Home Office

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Non-technical summaries granted during 2013

Volume 37

Project Titles and key words

- Cortical networks underlying primate choice

  Prefrontal cortex, neuronal networks, electrophysiology, lesions, non-human primate

- Physician aided reconstitution of immune system

  Transplantation Tolerance Alemtuzumab Immunomodulation Regulatory T cells

- In vivo physiological studies of brain circuits

  Hippocampus, neural dynamics, memory, space, reward

- Generation and regeneration of blood and the cardiovascular system

  Blood, Heart, Development, Fish, Frogs

- Physiological roles of phosphate changes in proteins

  Diabetes; hormones; metabolism; adipose tissue

- Disease ecology in wild columbiform bird populations

  Trichomonas gallinae, Turtle Dove, columbiform

- Molecular basis of pain and thermal sensation

  Pain, thermal sensation, analgesia, neuropathy

- Cardiovascular and respiratory function in disease

  Low oxygen; blood vessels; control of breathing; cardiovascular disease

- Kidney Cancer Biology and Therapy

  Kidney cancer; Genes; Proteins; Drug Targets; Biomarkers

- Trigeminal and spinal acute/chronic pain systems.

  Pain, spinal, trigeminal, dorsal horn

Cortical networks underlying primate choice
Prefrontal cortex, neuronal networks, electrophysiology, lesions, non-human primate

- Summarise your project (1-2 sentences)

  We do not yet understand how brain regions interact at a neuronal level while mediating cognition and therefore this project aims to discover general principles of neuronal network interactions during one type of cognition, namely choice-behaviour. The only way to do this is to use an animal model in order to record simultaneous neuronal activity from multiple individual neurons in multiple brain regions while simple decisions are being made.

- 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.

  The prefrontal cortex constitutes around one quarter of the brain, and damage to it in patients can cause severe disruption of the ability to cope with everyday life. The frontopolar cortex is the largest area within human prefrontal cortex. It is also an area that is considerably developed in primates compared to other mammalian species. Yet frontopolar remains one of the least understood regions of the brain in terms of its function. It is therefore essential that we understand how it operates in the coordination of cognition. This will be one major benefit of this program of research. Another major benefit is that our research will advance knowledge and understanding about general principles of neuronal network communication. This is important because the aim of neuroscience is to understand how the brain works as a system. Until very recently, most studies of human psychiatric and neurological disorders tried to identify a single malfunctioning area. Likewise, most neuropsychological (lesion) and neurophysiological (neuronal recording) studies tended to focus on one area at a time. However it is now becoming increasingly understood (e.g. from studies of changes in functional connectivity obtained via MRI) that human psychiatric and neurological disorders are often related to disturbances within extended networks of interacting brain regions. There are also changes in structural connectivity too (e.g. data obtained from studies of MRI based tractography techniques such as DTI). For these reasons, basic neuroscience research must now progress to providing knowledge about how brain regions interact at a neuronal level; this is the aim of this project. An additional aim is the assessment of the welfare impact of the procedures themselves on the animals.

- Outline the general project plan.

  A key aim of our research is to understand the general principles that govern interactions between connected regions in the brain. We will examine one system of interacting regions for which we already have expertise, namely that which underlies choice behaviour. The choice behaviour network is centred in prefrontal cortex, involves the frontopolar cortex, and extends posteriorly to medial regions such as cingulate and retrosplenial cortex, as well as posterior regions including parietal cortex, and temporal lobe areas. In order to determine both general principles of neuronal interaction, and specifics that may differ between particular kinds of interacting brain

areas, we will examine how a sub-set of directly connected regions within this network interact with each other to determine how causal influences are exerted between regions to mediate well organised and effective behaviour. To do this we will use electrodes and microelectrode arrays to record simultaneous neuronal activity at the millisecond level resolution from multiple individual neurons in multiple brain regions within the choice-network described above while simple decisions are being made. Then, to address the crucial question of how brain regions causally influence each other, we will compare this data to that of similar recordings made after lesions and/or inactivations have been made to precisely defined brain areas that occupy key nodes in the network. To assess the impact of the procedures on the wellbeing of the animals we can take measures of behaviour and physiology. We hope that these measurements can be used to help us identify the least stressful ways to carry out the research.

- 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.

  Adverse effects arise from the necessity for invasive recording from the brain, for daily recording sessions to measure neural activity, and for motivation by control of fluid intake:

  i) Repetitive restraint - animals may experience periods of stress related to behavioural testing because the nature of the procedures requires prolonged head-restraint during training and testing stages (several hours per day, several days per week, for 3-5 years, albeit with some days off per week and with some interspaced 'vacation' periods with no testing)
  ii) Fluid control – some level of fluid control/restriction is likely to be required on most days to help motivate animals' training and performance. There is therefore a moderate risk some animals may experience transient periods of mild dehydration, particularly during the initial stages of training or when tasks suddenly increase in complexity; and transient weight loss may occur when starting on fluid control. This is mitigated by using only the minimum necessary amount of fluid control throughout, by giving some ad lib access to water at weekends, and by giving ad lib access to water on all days in the 'vacation' periods.
  iii) the potential for stressful motivational/training techniques such as the rare use of pole-and-collar. This will be mitigated by only using pole-and-collar only on rare occasions and only for short durations.
  iv) the necessity to use implants means that there is a moderate risk that their implants may become mildly infected; there is also a small risk that the implant may become damaged/fall off and require repair or replacement or become damaged.
  v) there is a very small risk of seizure, infection, and/or haemorrhage associated with neuronal recordings, introduction of needles for injection of substances, and surgical implantation of recording devices.
  vi) the introduction of lesions requires invasive surgery but the animals are given appropriate analgesia in the post-operative recovery period and animals typically return to physical and physiological normality over the course of a few days; behavioural normality is also expected to resume as any cognitive deficits will not be readily observable and require sensitive cognitive tests even to discern.

  At the end of the experiment the animals will all be humanely killed and perfused so that their brain tissue can be examined.

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

There are around 80 billion neurons in the brain and each neuron may have tens of thousand of connections to other neurons. In order to understand how such a complicated network operates it is helpful to divide up the brain into different areas according to general patterns of connections or anatomy that characterise and differentiate those areas. It is becoming more generally understood that multiple areas contribute to any given cognitive function. Yet neuroscientists currently understand very little about how populations of neurons in one area influence the activity of populations of neurons in other areas and how these causal influences underlie behaviour. Therefore we expect this project to yield novel insights as to how neurons causally influence each other within interlinked networks of brain areas. We will focus our research on understanding the causal influences that brain regions in the prefrontal network, which is known to be involved in choice behaviour, have upon each other and upon areas to which they are directly connected outside of prefrontal cortex. We expect to increase basic scientific understanding of how prefrontal cortex operates, both normally and following insult. This will have immediate widespread benefit to the neuroscience community, particularly those interested in neuronal communication. This will provide benefit to clinical researchers and clinicians whose interests are focussed on neurological damage and/or neuropsychological disorders associated with prefrontal cortical dysfunction or disturbed network behaviour (many neurological conditions are increasingly understood to relate to disturbed interactions between multiple areas). Over the course of this project, we expect to develop new and/or refined technological approaches in surgical techniques and neuronal recordings. Not only will we be training early career neuroscientists in these techniques, but we will also share this knowledge with other neuroscientists (both in the UK and abroad) through meetings (e.g., NC3Rs Primate Welfare Day), conferences, and publications. We expect this to contribute to the more widespread use of these techniques, which will bring both scientific advances and experimental refinements.

- 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.

  We estimate we will use up to 18 macaques in total; an initial program of work will use up to 6 animals to fulfil its specific objectives focussed on frontopolar cortex networks and choice-behaviour. The additional animals will constitute independent yet related programmes of work (i.e. focussed on different behavioural tasks and concerning overlapping but not identical networks of brain areas). Achieving the scientific objectives of this research project requires that we measure neuronal activity in awake, behaving subjects, which involves invasive techniques that cannot be performed in humans. We need to use macaques because non-primate laboratory species (e.g. rodents) do not possess some of the prefrontal cortical areas we are investigating. Our research depends upon the extensive published literature of brain connectivity which is greatest for the macaque monkey. Our research also depends upon the use of some established behavioural tasks for macaques in the literature which macaques are the only laboratory species demonstrated to be able to learn to perform them to the level of competence our neuronal analyses require.

  Reduction in this project is achieved by: a) reducing the number of animals necessary; b) reducing the duration of each daily experimental session; and c) reducing the number of experimental sessions (thereby reducing the time an individual animal spends on study). Our experiments are based on a “within-subject” design,

such that research questions are based on comparisons in one experimental condition vs. another, which can be embedded within the same behavioural task. Therefore we are able to use the minimum number of animals per experimental objective (typically 2 or 3 animals) sufficient to show replicability of the existence of newly discovered neuronal properties and mechanisms between animals. We design the behavioural tasks to be as simple as possible given the experimental objective. This reduces the duration of individual experimental sessions as much as possible and ensures that few (if any) animals need to be removed from study for failure to learn/perform the task. We also design the tasks to enhance differences between experimental conditions (wherever possible) to reduce the amount of data necessary to achieve statistical reliability. Finally, we design our experiments such that a single animal/experiment can contribute to multiple objectives (without increasing the cost to the animal), thereby reducing the total number of animals necessary.

- 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.

  Replacement: Achieving the scientific objectives of this research project requires that we measure neuronal activity in awake, behaving subjects, which involves invasive techniques that cannot be performed in humans. In very rare circumstances some human clinical conditions require implantation of electrodes in sites dictated by clinical need; where possible we recruit such patients and record limited human neuronal activity in similar cognitive tasks. These studies can show generalizability to human brain function albeit without the possibility to sample many neurons from many areas over extended periods of time, and without the possibility to intervene to determine causality. Computer modelling of the kinds of neuronal network interactions we are investigating is not possible without first knowing the basic parameters of neuronal function which is what our work provides; we collaborate with computational modellers who model neuronal data from our work and the work of others who also use animal models to provide vital information about neuronal activity and function underlying cognition.

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

  Refinement: We have taken every measure possible and established multiple intervention points to ensure that each individual animal will experience the minimum potential possible for any adverse effects (pain, suffering, distress, or lasting harm) and only when it is absolutely necessary to achieve the scientific objectives; and to ensure that that no more animals are used than is absolutely necessary. We have designed the scientific programme in order to maximise the amount of data that can be obtained with each experiment, with many experiments contributing data to more than one objective. The individual experiments are designed to build upon one another to ensure that they are conducted with the most up-to-date information possible and to achieve maximum scientific benefit from each animal. We have incorporated flexibility in terms of recording techniques to ensure that the best available recording technique will be used for each scientific objective. This will maximise the quality of data and minimise the amount of time the animal is required to be on protocol. We have established intervention points between each new procedure to ensure that no new procedure will be performed unless the NVS, NACWO, and researchers agree that the animal is not likely to experience an unreasonable increase in risk for adverse effects. This will not however, compromise the utility of the data obtained prior to these intervention points should it be deemed inappropriate for the animal to continue. We have developed a progressive method of instituting reinforcement training/fluid control

(and monitoring its efficacy through behavioural monitoring) that will serve as the basis for developing individualised programmes for each animal (to be done with the NACWO). This will ensure that no animal will experience any more restriction than necessary to overcome specific hurdles in training. We will use the latest technology/methods to increase the quality of the data thereby minimising the duration of experiments and the time each animal is required to be on protocol. We have specifically designed (and continue to refine) our restraint systems (both headposts and restraint chairs) in order to minimise the discomfort experienced by the animal during prolonged periods of restraint. Finally, we continually liaise with the NVS/NACWO to refine our behavioural and health monitoring procedures.

Physician aided reconstitution of immune system
Transplantation Tolerance Alemtuzumab Immunomodulation Regulatory T cells

- Summarise your project (1-2 sentences)

  The aim of this project is to determine how we can translate protocols that induce immune tolerance in mouse models into clinically applicable therapies for human transplantation and autoimmune diseases. Current treatment for these conditions involves the long-term use of immunosuppressive drugs that can have serious side effects, increasing the risk of infection, cancer and heart disease, which could be avoided if we could induce specific immunological tolerance in these patients.

- 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.

  Our previous work in mice showed that a brief treatment with certain monoclonal antibodies can induce lifelong immune tolerance to organ grafts and can cure the mouse model of “childhood” (autoimmune) diabetes. What is now required is to develop a sufficient understanding of how these monoclonal antibody treatments work in the rodent models to be able to translate them to the human clinical situation.

  We have previously developed a monoclonal antibody (CAMPATH/Alemtuzumab) that is able to improve the clinical outcome for transplant recipients and patients with multiple sclerosis in the short term (eg. 5 years), but immunological tolerance holds the potential for long term or even lifelong improvements with minimal or no further requirement for chronic drug administration. Our objective is to identify other immune modulatory drugs that are either in advanced stages of clinical development or are already available, that can encourage the development of regulatory T cells, which are required for the generation of transplantation tolerance and can re-establish self tolerance in autoimmune diseases.

- Outline the general project plan.

  We always start from in vitro experiments, using established tissue culture cell lines when possible, but where this is not appropriate we have to use tissues obtained from experimentally or genetically manipulated mice and transplantation studies in the whole animal. We will use a number of different mouse models of transplantation, but mostly skin grafts given to mice that have been genetically altered so that they only have one type of lymphocyte able to recognise and reject the skin graft. This allows us to most accurately follow the cellular and molecular mechanisms of tolerance or rejection. The other main model we will use is where mice have been genetically altered so that they express the human CD52 molecule on their lymphocytes, which can then be targeted for depletion by the clinically applicable monoclonal antibody CAMPATH/Alemtuzumab. We will then search for other clinically applicable drugs that, alone or in combination, encourage rather than interfere with, the development of regulatory T cells and tolerance induction during the reconstitution that occurs after CAMPATH mediated depletion.

- 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.

A large part of this project will involve the breeding and maintenance of genetically altered strains of mice, particularly strains that have only one type of lymphocyte and where every lymphocyte recognises the difference between male and female tissues. In the vast majority of cases we do not expect any adverse effects to be caused by the genetic manipulations or the normal process of breeding. Very small numbers of mice (max. 1550 over five years) may have minor surgical procedures required for the maintenance of the lines or for generation of embryos for cryopreservation. A small proportion of the total number of mice (approximately 5-10%) will have up to 3 minor surgical procedures under general anaesthesia, such as grafting skin onto their flank, and may also have blood samples taken from a superficial vein on up to 5 different occasions.

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

  This project will considerably advance our understanding of how regulatory T cells and immunological tolerance works in our mouse models, and if we can identify clinically applicable drugs that can guide the immune system to tolerance after treatment with CAMPATH/Alemtuzumab, will provide a route to translate this knowledge into better treatments for human transplant recipients and patients with autoimmune disease.

- 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.

  In order to perform all tests with sufficient statistical confidence we will need to use up to 2000 grafted mice per year, while to maintain the genetically altered strains we will need to breed up to 8,000 mice per year. The vast majority of mice will be bred to provide tissue samples or for maintaining the strain, and the majority of genetically altered mice are indistinguishable from normal mice in their overall health status.

- 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.

  Mouse models are the only option to perform many of these experiments for a number of reasons. First, immune tolerance can only be defined in an intact immune system within a living mammalian animal. Second, at present our ability to induce immune tolerance in transplantation and autoimmune disease has only been formally demonstrated in rodents, and only the mouse provides us with the degree of control and the ability to manipulate the genetic makeup that we require to identify molecular mechanisms. Finally, we already have a large body of background information, reagents and monoclonal antibodies with predictable properties in mouse models.

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

  All experiments will use the most refined techniques as recommended by veterinary advice and the Institute for Animal Welfare (NC3Rs). Appropriate anaesthesia and analgesia will be given whenever there is a potential for pain to be caused as part of a

procedure. The procedures are all classed as moderate or mild. All mice will be kept in a high quality and specific pathogen free facility with environmental enrichment provided.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>In vivo physiological studies of brain circuits</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Hippocampus, neural dynamics, memory, space, reward</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)[1]</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>
<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[2]</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>This project aims to determine how learning and memory processes rely on the precise coordination of neuronal activity across multiple brain regions for the purpose of goal-directed spatial behaviours. We will study how and why neuronal activity is structured in time and space across three interconnected brain regions: (1) the hippocampus, a key circuit for spatial memory, (2) the ventral tegmental area, a key circuit for reward processing, and (3) the nucleus accumbens, a key circuit for action selection and goal-directed behaviours.</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>A better understanding of the brain circuits we are studying will not only increase our understanding of normal brain function but also help in understanding what goes wrong in neurological and psychiatric diseases and thus, may give clues how better to treat them. The knowledge gained from the approaches developed in this project will help in the future to test the effect of memory-enhancing drugs and could lead to new therapies for both memory disorders and reward-seeking pathological behaviours.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>In this 5-years project we are planning to use up to 100 rats and 800 mice for in vivo neurophysiology experiments and up to 2000 mice for breeding and maintenance of colonies.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse</td><td>Possible adverse effects may include discomfort and pain. Animals under procedure will be daily monitored. Based on our past work, we expect</td></tr>
</table>

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

effects and the likely/expected level of severity? What will happen to the animals at the end?	signs of no more than mild distress after 24hour post-operation. During anaesthesia provision will be made for continuous warmth of the animal and regular fluid support. Analgesic will be provided to prevent pain prior to, during and/or after surgery. Aseptic techniques will be used during surgery to avoid postoperative complications with infection. If doubts occur the University Veterinary Officer will be contacted. At the end of the experiments animals will be humanely killed using either schedule 1 procedure or terminal general anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are planning to use animals because at present there is no possible replacement for experimental work aiming at defining how neuronal networks operate within the brain in health and disease. Moreover many experiments require a behavioural analysis. It is not possible to perform large-scale brain recordings in behaving human beings. In vitro recordings will not allow us to carry our research project as the integrity of brain large-scale networks will not be preserved. In silico simulations cannot be used as they do not capture brain circuit dynamics. Hence we have not found an alternative model for the recording of brain activity but the electrophysiological data set we collect in our experiments is available to computational modellers to assist them in designing and testing their in silico simulations of brain networks so that they become more biologically realistic and their successful development will eventually lead to a reduction.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In this project we have reduced the number of protocols to two instead of the previous four. Moreover we will use a chronic large-scale recording approach that leads to more data collected from the same animal than with traditional preparations. The consequence is that fewer animals are needed or used as compared to traditional preparations. Hence we will use a small number of animals in this project. The numbers are also kept small because of the multifaceted data analysis techniques we perform on these large-scale physiological data collected from each animal that take many months for each recording day.
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 because: (a) they are the most widely used animals that have sophisticated spatial and other types of behaviours; (b) the anatomy and physiology of the rat and mouse brains are well described; (c) the proposed procedures have been specifically designed for and successfully used in these species; and (d) some of our scientific objectives rely on animals expressing genes of interest and such transgenic approaches

	are best established in mice (and rats to a lesser extent). We will keep abreast of the latest technical advancements in the field, both in terms of recording methods and statistical analysis techniques, to ensure that the minimum number of animals is used and to further reduce the incidence and level of any discomfort that may occur during the procedure. Accordingly, we will constantly monitor the literature and maintain contact with other investigators of the field both in scientific meetings and laboratory visits. If required, the project licence will be updated to reflect the refined protocols. Moreover, our anaesthesia, analgesia and surgical practice under the previous licence have been specifically and regularly reviewed with the NACWO and NVC, by way of seminars and round table discussions. This process is planned to continue to ensure the constant refinement of our protocols.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Generation and regeneration of blood and the cardiovascular system</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Blood, Heart, Development, Fish, Frogs</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)[3]</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[4]</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">Our overall aim is to understand how vertebrate embryos make a blood and cardiovascular system as they develop or regenerate, and how the genetic programming is corrupted in disease. Specific objectives:
1. To characterise the programming of tissue-specific gene expression in the blood and cardiovascular systems in developing Xenopus and zebrafish embryos.
2. To determine the cellular hierarchies that lead to the formation of blood, vascular and cardiac tissues, including their stem cells
3. To conduct functional analysis of candidate stem cells in an appropriately conditioned recipient host.
4. To define the cellular hierarchies and genetic circuits involved in heart regeneration.</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 list here the expected benefits from each of our main objectives (Primary purpose: advance science; secondary: disease control):
1 and 2. These will shed crucial light on congenital and developmental human diseases, and they will also facilitate tissue engineering, disease modelling and drug screening in the future.
3. Identifying adult stem cells and elucidating their programming will enable a better understanding of their normal and defective behaviour and its control. We will directly compare normal blood stem cells with their counterparts in leukaemic models in zebrafish to identify novel targets for therapy. It will also facilitate the production in the lab of normal adult stem cells, which in the case of the blood, could provide a solution to rejection of other tissues</td></tr>
</table>

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

	generated in the laboratory. 4. Comparing the genetic circuits that control heart regeneration with those that control heart development will enable the key controls to be better understood. In the longer term, it may become possible to manipulate these controls after ischaemic heart damage to facilitate regeneration of cardiac muscle and to ultimately translate this knowledge to humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Over a 5 year period we will use six thousand five hundred Xenopus and around eighty five thousand zebrafish, a large majority of which will be used to generate embryos for breeding and maintenance of 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?	In order to achieve the aims of this project we need to introduce various reagents into developing embryos, in some cases permanently so that cells are marked for tracking, or gene activities can be controlled. In general, the perturbations to normal life will be mild or non-existent until induced, or the perturbed embryos will be terminated before free feeding. The most extreme damage will be the removal or freezing of part of the heart muscle, which the zebrafish will in time replace. Here a small proportion of the experimental animals (<10%) may suffer adverse effects during/after treatment and require euthanasia but the majority will recover after a few hours and exhibit normal swimming and feeding behaviour. All investigators are highly trained and competent to perform the techniques in the animals, and we have substantial experience of these techniques. The protocols are continually refined to cause the least suffering. Animals are monitored daily and more frequently throughout experiments for signs of distress or disease. Any animals showing signs of distress are isolated and treated appropriately or where required the advice of the vet is sought. Where necessary we have established a detailed set of humane endpoints. At the end of the study animals are humanely euthanized.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to study tissue generation, it is essential to determine what happens in a developing embryo and then to find out how this translates into the adult in the context of regeneration. Generation of the blood and cardiovascular system occurs in a 3D matrix of cells and these cells communicate with each other. Such a matrix has not thus far been fully recreated outside of an animal. However, what we discover in this project will bring that goal closer.

2. Reduction Explain how you will assure the use of minimum numbers of animals	To ensure the fewest amount of animals are used we will use minimal group sizes that will give us statistically significant results. Good experimental design and collaborations within the group aid reduction of animals by decreasing duplicate analyses.
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 the lowest life forms available to us that generate a blood and cardiovascular system similar to that of humans. We are using amphibian and fish because their embryos are available in large numbers and develop externally, and are easy to manipulate. At the same time their genetic circuitry controlling blood and cardiovascular development is conserved with mammals (humans). In addition, adult zebrafish regenerate their hearts after damage, a property not shared by adult mammals including humans. There is also a large number of pre-existing zebrafish genetic lines available to the community. Furthermore, its genetics allow easy manipulation to create new lines in house. Genetically altered animals are important for understanding fundamental biological processes and the way they are implicated in human disease. All investigators are highly trained and competent to perform the techniques in the animals. The protocols are continually refined to cause the least suffering. Animals are monitored daily and more frequently throughout experiments for signs of distress or disease. Any animals showing signs of distress are isolated and treated appropriately or where required the advice of the vet is sought. Where necessary we have established a detailed set of humane endpoints.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Physiological roles of phosphate changes in proteins</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Diabetes; hormones; metabolism; adipose tissue</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>May be up to 5 years</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>
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<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>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Phosphate groups can be added to and removed from specific sites in proteins very rapidly and they can alter the function of the protein. Phosphates are removed by enzymes called protein phosphatases. The objectives are to determine the in vivo function of certain protein phosphatases and whether modifying their activity may be beneficial for the treatment of diabetes, cancer or disorders involving cellular stress.</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>There are a large number of genes encoding protein phosphatases. We have created specific changes in the genes for a number of these enzymes, with the aim of understanding their importance in the control of normal glucose metabolism and their roles in influencing responses to hormones and in controlling the activity of other enzymes that are known to be important in metabolic diseases such as diabetes and in cancer. If specific protein phosphatases can be identified as being of similar importance, then they too may become targets for future therapeutic interventions,</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice- approximately 500 per year. Most are used in breeding programmes only, in order to generate animals with the precise genetic changes that will be informative in our analyses.</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 level of severity? What will</td><td>None of the genetically modified mice we have engineered have so far had any obvious adverse effects. The usual level of severity is mild. In the majority of cases, the animals are killed humanely and the scientific analyses are carried out on</td></tr>
</table>

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

happen to the animals at the end?	tissues or cells harvested after death. In a minority of cases, it will be necessary to intervene during life, e.g., to feed an altered diet and/or to measure an animal's sensitivity to glucose or insulin. These interventions are also expected, in the most part, to be mild, with a severity limit of moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We try to reduce the number of animals by culturing embryonic fibroblasts, hepatocytes and pre-adipocytes for analysis of the levels and activity state of some proteins. However, the alterations that occur in diabetes and other human disorders involve many tissues and therefore cannot be studied completely in cell cultures. For example, increases in the amount of adipose tissue or changes in glucose tolerance and insulin sensitivity can only be investigated within an animal model. No alternatives are available.
2. Reduction Explain how you will assure the use of minimum numbers of animals	I have previously carried out similar studies to those described above, with different mouse lines and this information will be used to assess the number of mice required. The normal group size is about 5 animals, though statistical analysis will always be undertaken to ensure that this is sufficient and, if not, what the correct larger number of animals should be.
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.	I use the mouse as a model system to test because it is a small mammal that can be easily bred and tested in laboratory conditions. Non-mammalian, lower organisms do not provide a suitable model for the biochemical processes being analysed as they do not share the same control mechanisms (e.g., for the regulation of glucose metabolism, involving brain, pancreas, liver, muscle, fat and other tissues). The genome of mice has been well studied and there are good methods available for engineering the required genetic alterations in mice. As noted above, most animals will be used for breeding only (with scientific analysis occurring after death). For other interventions during life, humane endpoints have been set to provide severity limits of no more than "moderate", though in most cases any deviation from normal welfare will be no more than "mild".

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Disease ecology in wild columbiform bird populations</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Trichomonas gallinae, Turtle Dove, columbiform</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</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>Yes</td><td></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></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 project has five aims:
1: Examine the genetic diversity of disease in UK Columbiformes.
2: Determine whether parasites are shared between columbiformes, passerines, gruiformes and galliformes at shared food and water resources
3: Determine the impacts of infection upon nestling growth, fledging success and post-fledging behaviour.
4: Determine the diet of nestling Turtle Doves, and the influence of diet on adult and nestling oxidative stress
5: Determine whether food supplementation or lack of disease is associated with reduced stress levels in all five species

Populations of four of these species are increasing whereas one is declining, and recent work has found parasites to be present at a high prevalence in the declining species. This work aims to establish the significance of disease for the ecology of this species.</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">The results of this work will further the knowledge of the ecology of British dove and pigeon species, and allow informed conservation strategies to be established for the species of conservation concern.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td colspan="3">In order to gain an accurate estimate of disease prevalence, along with an accurate picture of the diversity of the diseases affecting these species, we</td></tr>
</table>

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

over what period of time?	need to collect samples from a large number of individuals although, where possible, we will minimise sample sizes through the use of statistical models to aid analysis. We estimate that we will sample up to 200 of each age group (adults and nestlings) of the focal columbiform species, and up to 50 adults of each of a number of additional passerine and gallliform species known to be present in the same environment and to share the same food resources, over a period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The protocols we will use are well established and will cause minimal suffering. Birds will be sampled at their site of capture and re-released into the wild without delay once their health status has been deemed satisfactory. No adverse effects are considered likely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We wish to further understand the ecology of disease in both declining and stable columbiform populations; we cannot achieve our objectives without the use of wild caught birds.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To obtain accurate estimates of disease prevalence, we need to sample a relatively large number of birds; however, where possible we will reduce the required sample sizes through the use of statistical 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.	Previous work has established that declining columbiformes in the UK have a high incidence of parasite infection. The work we propose here will assess the ecological impacts of infection. All samples will be collected using established procedures, which are minimally invasive and can be collected from each individual within a short space of time at the capture site. Pain caused by these procedures is minimal, and no adverse effects are expected. Birds will be assessed to ensure their health status is satisfactory prior to release.

Project Title (max. 50 characters)	Molecular basis of pain and thermal sensation
Key Words (max. 5 words)	Pain, thermal sensation, analgesia, neuropathy
Expected duration of the project (yrs)	5
Purpose of the project (as in Article 5)[9]	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[10] Yes
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective is to understand the fundamental mechanisms of how individual proteins, typically ion channels or other proteins which modulate ion channels, cause or enhance pain and how these and other proteins mediate thermal sensation and regulation of bodily temperature. We will also seek to develop and test novel drugs which modulate the actions of proteins such as ion channels.
What are the potential benefits likely to derive from this	Pain causes much suffering in humans and in animals and is poorly treated by current analgesics,

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

project (how science could be advanced or humans or animals could benefit from the project)?	which have many side effects. One benefit will be to enhance our understanding of pain and so to lay the foundations for future development of more effective analgesics. Part of the work involves drug discovery in which we will seek to develop and test novel analgesics. A better understanding of thermal regulation will assist in understanding medical conditions in which thermal regulation is abnormal.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use rodents (rats and mice). Approximately 6000 mice and 1000 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?	A number of different procedures are proposed in this work. We will use standard methods for creating transgenic mice and cross-breeding them to produce novel strains, with the objective of studying the effect of particular genes on inflammatory and neuropathic pain and on the ability to detect temperature and to maintain bodily temperature. In the large majority of cases the genetic manipulation causes no observable alteration in the behaviour of the mice as they feed, groom, socialise and reproduce normally (mild severity level). In some cases adverse effects can be seen, and in one strain of particular interest to us seizures are sometimes observed (moderate severity level). These animals will be closely observed and in the event of seizures occurring more than 3 times daily or weight loss approaching 20% they will be humanely killed. In a second part of the project the effects of gene deletion or drug treatment on pain will be observed. We will induce inflammation or neuropathy (nerve injury) by injection of inflammatory substances or by damaging nerves surgically or with drug treatments in order to mimic common painful conditions seen in human patients. Some painful conditions such as experimental neuropathy cause lasting discomfort, as shown by guarding of an affected paw (moderate severity level) but the animals continue to feed, socialise, groom and breed normally. We will test the pain responses of animals with painful conditions and will observe the effects of gene deletions or drug treatments on the pain responses of the animals. Pain testing involves mainly threshold tests in which the animal is free to withdraw as soon as it begins to feel discomfort. We will also test thermal sensation in genetically modified or drug treated animals. In these tests the animal has a choice of environments in which to spend its time (thermal preference tests, mild severity limit). Finally, in many aspects of the work we will monitor vital signs such as heart rate and

	body temperature. These tests involve external monitors or in some cases a subcutaneous implanted probe and the animals feel no more than minor irritation (mild severity limit). At the end of procedures most animals will be killed by a schedule 1 method. In a few cases animals may be killed by perfusion fixation in order to preserve tissues for histological examination or by exsanguination in order to obtain blood for subsequent analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project will make extensive use of testing in cell culture and computer modelling. These approaches have greatly reduced the need for animal experiments, for instance by eliminating many unsuitable drugs in cell culture testing and so reducing the numbers that need to be tested on animals. However, in order to understand pain, which depends on consciousness, it is essential to observe the effect of potential analgesic treatments or gene deletions at the level of the whole animal. Thermal sensation and regulation of body temperature are also whole-animal phenomena which cannot be understood apart from by using intact animals. We seek to understand pain and thermal sensation in mammals, with a view to developing novel analgesics which will be effective in humans and in mammalian species and therefore the use of single cell organisms or non-mammalian species is not appropriate.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We aim to use the minimum numbers of animals needed in order to obtain a clear experimental answer to our questions. It is vital to use the minimum number of animals to achieve a statistically significant result. It is widely accepted that 5% significance level is adequate to regard an experimental result as valid, though not completely beyond that which could be achieved by chance fluctuations alone. For this reason testing will be carried out to achieve this widely recognised level of significance and we will cease a particular experimental protocol once this is achieved.
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 only species on which, for technical reasons, the genetic manipulations which are essential for this work are capable of being carried out. For some behavioural experiments not involving genetic manipulation, however, rats have been found to be more reliable subjects (i.e. to give experimental results of lower variability) and for this reason we plan to use a small number of rats in our work. In terms of the experimental models of inflammation, neuropathy and temperature sensation that we will employ, our models are in common use amongst other experimenters, which

	has the advantage that they have been well validated and are reliable, that they are known to cause minimum discomfort, and that the results we obtain will be readily comparable with the work of other labs.

Cardiovascular and respiratory function in disease
Low oxygen; blood vessels; control of breathing; cardiovascular disease

- Summarise your project (1-2 sentences)

This project will examine the importance of a new mechanism underlying the dangerous thickening of blood vessels due to growth of muscle cells in the vessel walls in response to hypoxia (low oxygen), a common clinical problem. Using non-animals studies, we have demonstrated the importance of this mechanism to the growth of these muscle cells and we now need to find out if this mechanism is working in living creatures.

- 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.

Cardiovascular diseases cause millions of deaths worldwide. Many of these involve proliferation of smooth muscle cells which form the walls of blood vessels, causing vessels to thicken, therefore restricting or even preventing blood flow. This proliferation can be triggered by low oxygen levels (hypoxia). Understanding the mechanisms by which smooth muscle cells proliferate will allow design of new therapies to prevent such diseases. My group has discovered a novel pathway in which the gas carbon monoxide is generated within cells by an enzyme called heme oxygenase and controls proliferation by regulating ion channels which dictate the rate at which cells proliferate or die. Our data so far have been obtained using cultured cells. In order to develop our observations towards designing new therapeutic approaches to vascular diseases, we now need to investigate whether the signalling pathway is active within mammals. This project will examine this directly.

- Outline the general project plan.

We plan to expose animals to hypoxia (either sustained or intermittent) to mimic common cardiorespiratory diseases which lead to vascular thickening in humans. We shall monitor the thickening of blood vessels triggered by hypoxia in normal animals and compare results with those from genetically altered animals which lack (or have excess amounts of) the enzyme which normally generates carbon monoxide, and in animals which lack the target ion channels which control proliferation. We shall also test the effects of introducing small amounts of carbon monoxide into the atmosphere that the animals breath. Similarly, we shall examine how hypoxia (with or without carbon monoxide) alters the ion channels and activity of the carotid body, and organ which senses oxygen levels and influences development of vascular diseases in response to hypoxia.

- 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.

Animals (mice and rats) will be exposed to atmospheres in which the oxygen levels are reduced and, in some cases, with the addition of small amounts of carbon monoxide. There are no adverse effects of such treatment. Immediately after the period of exposure, animals are sacrificed for assessment. For some animals, the carotid body will be removed whilst the animal is under deep anaesthesia, from which it will not recover.

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

Results from these planned studies will help us better understand how vascular diseases progress, and will explore a possible mechanism by which progression can be controlled. The key elements of this mechanism (ion channels and heme oxygenase) can be regulated by newly emerging drugs. Our results will determine whether such drugs might be useful in the treatment of vascular diseases.

- 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.

Over the course of 5 years, we aim to use approximately 1500 mice and 600 rats. We use mice because they can be genetically modified so that the key targets (ion channels and heme oxygenases) can be deleted or augmented – this is a means by which to test our hypotheses which is far more accurate that application of drugs to block the targets. Rats will be used for some experiments (carotid body investigations) because their properties are extremely well defined already, and so differences caused by hypoxia etc will be more rapidly identified. We use statistical analysis (advised by experts within the institution) to validate any observed changes found in our experiments and to ensure statistical significance whist using the smallest numbers of animals possible.

- 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 animal experiments we have designed are based very carefully on substantial results obtained in non-animal experiments (some of which are already published), so we believe they are very directed, ensuring minimal numbers of animals are used. Additional non-animal experiments, which will be conducted in parallel, will further refine our animal work thus ensuring no unnecessary animal experimentation is undertaken.

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

To investigate the effects of low oxygen (with or without small levels of carbon monoxide), animals must be exposed to such conditions. This exposure is well tolerated and does not cause distress or suffering, merely a degree of lethargy. After such exposure, animals are either sacrificed immediately, or rapidly and deeply anaesthetised for removal of carotid bodies, after which they are sacrificed whilst still under deep anaesthesia. These approaches ensure that little or no suffering occurs at all.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Kidney Cancer Biology and Therapy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Kidney cancer; Genes; Proteins; Drug Targets; Biomarkers</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>Kidney cancer is the 8th commonest cancer in the UK, with around 9000 new cases each year. It is characterised by being highly variable in its aggressiveness and response to treatment. Current therapies do not work in all patients, they are toxic and they usually stop working after a few months. The median survival for patients with advanced disease remains in the order of two years. We therefore need tests to help us identify which patients have aggressive cancers that are likely to spread and which tumours will respond to existing treatments. We also need to urgently discover new ways to treat kidney cancer. These are the aims of this project.</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>The project has a clear path towards patient benefit. By identifying important genes and proteins that regulate tumour growth and metastasis (cancer spread) we can develop tests to identify which patients are at higher risk of relapse after their cancer has been removed, for example. Such tests do not currently exist. In addition, we may also identify ways to predict which patients may be resistant to currently used drugs, so that alternative therapies can be tried. Finally, we hope to identify new ways to treat kidney cancer, which is crucial to improve the outlook for patients with this disease. We will use tumour and blood samples from patients with kidney cancer to help validate our findings in mice.</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?	We expect to use approximately 750 mice during the course of this five-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?	Tumour growth will be examined by growing tumours both under the skin of mice and within the kidney itself, by direct injection of cancer cells at these sites. Animals experience only transient discomfort from injection of cells under the skin. Injections into the kidney require surgery and may cause moderate discomfort. Mice will be anaesthetised during these procedures, and painkillers will be administered before and after, as required. Tumours will not be allowed to grow beyond a fixed size limit and below this size tumours have minimal effects on the animal. The growth of metastatic tumours (spread to other parts of the body) may lead to the onset of distress in mice. If a mouse is seen to develop any signs of this it will be humanely killed. Mice may also be administered substances by mouth or by injection into a vein or under the skin. These procedures cause only transient discomfort. All animals will be humanely killed at the end of an experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Mouse models of renal cancer are well established and we are not aware of any superior models or replacement alternatives. Studies in the laboratory can provide some information with regards to tumour biology and this will be examined and used to inform our animal work. However such information is limited. Kidney cancers are complex and some of the drugs used for treatment act on blood vessels, rather than the tumour itself, which means this can only properly be studied in animals. Processes such as metastasis (spread of cancer) also cannot be modelled adequately in the laboratory. Moreover, potential new drugs need to be tested in an animal setting before they can enter the stage of clinical trials in human patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are committed to minimising the numbers of mice used in our experiments. We will prioritise and rationalise experiments based on initial experiments in the laboratory (*in vitro* studies). Small, pilot experiments will be conducted, where necessary, prior to carrying out larger experiments. Multiple parameters will be

	analysed in each individual experiment, to provide maximum information from the minimum number of animals. Statistical analyses will also be conducted to determine the minimum number of animals required to achieve a significant result.
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 tumour models used are the lowest form of mammal recognised as relevant for human cancer. The kidney cancer models that are proposed have been used by others and their relevance to human biology has been demonstrated. Discomfort and distress to animals will be limited to unavoidable procedures required for the conduct of sound research. We will take all reasonable steps to reduce discomfort or suffering to mice during the experiments. Temporary discomfort will result from the surgery and anaesthesia required for injections into the kidney. This will be minimised by administration of painkillers. Only one kidney in any given mouse will be injected, to avoid any suffering from inadequate kidney function. Tumour growth will be closely monitored. For tumours in the kidney, we will use imaging techniques to monitor growth from an early stage, avoiding tumours reaching a size that may be distressing to the animal. When grown under the skin, mice will be killed if *a tumour exceeds a mean diameter of 1.5 cm*. The growth of subcutaneous tumours may sometimes lead to ulceration at the tumour site. It may be necessary to humanely kill mice that develop ulcers and this decision will be made by using an ulcerated tumour scoring sheet**.** Any mouse that shows any signs of distress or suffering, in any experiment, will be immediately humanely killed.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Trigeminal and spinal acute/chronic pain systems.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Pain, spinal, trigeminal, dorsal horn</td></tr>
<tr><td>Expected duration of project</td><td>5 yrs</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes
X</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes
X</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No
X</td></tr>
<tr><td>Protection of natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No
X</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No
X</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No
X</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No
X</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</td><td>Yes</td><td>No
X</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>Pain that won’t ‘go away’ is the most common reason that drives us to seek medical help. It is an affliction with a big socio-economic impact and accounts for >500 million lost working days p.a. at a cost of >€34 billion. Clinical management of chronic pain is poor -many sufferers report a lack of relief and being left to ‘soldier on’. This is unacceptable and inhumane. Our studies seek to understand the neurobiological processes that cause an acute pain that ‘heals’ to become a chronic condition which can last a lifetime.</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>An understanding of pain, especially chronic pain, will aid the future development of better treatments for many current intractable pain conditions. The results from these studies will provide us with important information relating to how the body processes pain signals. Such studies are already leading us towards targets for new therapies which will benefit those people and animals suffering from chronic pain conditions.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Rats – 1,300
Mice – 2,700</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>Most experiments will be carried out under anaesthesia and the animals will not wake up from the anaesthetic. In some cases it will be necessary to induce on-going pain by inflammation or by</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?	inducing nerve injury. It should be noted that these procedure will be performed under anaesthesia but that animals will recover for further study at a later time. These animals do not experience debilitating on-going pain, they perform normal daily behaviors e.g. feeding, drinking, grooming & socializing. However in behavioral tests where stimuli are applied to the affected area, they show localized increased sensitivity to mild mechanical and thermal probing. Any animal displaying noticeable distress/loss of normal behaviors as a result of the procedures are immediately sacrificed using an approved humane procedure. All animals are sacrificed humanely 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 complicated and poorly understood. Unlike senses such as 'seeing' or 'hearing', there is no well-known brain area dedicated to pain. We are beginning to understand more about some key players e.g. spinal cord & trigeminal brainstem. However, we are not yet in a position to fully replicate pain processes in models such as cultured neurones or to apply computational simulations. Therefore there remains a requirement for animal models approved for use by the Home Office. However, in part of this project 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 animals	In considering the needs of each study, we are able to use approved methods (Power Analysis or Resource Equation) to work out how many animals are strictly necessary. 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 not so many that the lives of animals are sacrificed to no purpose.
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 are the rat & mouse. Both species are good models widely used in Neuroscience research and studies of pain. Small animal models like this have almost fully replaced the use of larger mammals such as dogs and cats for this type of basic science research. The spinal cord and trigeminal brainstem (two 'pain' areas that we are interested in) are very similar across different types of mammals including humans. Lower species such as frogs, insects or crustacean do not have the complexity of the mammalian central nervous system and, whilst they are used for some types of neurobiological studies, they are not used broadly for pain research. This is because we still need to learn more about what exactly is 'pain' for these simpler animals. To minimize the

	welfare cost of our work, anaesthetics are used usually in a way that means the animals do not wake up. Where animals recover, a careful eye is kept on them to make sure that they are behaving normally and not showing signs of distress. If needed, local anaesthetic cream and post-surgery analgesia will be used to reduce local soreness.