) as a novel treatment for depressive illness in man. In both cases, new knowledge of melatonin action will likely offer novel insight into the cellular and genetic mechanisms involved, with potential for further refinement of pharmaceutical products of benefit to man based on this new knowledge. *Use of sheep as model animals:* Sheep offer an ideal research model to test these ideas. The strong well-characterised seasonal nature of the animal – driven by melatonin – and relatively large brain has allowed our laboratory and other researchers to obtain the necessary anatomical insight into specific pituitary regions where melatonin acts and also obtain PT tissue by dissection allowing gene array studies (see below). By using sheep, we will be able to minimise the numbers of animals used as the large size of the

	PT means that we will be able to obtain sufficient research material from many fewer animals than if we had used rodents. We have to use animals for this research as our goal is to define how intact tissue connected to the hypothalamus responds to melatonin signals. This cannot therefore be achieved using cell or tissue culture models or approaches.
What species and approximate numbers of animals do you expect to use over what period of time?	300 sheep 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?	Blood sampling for measures of hormone changes may cause mild pain, and very rarely result in a local haematoma, which can be addressed by sustained manual pressure to the site for a few minutes. Implantation of hormones can cause transient local pain at the time of application, but is likely to be less painful than ear-tagging, which is undertaken in routine agricultural practice. Animals will be euthanased by schedule 1 procedure (normally barbiturate over-dose).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	These studies require the use of living animals due to the complexity of the cellular and tissue responses
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our prior extensive expertise means we have optimised laboratory measures and protocols, and this allows us to reduce animal numbers used as replicates within experimental cohorts.
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 the only suitable species for such studies since they are the only species of seasonal mammal with a sequenced genome. The husbandry conditions at the facility where the animals are maintained are outstanding, and the staff highly experienced, thus allowing us to minimise harm to the animals during periods of housing in artificial photoperiods.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Visualising NF-kB pathway dynamics in IBD in mice.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Inflammatory bowel disease, NF-kB, oscillation, IL-10</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 Years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gut, with the prevalence of western European countries being about 400 per 100000 inhabitants. Altered microbiota, impaired intestinal barrier function and dysregulated immune response play an important role in the development of the disease. The treatment is mainly based on immunosuppressive treatments which aim to dampen inflammatory signalling pathways such as the NF-kappa-B pathway, though many patients still require surgery. NF-kappa-B activity regulates multiple cellular functions such as cell development and growth, however increased NF-kappa-B activity drives pro-inflammatory functions in immune cells in the course of IBD. General targeting of the NF-kappa-B pathway may have detrimental side effects. We aim to generate transgenic mouse models that will allow the visualisation of NF-kappa-B dynamics in healthy and inflamed tissues. We will isolate different cell types as well as whole tissue to visualise the NF-kappa-B oscillations using state of the art microscopy.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">IBD is used as a paradigm for multi-factorial disease. The project will aim to develop a multi-scale model of chronic inflammation. It is vital to understand how imbalance in the immune response results in chronic inflammation and explore how modulating the pro- and anti-inflammatory cues can lead to resolution of this response. This knowledge will lead to improved treatments, as the present</td></tr>
</table>

[7] Delete Yes or No as appropriate.
[8] At least one additional purpose must be selected with this option.

	scope of treatments is limited and it is clear that we need a better understanding of the interplay between different risk factors in order to improve the effectiveness of treatment.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use 13250 mice in 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of the animals will not have any regulated procedures that will cause them distress undertaken on them, as we will cull the mice then we will be isolating cells. The likely severity will be mild. Additionally we will be imaging the gut on anaesthetised mice, therefore the mouse will be unconscious throughout the procedure, therefore the expected severity will be mild. The mice will then be culled. A minority of mice will be infected with parasites to induce colitis, which may result in intestinal discomfort, or rarely diarrhoea. The mice will be culled up to 40 days post infection, then the tissues will be analysed. The expected severity is thought to be moderate. Colitis will be induced by giving the mice dextran sodium sulphate (DSS) in their drinking water, in a minority of mice. DSS is directly toxic to gut epithelial cells and disrupts the epithelial barrier. Mice may experience diarrhoea and weight loss. These mice will be closely monitored, and rehydration therapy will be used if required. The mice will be culled upto 14 days after treatment, then the tissues will be analysed. The expected severity is thought to be moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Studying the immune system is not possible except in organisms such as mice. It is not ethical to perform infections and treatments on humans therefore there is no alternative to entirely replace a living animal that would allow our objectives to be met. We have used non-animal alternatives in using cell culture to guide our animal work, for example NF-κB dynamics have already been verified in cell culture. Additionally, blood from patients will be analysed along side analysis performed in mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce the number of mice used in the project, our initial work was undertaken *in-vitro* to ensure that the vectors constructed were functional. We will also test various immunomodulatory compounds in cell culture, and any promising compounds will then be tested mice. This will reduce the number of mice used as only a few select compounds will be used mice. To prevent redundancy in experiments, all data will be available online to all consortium partners via

	the project wiki site. Results will be published with as little delay as possible in peer-reviewed journals.
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.	<u>Choice of species</u> We have chosen to use mice. Mice are a common choice of species to study the immune system as their immune system is highly similar to the complex networks of the human. <u>Choice of model</u> We have chosen to use DSS, T.muris infection and genetically susceptible models (IL-10 and IL-10R) to study NF-kappa-B network during IBD. DSS induced colitis and T.muris infections are well used and optimised in the laboratory, therefore no further optimisation will be necessary, thereby reducing numbers and unnecessary pain and suffering. Likewise the IL-10ko mice are well known and studied model of colitis, having been studied for 25 years. To minimise the suffering of the mice, if the mice suffer from colitis they will be given rehydration therapy or culled humanely. The mice will be checked regularly, both day and out of hours. Should any sign of distress be noted as a consequence of treatment, it will be reported immediately to the BSU staff or veterinary officer, treatment ceased and, if advised by the veterinary officer, the animals will be humanely killed. <u>Choice of method</u> We plan to study oscillations of NF-kappa-B in and out of the nucleus. We will image the mice by molecular imaging, which allows the imaging of cellular functions in living organisms. As this will be undertaken while the mouse is anaesthetised, then this is the most suffering they will endure.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Control of mammary gland function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Mammary, breast, integrin, adhesion, stem</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
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<tr><td>Regulatory use and routine production</td><td></td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>
Our research focuses on the adhesion of cells to their local environment within tissues. We are interested in how adhesion controls cell behaviour in the mammary gland, and how this becomes altered in diseases including breast cancer.

The objective of the project is to determine mechanisms by which the adhesion of cells to an extracellular scaffold of proteins, called the extracellular matrix, regulates cell behaviour in the mammary gland. We will characterise the role of key molecular components of adhesion signalling pathways, e.g. extracellular matrix proteins, extracellular matrix receptors on the surface of cells, called integrins, and integrin-signalling proteins, in the control of mammary gland development, function, and neoplasia. The importance of this work is that understanding how adhesion-related proteins determine cell behaviour in mammary tissue will ultimately lead to better strategies for breast cancer prediction and novel targets for breast cancer intervention.

Examples of specific current projects include:

The role of adhesion signalling in mammary development and function. We will determine how integrins and integrin signalling proteins, control cell cycle, survival, migration, and differentiation of mammary epithelial cells.

The role of adhesion signalling in mammary stem cell function. We will determine how deleting, or otherwise
</td></tr>
</table>

[9] Delete Yes or No as appropriate.
[10] At least one additional purpose must be selected with this option.

	altering the function of integrin signalling proteins affects the ability of stem cells to form mammary gland ductal networks following implantation of cells into mammary glands. *The contribution of adhesion signalling in the genesis of neoplasia*. We will use genetic strategies to determine the integrin-signalling pathways that are needed for breast oncogenes to form primary 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)?	This project will provide a greater knowledge of basic processes involved with the normal development of glandular epithelium, a greater insight into the causes of breast cancer formation, and may ultimately lead to the identification of new biomarkers for detecting cancers and possibly molecular targets that would be useful to target therapeutically. This work will have significant mechanistic implications for many scientists studying the role of adhesion, because what we find out using the mammary gland is likely to represent universal principals for other epithelial cells and tissues. In addition, experimental evidence that altered adhesion-signalling proteins perturb normal mammary gland development and function, or contribute to neoplasia *in vivo*, will have significant implications for the development of future breast cancer therapeutics.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use ~17,000 mice over the 5-year duration 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?	*Protocol 1*. Breeding and maintenance of genetically modified mice. Mild. No phenotypes or adverse reactions are expected in tissues other than mammary gland. Any defects arising within the mammary gland are unlikely to cause disease or mastitis, though they may cause cessation of lactation. *Protocol 2*. Transplantation into cleared mammary fat-pad. Moderate. Recipient mice maintained under this protocol are not expected to exhibit harmful phenotypes. *Protocol 3*. Breeding and maintenance of genetically modified mice. Moderate. No phenotypes or adverse reactions are expected in tissues other than mammary gland. For tumour-bearing mice we will closely monitor tumour growth in the mammary gland and use non-invasive techniques to minimize suffering whenever possible. If symptoms of metastasis are observed, affected mice will be killed. In each protocol, animals will be killed by a Schedule 1

	method at the designated establishment.
Application of the 3Rs	
Replacement State why you need to use animals and why you cannot use non-animal alternatives	*Protocol 1*. Developmental processes occur in tissues in vivo. In order to study how the protein products of genes work in developmental processes, it is necessary to alter the function of genes, which is done through transgenic means. Alternatives to studying true developmental processes are not possible ex vivo because development occurs in the context of multiple cells within organs and an endocrine milieu, which cannot yet be fully recreated in culture models. *Protocol 2*. The only true way to study mammary stem cell function is to determine whether stem cells are able to fully regenerate mammary ducts in vivo. Alternatives include the use of culture models for stem cell function and FACS analysis using stem cell markers. While these are approaches that we routinely use, it is important to complement culture studies with in vivo analyses of stem cell behaviour. *Protocol 3*. Studying mechanisms of neoplasia requires the analysis of tumours that develop in the orthotopic organ. Alternatives include the use of culture models, however such models frequently do not reflect the behaviour of tumour cells in vivo.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have carefully considered all the aspects of mouse colony management and optimized experimental design to minimize the number of mice to use in order for us to: i) maintain stocks of various mouse strains, and provide female mice whose mammary glands will be used for *ex vivo* studies; ii) provide female mice for *in vivo* implantation; iii) provide female mice for genetic analysis of mammary gland development and neoplasia.
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 study of mammary gland function requires the use of mammals. In all protocols, mice are the most appropriate species for genetic analyses using conditional-null gene deletion. In order for the new proposal to yield meaningful conclusions, we need to compare our future data with those results obtained and published in the past. *Protocol 1*. We do not expect that these mice will show harmful or abnormal phenotype during the course of their housing, because the use of mammary-specific promoters will ensure that resultant phenotypes only occur in mammary epithelial cells of post-pregnant female offspring. *Protocol 2*. Following surgery, soft water gel pads will be provided to keep mice hydrated, and mice will be monitored closely over the first 48 hours, We do not expect that these mice will show any harmful or abnormal phenotype following implant of cells into mammary fat pad.

	Protocol 3. Mice will be checked routinely for the appearance of mammary or other tumours and killed if lesions become greater than 1 cm diameter (UKCCCR guidelines). During the course of this project, we aim to develop non-invasive imaging techniques to monitor tumour growth, and minimize the number of mice to use and their suffering. We will sacrifice tumour-bearing mice if we determine that mice have health related issues that warrant immediate attention.

Type 2 Inflammation in Health and Disease

Inflammation, immunity, parasitic infection, allergy

- Summarise your project (1-2 sentences)

The overarching aim of our research is to identify which immune cells are important, and which mechanisms and pathways they use, to initiate, maintain and regulate Type 2 inflammation, which is responsible for widespread suffering in allergy, as well as being a hallmark of infection with parasitic worms (helminths).

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

There is a global need to cope with helminth infection and allergies. Helminth infections impact 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 should help our understanding of all these conditions as well as their relationship to one another.

The intention of the work outlined in this application is to take the experience that we have gained in studying the role of immune cells in coordination of inflammation in helminth settings, build on this, and begin to ask what fundamental mechanisms underlie induction of immunopathology in allergic settings. Although this is not a novel question, it remains unanswered and important to understand for future development of therapeutics against debilitating conditions such as asthma. A detailed understanding of the molecular mechanisms of Type 2 inflammation that are shared by or unique to allergens or helminths should enable us to determine how best to address and correct dysregulated or aberrant Type 2 immunity, as is found in allergic settings. We will bring a fresh perspective to this issue by directly contrasting immune responses against helminths and allergens focussing on mechanisms of induction, maintenance and regulation that are as yet poorly understood.

Our ultimate goal is identification of cellular and molecular targets for rational development of therapeutics.

- Outline the general project plan.

To achieve our objectives, it will be necessary to utilize mice that have been genetically altered (GA) with regard to immune function. These include mice with specific deletions in immune function genes or transgenic expression of immune receptors, as well as mice bearing transgenic reporter constructs such as Green Fluorescent Protein.

We will use normal and GA mice to study the function of specific immune cells or proteins following murine challenge with substances that provoke Type 2 inflammation. We will also be able to intervene before or during immune challenge with specific antibodies, cytokines, neutralising proteins, inhibitors and ligands which will deplete, neutralise or stimulate given subsets of the immune system. In particular, the depletion of cell populations (e.g. using antibodies or drugs) at specific times during immune responses is a powerful tool that mirrors the clinical intervention using drugs.

Studies on immune and inflammatory aspects of schistosome infection will be carried out

using a well-established murine model of this medically important parasitic infection. This model provides a powerful and relevant system to interrogate immune mechanisms involved in pulmonary, hepatic and intestinal Type 2 inflammation. We will be able to dissect the important cells and mediators involved during schistosomiasis by intervening before or during infection with specific antibodies, cytokines, neutralising proteins, inhibitors and ligands which will deplete, neutralise or stimulate given subsets of the immune system. The depletion of cell populations (e.g. using antibodies or drugs) at specific times during infection is a powerful tool that mirrors the clinical intervention using drugs.

To complement these approaches, we will use murine models of allergic pulmonary inflammation, and defined non-helminth models of intestinal inflammation, to assess the fundamental relevance of our studies using schistosome infection or challenge with substances that promote Type 2 inflammation.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

Our work will involve using models of parasite infection, and lung and intestinal inflammation, where symptoms are no more than moderate. Substances will be administered to mice, normally by injection, which will usually cause little if any suffering. Mice will be infected with schistosomes under sedation. Although infection and inflammation can cause weight loss, impaired mobility or lack of response to stimuli, the majority of animals will experience only mild adverse effects. A minority of animals succumb to schistosome infection with no overt warning signs or evidence of suffering.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

The primary benefit of our work will be discovery of new knowledge about the initiation, maintenance and regulation of Type 2 inflammation. Although we do not anticipate immediate therapeutic benefit, our work could lead toward treatment. For example, identification of key proteins produced immune cells could in the long-term lead to development of blocking strategies for therapy. The work on understanding the key cellular players involved in promotion or regulation of Type 2 inflammation also has the potential to direct drug development. We primarily believe that in the short-term our work will increase our fundamental understanding of mechanisms underlying Type 2 inflammation, which will facilitate the development of intervention strategies in the longer term.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

Mice are the most established animal model for study of the parasite that we work with (schistosomes). Parasite migration, maturation, fecundity, and pathological consequences of infection in the mouse are similar to the processes in humans. Throughout the project we will liaise with an expert in experimental design and statistics to ensure that the minimal number of animals are used in experiments to obtain significant results. We estimate we will use approximately 16,000 animals in our studies throughout the proposal (~3000 mice per year), which will enable us to perform parasite infection, immune challenge, airway challenge for intestinal inflammation experiments.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

The use of animals is imperative to the success of the project. 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* or zebrafish is not feasible, as such organisms 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.

Where possible, we use *in vitro* systems to address specific questions. For example, when we identify the induction of particular proteins in a particular cell type *in vivo*, we see if we can replicate this *in vitro* using related cell lines and the mediators observed *in vivo*. If we can replicate a specific aspect of our data, detailed analysis of signaling pathways is undertaken *in vitro*.

- Explain why the protocols and the way they are carried out should involve the least suffering.

Our work will involve using models of parasite infection, and lung and intestinal inflammation, to determine important cells/molecules at the heart of the regulation of immunity. In all of these models, symptoms are no more than moderate and animals are carefully monitored to limit or prevent suffering. These models are wholly necessary for our project, as they will enable us to determine important interventions that can alleviate symptoms of inflammation in parasite infection and allergies, thus identifying potential therapeutic targets for treatments for people.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Embryo Development and Implantation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Embryo, implantation, chondrogenesis</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[11]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Our work addresses understanding of how early embryos develop and how the embryo implants into the uterus at the blastocyst stage (about 30-60 cells). We use the mouse as a model as it is a mammal like humans with a similar reproductive strategy and its genetics are well established. We aim to find critical molecules which function in the uterus to allow the embryo to implant and decipher further the key molecular events in generation of the blastocyst. In parallel we apply our findings to human reproduction and development e.g. to determine if critical components are present in human embryos (obtained after informed consent from the IVF lab). We use human stem cells to model cell specialisation and test if findings from mouse are relevant to human development. We make human stem cells become cartilage cells in a culture dish and then need to test whether these can form cartilage in the joint suitable for repair in humans.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Our work informs Assisted Reproduction Technologies (ART) for couples with infertility problems and we are working to develop new diagnostics for infertility. By understanding the process of early development and implantation we hope to develop therapies to overcome some infertility problems. We can generate cartilage cells from human stem cells and implant them into small defects in the rat knee joint to show they can repair these defects. Thus we produce preclinical data to help us develop a new cell therapy for use in humans for osteoarthritis/sports injury.</td></tr>
</table>

[11] Delete Yes or No as appropriate.
[12] At least one additional purpose must be selected with this option.

What species and approximate numbers of animals do you expect to use over what period of time?	Mice 880/5years Rats 70/5years
In the context of what you propose to do to 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 give natural reproductive hormones by injection with little adverse affect except soreness at injection site. Various surgical procedures will be used to manipulate the hormonal status of the animal to confirm the reproductive signalling systems discovered in vitro and interfere with those pathways. This will give some discomfort following surgery but infection is very rare. Implantation of cells to test if they can form a benign tumour containing a variety of tissues tells us if these cells are able to generate particular specialised cells. This will give some discomfort following surgery which will be mitigated by analgesics, but infection is very rare. Animals will develop the benign tumours which do not invade and spread and are small, but if cysts develop and cause discomfort then animals will be killed. Surgery will test if implanted cells can form mature cells of cartilage in an animal joint environment to test potential for cartilage repair. This will give some discomfort following surgery mitigated by analgesics, but infection is very rare. All animal 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	We cannot study the process of embryo-implantation into the uterus in the human for ethical reasons. We use the mouse because a mammal is essential for investigation of embryo implantation; it provides a model similar to human. The mouse is the least pain-sensitive mammal. There are no entirely satisfactory models in a dish for embryo implantation, so a combined approach using research outside the body (in vitro) and with animals is essential. Data on implantation obtained from mouse embryos in a dish (our main experimental model) must be checked in animals. We use human and murine embryonic stem (ES) cells to study how embryonic cells become specialised, avoiding animal use. In vivo protocols are only used when there is no alternative, such as to validate data obtained in vitro. This is essential because we can only examine the earliest stages of development/implantation in the dish. We have to use the gold standard method for testing hESCs for their potential to form specialised

	cells and for tissue formation, the teratoma (a benign tumour) assay in mice, because the cells cannot reproducibly produce tissues in a dish, or in alternative models (e.g. zebra fish as an alternative unsuccessful). For preclinical research on hESC-derived chondrocytes it is essential to test the cells for ability to make true knee-type cartilage in the joint. There is no reproducible model in a dish to test this. Thus we need to use the rat cartilage repair model, the rat being a mammal with sufficient body weight and depth of cartilage to provide a valid initial test system (not the case for mice). We cannot test human chondrocytes in non mammals to give meaningful data on joint repair.
2. Reduction Explain how you will assure the use of minimum numbers of animals	• Where possible experiments are undertaken with hESCs thus avoiding mouse embryos. This is not possible for embryo implantation. Here we carry out most studies with very early embryos: a ball of < 100 cells together with cells from the lining of the human uterus, in a dish. Only validation experiments will be done using mice. • Embryos: We will use the minimum number of female mice to generate the minimum number of embryos needed for statistical significance in implantation studies in culture. • Teratomas: are produced from stem cells by 2/ 5 SCID mice injected. Therefore a minimum to assure tumour formation of 5 animals are used for each human stem cell lines tested. • Cartilage repair; Cartilage repair from our cells is seen in around 66% of joints. Therefore we need a minimum of 5 animals at each time point to monitor the process of repair. We are developing an in vitro assay to assess cartilage repair but it is not yet reliable.
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 the mouse for studies on reproduction because a mammal is essential for investigation of embryo implantation and this provides a model similar to human. The mouse is considered to be the most tolerant, least pain-sensitive mammal, with the best understood genetics. • For surgical procedures, we use general anesthesia in purpose built operating theatres with best-practice operating techniques to avoid infection and surgical complications. We apply pain killers to minimize postoperative discomfort. • The rat is used for cartilage repair work (reasons above). We are developing imaging

	techniques to monitor cartilage repair in situ which will allow us to i) visualize repair in animals harmlessly; ii) monitor the time course of repair; iii) use fewer animals since animals can be viewed at different times and killed humanely after a final scan.

Project Title (max. 50 characters)	**Prefrontal-hippocampal function**
Key Words (max. 5 words)	Behaviour, electrophysiology, memory, neural networks
Expected duration of the project (yrs)	5
Purpose of the project (as in Article 5)	Basic research **Yes** Translational and applied research **Yes** Regulatory use and routine production **No** Protection of the natural environment in the interests of the health or welfare of humans or animals **No** Preservation of species **No** Higher education or training **No** Forensic enquiries **No** Maintenance of colonies of genetically altered animals **Yes**
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The ability to deal with environmental changes depends upon regions of the brain known to control and produce behavioural choices in light of memories of past experiences that can guide those behavioural choices. These brain regions are the prefrontal cortex (PFC) and hippocampal formation (HF), respectively. However, we still do not understand how these regions co-operate to produce the appropriate behavioural choices for a given set of circumstances (and previous memories related to those circumstances). The principal aim of the current license is to shed light on how PFC and HF interact by studying these regions in mice and rats. A second, related aim of the license is to determine (a) how damage to PFC and/or HF leads to behavioural deficits in the behaviours described above and (b) how current and new drug treatments might halt or reverse these problems. We will use current and novel models of human neuropsychiatric diseases where PFC and HF damage is well-established (e.g., Alzheimer's disease, schizophrenia). We will use multi-electrode recording arrays and various behavioural tasks to determine how PFC and HF networks interact to produce complex behaviour. Disease models will identify the crucial neural components of behaviour (they show where the system breaks down) and we will attempt to halt/cure function of PFC/HF circuits and cognition by pharmacological intervention.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We will add considerable knowledge to our understanding of the brain circuits responsible for many of the most important behaviours in man. We will also gain considerable insight into how these circuits deteriorate in common neuropsychological disease states and how current and novel therapeutics can help to halt or cure these devastating human conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use common laboratory species or rats and mice. We ask for 3600 animals in the current license 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 majority of adverse effects will be non-recovery or mild. Some animals will experience moderate severity recovery surgery for implantation of recording devices or injection of test compounds and these will be monitored closely for severe adverse effects. The likely benefits from this Project are substantial as we currently have little idea of how PFC and HF interact in health and disease. The license will address this by correlating physiological changes with behavioural deficits. These experiments are crucial if we are to understand the impact of states such as Alzheimer's disease on the brain and, ultimately, halt or cure these states. Most animals will be killed by terminal anaesthesia (any other animals by appropriate schedule 1 method). We will examine brains *post hoc* to visualize the location of recordings neurons/electrodes and provide detailed histological and biochemical data regarding disease progression and the effect of any pharmacological intervention.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex relationships within PFC-HF anatomy and physiology in producing cognitive function are poorly understood, so cannot modelled adequately *in silico*. The *in vitro* brain slice preparation provides some access to components of these circuits; however, this approach still requires animals and has the disadvantage that it cannot preserve the entire connectivity within a single brain slice. Hence, the present experiments have to be performed using animals *in vivo* where anatomical connectivity and circuit physiology is maintained.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We require at most 10-12 animals per group. In goal-directed tasks numbers will only be 8-10/group as motivation reduces response variance. We will monitor test power closely and alter group size as appropriate (with local expert statistical guidance). Variance will be minimised by careful animal handling, thorough habituation and good researcher training. We will use a repeated-measures design where possible - however, this is often not best practice due to confounds from repeated exposure to testing, carry-over effects (e.g., learning), etc..
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 license aims to reveal the complex interplay between PFC and HF. We will use rodents as these are of low neurophysiological sensitivity, yet show remarkable evolutionary conservation of PFC and HF compared to higher primates including man. Alzheimer's disease mice develop symptoms in a manner similar to human AD. We will only use mice once required by the experimental plan and we will use male and female cage-mates, minimising animal wastage. We will inject amyloid beta oligomers i.c.v. into some normal rats and mice as this effectively models the very earliest cognitive and neural deficits of AD progression. Our BDNF-KIV mice are bred as required. As they exhibit sex- and genotype-linked deficits, we will again use offspring of both sexes and all genotypes, minimizing animal wastage. The use of normal animals to produce models for impulsivity/ADHD and schizophrenia (NMDA antagonist model) are now well-established and validated. None of these models routinely produces severe side-effects, however, if present the animal will be killed immediately by schedule 1 method. Some behavioural tasks are motivated by either escape from shock/water or food restriction. In the latter, animals are kept at 85-90% of their free-feeding weight (by adding pre-weighted food amounts to each cage) and this will be maintained only during behavioural testing periods.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The aetiology of diabetic neuropathy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Diabetes, neuropathy, nerve, regeneration, sensory</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall aim for this programme of work is understand the mechanisms underlying the key functional changes that take place in the nervous system in diabetes and develop therapeutic strategies to reverse or prevent these changes.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Diabetes currently affects 230 million people worldwide, and by 2025 the World Health Organisation estimates 350 million people will have diabetes. Approximately 50% of diabetic patients will present with some degree of sensory neuropathy which may be accompanied by: tingling and burning sensations, over-sensitivity to normally innocuous stimuli, pain, numbness and ultimately a loss of sensation which can lead to tissue trauma and increased risk of amputation. It is a debilitating condition which impacts on patients quality of life. There is currently no effective treatment.

From these studies we hope that there will be a greater understanding of the mechanisms underlying diabetic neuropathy and a realistic prospect of development of novel therapies.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We estimate 1400 rats and 900 mice over a 5 year period</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected</td><td>We use rodent models of Type 1 and Type 2 diabetes. Animals are monitored regularly. Animals have free access to drinking water and food. Both of these will be provided in extra</td></tr>
</table>

[13] Delete Yes or No as appropriate.
[14] At least one additional purpose must be selected with this option.

level of severity? What will happen to the animals at the end?	amounts. Morbidity of persistent diabetes will be controlled by implantation of insulin delivery pellets to give a degree of control. Animals have raised blood glucose at levels that might be seen in poorly-controlled clinical diabetes. We may assess sensory and cognitive function over the timecourse of the study using tests that do not cause any tissue damage and rely on object recognition. Under terminal, non-recovery anesthesia we may assess how the central nervous system processes sensory information and determine whether there is a difference in how complex sensory information is processed between control and diabetic rats, and also measure nerve conduction speeds. Animals are euthanased at the end of the protocol and tissues collected for molecular and biochemical analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In our laboratory we use cell culture to both complement and inform our in vivo work, however since diabetes is a whole body disease, affecting the hormonal, cardiovascular and nervous systems which all play a role in progression of the disease, it is impossible to wholly mimic in a culture dish. The complications of diabetes are progressive and take time to develop and are the product of several consequences of poorly controlled diabetes. Hence there is a need for animal models of diabetes. The similarities between the pathogenesis of diabetes in humans, mice and rats justifies the use of animal models of diabetes to elucidate the mechanisms of the disease process, in characterising phenotypic changes in the nervous system and assessing the efficacy of therapeutic agents. We are currently trying to develop a model of diabetic neuropathy in the roundworm *C. elegans*, and if successful, hope this invertebrate model may prove useful for high throughput screening of potentially pro-regenerative and neuroprotective drugs. If any relevant non-animal alternatives become available during the course of the project, we will incorporate these in our studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments are designed on the basis of previous work and published data and we refer to the ARRIVE guidelines (http://www.nc3rs.org.uk/page.asp?id=1357). Full evaluation of previous/pilot data and power calculations are performed, such that the minimum

	number of animals required to provide valid data are used (typically 10 per group for therapeutic studies). A method we have been developing is the imaging of corneal nerves under general anaesthesia. This method is used on human patients in the clinic as an effective marker for diabetic neuropathy. Because the monitoring could be repeated several times on the same animal under anaesthesia, such an approach will enable the reduction in 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 animal of choice for this work is a lower order mammal, typically the rat although mice may also be used. We typically use the streptozotocin Type I model of diabetes and are familiar with its advantages and limitations as well as the specific requirements for animal welfare. Our results can be integrated with the continuously expanding body of research conducted on these species and model, and contribute to the emergence of a comprehensive understanding of the pathogenesis of diabetic neuropathy. Numerous strategies are in place to minimize animal suffering, we use appropriate anaesthetics and analgesia for any surgery. Sensory and cognitive testing do not cause tissue damage and animals are handled and acclimitised to minimise any undue stress.