Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 14

Project Titles and key words

- Assessment of mechanisms of stroke induced impairments in rodents

  Stroke, dementia, depression, recovery

- A Practical Course in Microvascular Surgery

  Microvascular Surgery

- Investigating the biology of musculoskeletal tissues in health and disease.

  Musculoskeletal tissues

- Diagnosis and Control of Bacterial Infections

  Vaccination, diagnosis, bacteria, infection, livestock

- Origin of male reproductive and related disorders

  Androgens, Diet, Environment/lifestyle, Fertility, Testicular germ cell cancer

- Behavioural and Cognitive Neuroscience

  Learning, memory, space, hippocampus, plasticity

- Motor Networks of the Spinal cord

  Spinal cord

- Recovery of function after nervous system injury

  Stroke, brain injury, spinal cord injury

- Regulation of Brain Function across the Lifespan

  Stress, glucocorticoids, early-life programming, cognitive ageing, mood disorders

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Assessment of mechanisms of stroke induced impairments in rodents</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Stroke dementia depression recovery</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[1]</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[2]</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 project aims to examine the long term time course of motor and cognitive impairments following a stroke, examining the role of comorbidities in the development of these symptoms and the response to treatment. In particular, the project aims to determine:

• whether the long-term changes in human motor, cognitive and psychiatric function that develop after an ischaemic stroke can be modelled in rodents (mice, rats), and develop / evaluate methods for assessing these symptoms.

• the role of co-morbidities (eg hypertension) and systemic inflammation in the development of the long-term changes in motor, cognitive and psychiatric function that develop after an ischaemic stroke

• the molecular, cellular and neuroanatomical mechanisms causing the development of the long-term changes in motor, cognitive and psychiatric function that develop after an ischaemic stroke

• the scope for modifying with treatments the development of the long-term changes in motor, cognitive and psychiatric function that develop after an ischaemic stroke</td></tr>
<tr><td>What are the potential benefits</td><td>This project will benefit the scientific community by</td></tr>
</table>

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

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	providing better methods for assessing stroke in rodent models. This will mean that treatments can be tested more rigorously. It also aims to establish the causes of some complication which arise following stroke such as dementia and depression and develop treatments for stroke.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice, totalling 1500 over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals which undergo experimental stroke surgery will develop neurological deficits, this will be the main adverse effect of the project. In some cases, the brain damage may cause mortality: though this is unavoidable, we aim to avoid any mortality by humanely killing any experimental animal which appears unlikely to recover. We require a neurological deficit which is detectable, but not so severe that it impairs the animal significantly. The long term impairments will not prevent the animals used for testing post stroke impairments from moving around the cage or prevent them from eating and drinking. It is expect that some animals will reach substantial severity limit. At the end of the studies the animals will be killed by a humane method in order to collect tissues for histological examination.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Due to the nature of the project there is no suitable alterative to assess complex behavioural functions. It is also not possible to use patient based studies, as it is difficult to determine in patients which aspects of comorbidities have the most significant impact on the development of complications, due to the heterogeneous population and lack of detailed information on patients prior to the stroke. In animals models it is possible to test each factor individually and establish the specific important role of each these factors
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical advice will be sought in order to keep the number of animals used to a minimum. In addition imaging techniques such as MRI, may be used which enable one group to be followed over time, rather than using multiple groups.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	We are using the “gold standard” model of stroke. Aseptic surgical techniques will be used and the most up to date methods of refinement to reduce weight loss after surgery will be used. Where appropriate pain relief will be given to the animals under the guidance of a veterinary surgeon. We will assess various aspects of motor and cognitive function using a variety of behavioural

(harms) to the animals.	tests. These tests were selected based on their ability to detect deficits neurological disease models.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">A Practical Course in Microvascular Surgery</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Practical Course Microvascular Surgery</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5yrs</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[3]</td><td colspan="3"><table>
<tr><td>Basic research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></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><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td><s>Yes</s></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 Microvascular Course provides hands on experience for qualified surgeons to learn the intricacies of microvascular anastomoses. This provision is necessary as surgeons of certain specialties are now expected to be able to reconstruct bone and tissue after disease or injury. The microsurgery expertise required to do this cannot be learnt adequately on human subjects and if attempted without training may result in failure. Once a surgeon has completed a carefully constructed training programme he has the dexterity and confidence needed to transfer to this to the operating theatre, along with the knowledge needed to critically assess the anastomoses and be able to predict the outcome.</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 vast majority of trainees, i.e. more than 95%, are expected to gain sufficient skills to be able to perform clinical microsurgical techniques under supervision and a number develop sufficient competence to perform them without supervision. 95% will be able to achieve a good end-to-end and end-to-side arterial and venous anastomosis using the femoral vessels, and 70-80% will be able to produce a functional free flap by the end of the course. This should enable several units to take on microvascular procedures which they could not otherwise consider with consequent considerable benefits in patient care.</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">Rats, approximately 350 over 5 years.</td></tr>
</table>

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mild discomfort associated with restraint and administration of anaesthesia. Rats are maintained under anaesthesia and euthanised without recovery from anaesthesia. Non-recovery
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In spite of the advances in models that allow trainees to practice operations in a simulated surgical environment, such as laparoscopic surgery, arterial access, and suture boards, there are no prosthetic devices that replicate vascular microsurgery. The acquisition, development and maintenance of such skills can only be achieved by instruction and practice. A variety of techniques have been used in the past to develop and maintain these skills with variable success (e.g. chicken legs, placental blood vessels). None of them has proved completely satisfactory. The surgeon needs to practice the key steps, critical for operative success, for example, mobilisation of the artery and vein from surrounding tissues, preparation of the vessel wall with minimal handling and trauma, accurate positioning and tension of sutures, management of vascular leakage (haemostasis), an awareness and appreciation of problems such as vessel spasm. One is forced to conclude that presently the only truly viable method of assessing the adequacy of microvascular anastomosis is to observe blood flow across the anastomotic site and this inevitably involves living tissue.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Practice will take place using prosthetic devices. Only once participants are deemed to have shown proficiency in the basic techniques of suturing under an operating microscope, using non-living materials, will they move on to the anastomotic techniques using vessels in terminally anaesthetised rats. Participants are not allowed to progress to terminally anaesthetised rats until approximately 10 perfectly placed sutures have been achieved in non-living material – as assessed by one of the consultant course tutors. Each participant will generally use one rat each 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	Rats are the most (only) suitable species. The vessels are an ideal size for the practice of division, anastomosis, flap and graft creation. Animals will be maintained under anaesthesia throughout by experienced licensees.

minimise welfare costs (harms) to the animals.	

Investigating the biology of musculoskeletal tissues in health and disease.

Summarise your project (1-2 sentences)
We propose a detailed study of disease mechanisms leading to abnormal cartilage growth (chondrodysplasias) and osteoarthritis (OA) using genetically tractable mouse models. This will lead to the identification of potential biomarkers and of novel treatment avenues which can be translated into the clinic.

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.
Cells in our bodies exist embedded in a scaffold of various proteins, so called extracellular matrix (ECM) which they lay down during the course of their lives. ECM determines the biomechanical properties of tissues and contributes to the diffusion of nutrients and signalling molecules, and thus tissue homeostasis. Mutations in genes encoding ECM components lead to disease (for example increased matrix deposition leads to fibrosis and increased breakdown of cartilage matrix leads to osteoarthritis). The purpose of this project is to determine the mechanisms by which mutations in genes encoding the ECM components lead to chondrodysplasias and how they contribute to the more common complications such as osteoarthritis and lower-limb weakness. Numerous ECM mutations leading to musculoskeletal diseases have been previously described; however, the mechanisms leading to these conditions remain unknown. Our research to date highlighted the role of cell stress in the disease mechanism. However, we still don't know whether the stress responses we detected are protective or destructive in our models.

Outline the general project plan.
We will use mouse genetic models of human disease to examine the role of cell stress in the disease progression. We will cross our mouse models with mouse lines in which stress pathways have been blocked (knockouts) and examine the effect on disease severity. We will also determine the impact of cell stress upon the onset and severity of mechanically-induced osteoarthritis. These studies will identify pathways which could be manipulated to reduce disease severity and potential therapies will be tested.

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.
The mouse strains included in this application exhibit a mild short-limbed dwarfism and in our experience suffer no obvious pain or discomfort. Most our studies involve analysis of tissues after humane sacrifice of the animal. For the study of osteoarthritis, the animal will undergo a small operation (under general anaesthesia) on one knee to destabilise the joint and cause a reproducible osteoarthritis; as a result it will develop a slight limp. Potential treatments may be administered by injection which can cause momentary discomfort.

Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.
These studies will advance our knowledge of musculoskeletal biology and disease. They will provide insight into how ECM contributes to tissue integrity and disease, and what role cell stress plays in chondrodysplasias and osteoarthritis. In addition, our experiments will identify potential therapeutic targets and test small molecule compounds which could be used in treatment of patients in the future.

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.
Species: Mouse (Mus musculus). Number of animals: 10,750 - Breeding and phenotyping 10,000; Treatment 500; Osteoarthritis 250.

Chondrodysplasia and osteoarthritis can be studied in dogs and in Hartley guinea pigs. We rejected these models due to unknown genetic causes of canine chondrodysplasias and guinea pig OA, and the extended timescale of such study. Mice are the ideal model for our studies. Their skeletal system develops in a similar fashion to humans; they also develop OA similar to that in humans in response to joint destabilisation in a relatively short time frame; and they are genetically tractable. We have previously generated genetic models of specific human chondrodysplasias. Moreover, we can genetically dissect the importance of various pathways by crossing our mice with genetic knockouts of specified stress-related genes and determining the effects on disease onset and severity.

To generate statistically robust data, we performed calculations to predict the exact number of animals to be used. For quantitative aspects of the programme, age and sex-matched animals will be used. Where feasible, the animals will be littermates to reduce variation. Moreover, in order to reduce the numbers of animals needed for bone measurements, imaging under anaesthesia will be employed to collect data. The most reliable and reproducible mechanically-induced model of OA will be employed to minimise experimental variation in onset and severity of disease. For qualitative aspects, breedings where all the offspring have the desired genotype will be employed to reduce numbers of animals with unwanted genotypes. The number of observations will be the minimum necessary to provide an adequate description (single or multiple samples from 3 animals per group). Lines that are not required in next 6 months will be frozen down (sperm freezing if possible to reduce numbers of animals used) rather than maintained as minimal colony.

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 essential in our investigations. Although several aspects of musculoskeletal biology can be modelled in cell culture, the systemic process of bone growth involves integration of many tissues and cannot be modelled in cell culture; moreover, transgenic mouse models allow us to genetically dissect various disease pathways. We have applied advanced statistical analyses to ensure we extract maximum insight from the minimum number of animals used. We will use cell culture models where possible, to dissect the disease pathways in parallel with the animal studies and to biochemically test the likely efficacy of specific drugs prior to their use in mice.

Explain why the protocols and the way they are carried out should involve the least suffering.
Animal suffering should be minimised during the procedures for both ethical and scientific reasons. Undue stress may affect the quality of data thus leading to larger sample numbers. Transgenic models of chondrodysplasias employed in our project all present with a mild short limbed dwarfism which does not impact on the quality of life of the animal. Potential treatments may be administered by injection which can cause momentary discomfort, however, the animals will be carefully monitored during treatment protocols by experienced technical and scientific staff, and appropriate anaesthetics and analgesics will be sued where necessary to ensure they do not experience undue suffering or distress.

	Under the osteoarthritis induction protocol, most animals will experience some inflammation, (and possibly pain), and eventually arthritis, in their joint; as a result they may develop a slight limp. Post-operative pain will be controlled by the use of appropriate analgesics and the animals will be closely monitored immediately following the surgical procedure, focusing on the animal physical activity, grooming and well-being, in accordance with the LASA guidelines.
2	**What checks are to be made on the animals and how frequently?**
	The transgenic mouse strains included in this project application exhibit a mild short-limbed dwarfism and in our experience suffer no obvious pain or discomfort. The animal wellbeing will be monitored frequently (every day or at least 3 times a week) by the research staff and animal technicians. We will monitor the weight of the animals and create relevant growth curves, we will also focus specifically on the monitoring the animal physical activity and grooming.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Diagnosis and Control of Bacterial Infections</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Vaccination, diagnosis, bacteria, infection, livestock</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>Yes</td><td></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>Despite the use of vaccines and antibiotics, bacterial diseases in farmed animals cause continuing and significant welfare and economic problems and, occasionally, are also a threat to human health. This work seeks to examine and define the ways in which bacteria cause disease in domestic animals in order to devise new and effective methods of diagnosis and control, using relevant experimental models and the most up-to-date equipment, methodology and scientific information.</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 work is responsive to national and international needs, contributes to biological, veterinary and medical knowledge and is in the public interest. It is designed to improve the well-being of animals in human care and to be beneficial to the environment. If the objectives are achieved the benefits will be to remove obstacles to sustainable agriculture, improve the health and welfare of farmed animals throughout the world and alleviate suffering in animals caused by infectious bacterial disease.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The animals proposed for use are the natural hosts for the diseases being studied. Laboratory animals are used for initial in vivo studies as an efficient and quick means of providing novel information on pathogenesis and bacterial virulence that can then be transferred to more limited work in the target species (cattle). The numbers used are restricted to those expected to produce statistically significant answers to the question(s) posed, using a range of statistical methods based on previous work and experience in conjunction with experts in the field.</td></tr>
</table>

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

	Over the 5 year duration of the project, up to 300 mice and 300 cattle may be used, although wherever possible the use of surrogate (non-animal) systems will be employed to address the research objectives.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Great care and attention has gone into refining the techniques used to monitor animal responses to the treatments used in order to reduce the degree and duration of any suffering incurred to a minimum. Work with mice and cattle is not expected to be of greater than moderate severity. Experienced observers, with access to veterinary advice and care at all times, monitor clinical signs of all experimental animals at regular intervals in order to identify quickly any animal requiring veterinary treatment. Any animal failing to respond to treatment is killed humanely. By necessity, experimental animals will be humanely killed at the end of procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever possible, the use of non-animal models will be employed throughout this work as an alternative to the use of animals. However, in order to fully understand the ways in which bacteria cause disease, it is sometimes necessary to study this process in animals. This understanding will help in the development of new diagnostic tests and vaccines to help prevent infection. However, the use of animals is an absolute requirement for the assessment of the efficacy and safety of any new vaccine.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The careful refinement of experimental models ensures that only the minimum number of animals required to deliver a statistically significant and biologically relevant outcome will be used. Independent advice on experimental design is provided from trained statisticians in advance of work being conducted. In addition, proposed experiments are reviewed by an ethical review committee to ensure that only the minimum required number of animals are used. Mouse models of infection have been developed to reduce the need for experiments using cattle, meaning that work conducted in large animal species is only used for more targeted objectives. In addition, continued use of non-animal models for much of the work further reduces the number of animals used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general	We have developed a relevant, reliable and reproducible disease model in conventional cattle, which has been refined to be the least severe necessary for valid results to be obtained. Great care and attention has gone into refining the techniques used to monitor animal responses to

measures you will take to minimise welfare costs (harms) to the animals.	treatment in order to reduce the degree and duration of any suffering to an absolute minimum. Trained teams of observers monitor animals at regular intervals, accurately evaluating the responses of individual animals and initiating veterinary intervention where necessary.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Origin of male reproductive and related disorders</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Androgens, Diet, Environment/lifestyle, Fertility, Testicular germ cell cancer</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></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">Male reproductive disorders that affect either newborn boys or young men are remarkably common – eg a low sperm count affects 1 in 5 young men. Other disorders, such as testicular germ cell cancer (TGCC) have become increasingly common and affects young men in their 20s-30s. Research in the past 10 years has shown that these disorders are likely to have a common origin in fetal life that involves impaired production or action of androgenic hormones (eg testosterone) by the fetal testes during the masculinisation process. However, studying exactly what goes wrong in fetal life and how this has consequences, such as low sperm count or TGCC, 20-30 years later, poses huge practical problems in humans. This stands in the way of identifying causes of these disorders (eg lifestyle, diet, environmental chemical exposures), which in turn hinders the development of strategies to prevent the disorders from occurring.

The overall aim of this project is to use laboratory animal models to provide understanding that can subsequently guide clinical and interventional studies in humans. The animal models have been developed (and validated) specifically to address these problems – for example to identify what environmental chemicals or painkillers can affect testosterone production by the fetal testis, and the pathways involved. We plan to refine and further develop these models and to apply them in new ways. For example, there are 2 key unanswered questions about TGCC – (1) how and why do the</td></tr>
</table>

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

	pre-cancerous germ cells develop in fetal life, and (2) why do these cells only develop into a tumour after puberty, and not in childhood? As there are no animal models for TGCC, we are using a mouse model bearing xenografts of (1) normal human fetal testis tissue or (2) pre-cancerous germ cells from the testes of men with TGCC. Another important, and new, objective of our animal models is to identify how fetal and early postnatal androgen exposure can affect testosterone levels in adulthood. This is important because low testosterone levels in adulthood can predispose men to develop modern 'Western' diseases (obesity, type 2 diabetes, cardiovascular diseases), that are increasingly common. Lastly, there is emerging evidence that diet affects the reproductive processes/disorders in which we are interested, and might also have effects across generations – in other words 'you are what you eat, or maybe what your father or grandfather ate'. We will use a new animal model to study this that allows us to follow effects between generations and to identify critical time periods when diet can cause effects.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	In the short-term, our studies will identify the pathways via which male reproductive disorders arise, which in turn will enable us to assess experimentally what factors can affect these pathways adversely. For example, we will study how common painkillers (eg paracetamol) used by women in pregnancy, can affect these pathways, including effects on testosterone production and on germ cell development, or how the mother's diet (or that of the father) can affect these. In the longer-term, this will enable the development of preventative measures or guidance (to pregnant women) on how to minimise the chances of there being adverse reproductive effects. For TGCC, we have a unique, world-leading xenograft model that enables us to study hitherto hidden events that lead to development of TGCC. These studies have the added benefit of telling us about how normal germ cells develop, information that will be applied to develop means of preserving these cells in boys who are treated for cancer, as the treatment can destroy germ cells and thus their future fertility. Our past use of the animal models referred to above has identified the critical time period in fetal life (2-3 months of human pregnancy) when

	androgen action is all-important for ensuring normal reproductive development and function. They identified a non-invasive measurement that can be applied in human males at any age to 'measure' how much androgen exposure occurred during the critical fetal time window. A general aim of our studies is to identify and validate other such 'biomarkers' that can be used practically in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the next 5 years we expect to use 2450 rats and 800 mice.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of our studies involve only administering compounds to rats or mice during pregnancy and/or after birth. We do not use doses that cause general toxicity as this would confound our studies. Our most invasive models involve xenografting of tissue or the transplantation of germ cells, procedures which require anaesthesia and surgery (including castration in some instances). These all use well established and reliable procedures. Adverse effects of surgery, such as pain and risk of infection, are short-term (2-3 days) and are managed with appropriate analgesics and antibiotics according to local practice. At the end of procedures all animals are humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Studying of the fetal origins of later/adult disorders is impossible to study in vitro and is not amenable to study in humans in other than a descriptive way. 'Experimenting' on human fetal testes during normal, dynamic development (over weeks/months), which is essentially what our xenograft model enables, is otherwise impossible; in vitro studies only work for a few days and results are considered non-definitive by regulators.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our extensive experience guides our choice of animal model and numbers and experiment design. Collection and preservation/archiving of multiple tissues (reproductive and non-reproductive) is one way in which we facilitate future studies without new experimentation.
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.	Some aspects of development, for example of fetal germ cells differ in rodents and humans, hence the need for a xenograft model. The latter also enables us to compare function, regulation and susceptibility of rodent and human fetal testis, which is invaluable in experimental planning and design/choice of appropriate model. For xenograft studies in nude mice, we use rigorous sterile techniques and mainly subcutaneous grafts (via cannula) to minimise surgical intervention.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Behavioural and Cognitive Neuroscience</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Learning, memory, space, hippocampus, plasticity</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</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>
<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>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</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>One of the most important features of human life is our ability to learn, to remember and to forget. It is essential for everyday of our life, and particularly during critical periods such as a child learning at school or an older person living alone remembering things around the house. Our aim is to understand the neural mechanisms of learning and memory to a sufficient depth that we can advise on new procedures that might help learning, and/or new drugs or other treatments that might aid memory or prevent its loss.

Our past research has been a significant contribution to the current worldwide effort in this scientific domain, one of the so-called Grand Challenges of Contemporary Neuroscience.

Some components of our research involve human subjects and non-interventional experiments. Other parts involve in vitro approaches. But the need to make interventions in the brain to fully understand the underlying mechanisms requires the use of animals in our experiments.

Our more specific aims are to conduct state-of-the-art research on memory, spatial function, neuronal plasticity and related work, using animals models, focusing on better understanding of genetic disorders of brain development in children.</td></tr>
<tr><td>What are the potential benefits</td><td></td></tr>
</table>

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

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Research is inherently unpredictable, but our ambitions are to secure a much better understanding of the important process of memory consolidation (i.e. what makes memories last), to understand why our ability to remember events from one moment to the next ties in with memory for the spatial environment in which we are moving around (the "what and where" of memory), and whether forgetting is an active or passive process. The likely benefits of having answers to these questions are developments in (a) education and (b) therapeutics for cognition. For example, our work on 'schemas' using the event-arena paired-associate task is already perceived as having educational implications. The work on forgetting is relevant for the development of drugs that could enhance cognition.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice (N = 10,800 over 5 years)
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals are extremely well treated in our laboratory - an extensively modernised and state-of-the-art laboratory in which particular attention has been paid to the care and maintenance of the animals. The animal keeping quarters have very recently been re-furbished. The procedures are arguably the best in the country for behavioural neuroscience. The behavioural tasks we are using are very unlikely to have any adverse effects – in fact there is reason to believe that the animals are well motivated to participate because they secure food by doing so. There are potential adverse consequences of the surgery during which we either deliberately damage a very small part of the brain, we place very small electrodes to allow stimulation of or recording from specific brain areas, or we put in small tubes through which we can deliver very specific drugs. We provide the animals with pain-killers after the surgery and after they recover from the surgery they show few if any side-effects other than in behaviours related to learning and memory. At the end of a study, it is generally vital to check using chemical techniques that we have, for

	example, placed the drug infusion tubes in the correct places. This requires that we humanely kill the animals, take out the brain and process it using appropriate tissue staining methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals as we plan to make interventions that are not ethical for humans. Some experiments, as noted, can be done on humans and we will do this where a non-interventional method is feasible. However, recording the activity of brain cells deep in the brain is only possible at present with animals as is the use of light to activate brain cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are very keen to use the minimal number of animals to see a statistically robust effect. We can often do this by enabling a single animal to participate in a study that is conducted in stages rather than use far more animals who do these stages separately. The stages of these experiments are explained in protocols.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have been at the forefront of refining behavioural tasks for animals in a manner that makes them much more like tasks which humans perform daily. Thus, whereas some labs overseas use conditioning with electric shock, we see no need to do this and believe that an animal will participate much more effectively and with minimal stress in a study in which it works for its daily ration of food. The implication of our approach is that we are building in 'welfare' considerations to the design of our protocols.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Motor Networks of the Spinal cord</td></tr>
<tr><td>Key Words (max. 5 words)</td><td></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)[11]</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[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>The spinal cord is an integral component of the central nervous system. It is responsible for providing the brain with information about the internal and external environment and controlling the neurons that activate muscles to produce movement. In this project we will study the organisation of neuronal pathways that project between the brain and the spinal cord which influence movement.
The organisation of the pathways we will study is poorly understood. It is known that the pathway from the spinal cord to the brain has a profound influence on the cerebellum, a structure which has an important role in organising muscle activity to produce coordinated posture and movement. The pathway we are interested in may have a role in providing the cerebellum with information about injury to peripheral structures, (e.g. stubbing a big toe) so that appropriate modifications of movements can be made to compensate for injury. The pathway from the spinal cord to the brain also operates in tandem with a reciprocal pathway which projects from the brain to the spinal cord. This pathway may form a reflex arc which directly adjusts posture in response to information provided by sense organs in the skin. The evidence that underlies these proposed functions is partial and the aim of this project is to perform a rigorous analysis of the organisation of ascending and descending spinal pathways which will provide greater insight into their functions</td></tr>
<tr><td>What are the potential benefits likely to derive from this</td><td>This project will provide new information about an important network of nerve cells that control</td></tr>
</table>

[11] Delete Yes or No as appropriate.
[12] 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)?	movement. It is known that when part of this pathway degenerates as a consequence of a hereditary disease (Marcado-Joseph Disease) that failure of muscular coordination occurs. It is anticipated that this study will shed new light on the organisation of the pathway and may lead to a better understanding of what goes wrong. In addition, there is evidence that the component of the network that projects from the brain to the spinal cord can compensate for the loss of the major motor pathway from the cerebral cortex to the spinal cord following stroke or spinal injury but the basis of this compensation is poorly understood and we need more accurate information about the organisation of this component in order to assess how compensation occurs.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats will be used in these experiments. The rat is the standard laboratory animal for studies of the spinal cord and there is extensive literature on this species. The rat provides a good model of a mammalian nervous system which has much in common with species higher up the phylogenetic scale. We intend to use approximately 150 rats over a five year period. Our policy is to obtain the maximum amount of data from each animal; experiments are designed to provide material for several parts of the study from the same animal.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Some parts of the study involve injection of tracer substances into the brain, spinal cord or peripheral structures such as muscle or nerve. These procedures are performed under general anaesthesia but the tracer substances can take several days to be transported to their target neurons or nerve cell endings so animals are allowed to recover from anaesthesia for several days following the procedure. Usually animals recover from these procedures uneventfully and are routinely given drugs to control pain and/or inflammation. If any animal shows unpredicted adverse effects following these procedures, it will be killed humanely. At the conclusion of all experiments, animals will be given an overdose of anaesthetic and tissue will be prepared for microscopic examination.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of living animals is necessary for this project because meaningful data can only be obtained by studying an intact, fully functional nervous system. It is not currently feasible to study the systems we are interested in in reduced preparations or isolated brain slices where much of the central and peripheral nervous system has been removed. In addition, most of the techniques we will use only work in a living nervous system.

	There are no computer simulations of the systems we propose to study but data generated from our study will be useful to construct such models. Imaging techniques such as functional MRI cannot be used for this type of work because they have low resolution and do not provide information about individual nerve cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experimental design for this study has been refined so that we can obtain the maximum amount of data from a relatively small number of animals. In many cases tissue from a single animal can be used to satisfy more than one of the objectives of the study.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Adult rats will be used in the study. The rat is the standard laboratory animal for this type of work and there is an extensive literature on the rat nervous system. The rat nervous system has a similar organisation to higher mammals including humans. The protocols for this project are designed to minimise suffering. In some procedures animals will be allowed to recover from general anaesthesia following surgery. Such animals will be closely monitored and, where appropriate, will be given pain-killing and anti-inflammatory drugs. Animals showing unpredicted disability and/or pain will be killed humanely.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Recovery of function after nervous system injury</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Stroke, brain injury, spinal cord injury</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[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>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 studies the consequences of different types of nervous system injury. It will develop new tools for manipulating gene expression in the nervous system. It will also test potential therapies for restoring function lost after nervous system injury. This is important because there are no fully restorative therapies for disabilities resulting from stroke, spinal cord injury or peripheral nervous system injury.

<u>Stroke:</u> Cerebral ischemia (stroke) kills about 4.5 million people every year and there are over 9 million survivors left afflicted. In the UK and USA, stroke is the third greatest killer and the leading cause of disability. In the UK, stroke services are estimated to account for up to 6% of the NHS budget, not including costs to social services and carers. Treatment for stroke after the acute phase is currently limited to rehabilitation. There is a particular need for stroke research using <u>aged</u> animal model systems: stroke is most prevalent and disabling in elderly humans yet few preclinical studies use aged model systems. This may explain in part why treatments that showed pre-clinical promise failed in clinical trials: it is now recommended that aged animals be used in some preclinical studies. There is a need for therapies that work when initiated hours or days after stroke because of delays to hospital admission and diagnosis: this is especially the case for existing survivors of stroke. We have established a model of stroke in aged rats and will now test therapies for improving limb function at different times after</td></tr>
</table>

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

	injury. Spinal cord injury: Human spinal cord injury (SCI) is often incomplete, leaving some sensory and motor circuits partially functional. Optimising the function of surviving neurons and nerve fibres is desired because it can lead to improvements in function. We will use models of partial SCI together with manipulation of gene expression in spared (uninjured) tracts using new gene therapy techniques. We will develop and use viral vectors to increase or decrease expression of potentially therapeutic molecules in neurons to determine whether limb function improves. Peripheral nervous system injury: Peripheral nerve cells can sprout new processes after injury but the mechanism is not well understood. A better understanding could lead to the development of novel therapeutic strategies after nervous system injury. We aim to identify the transcripts and proteins involved. The eventual aim is to develop therapies that restore function lost after nervous system injury.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The work of my laboratory aims to advance science and quality of life across two fronts: 1. Provision of new research tools: Our viral vectors could be used to modulate gene expression in other cell types with a view to improving outcome after different types of nervous system injury. Naturally, we will make these tools available to other researchers. 2. Testing new therapeutic targets: We aim to determine whether anatomical and functional recovery can be obtained following different types of nervous system injury when novel therapeutic strategies are employed and tested. With this program of work we hope to identify methods for improving function after nervous system injury.
What species and approximate numbers of animals do you expect to use	*Per annum*, we expect to use 100 adult rats and 100 aged rats, although this will depend on the success of each study.

over what period of time?	*Per annum*, we expect to use twenty breeding pairs of adult rats to provide pups for cell culture studies. *Per annum*, we expect to use 40 normal adult mice and 40 genetically modified mice. Breeding and stock control can lead to larger numbers of animals being used. For example, when breeding genetically altered mice, not all pups in a litter will possess the desired genetic alteration: one therefore has to breed a larger number of mice to ensure sufficient numbers of the correct type are obtained.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	1. Cell culture work involves removal of tissues after humane killing; no additional adverse effects are expected. Severity level is mild. 2. Stroke and spinal cord injury work involves surgeries performed with anaesthesia and analgesia. We use models that only cause clumsiness in the animals rather than severe disability. Severity level is moderate. Animals will be humanely killed at the end using an approved method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals need to be used in all these studies because many techniques are not possible or feasible at present in humans. Cell culture and computer simulation techniques are also not sufficiently advanced that they can model the integrated actions of the nervous system. This is largely because our understanding of mechanisms within the nervous system is insufficient to allow effective modelling. Thus, we will undertake some of our work in animals. Mostly we are interested in animal models of human disease or pathology, and so some of our experiments will make use of such models. Some of these models are short onset and short duration and can therefore be studied acutely in animals. Other models, like their human counterparts, develop and change over time and so some of these experiments may last weeks or even months. The prolonged time course of some experiments, and the fact that one of the most important outcome measures in our work is behavioural assessment of the animal, means that only some work can be done on animals under terminal anaesthesia: the remainder will require the use of recovery protocols.
2. Reduction Explain how you will assure	We aim to measure many different variables in each rodent, thereby reducing the number of

the use of minimum numbers of animals	animals used, and improving the power of the study. As a generalisation, each rodent will receive a nervous system injury under anaesthesia. A potential therapy will be administered and tract tracing performed to identify whether nerve cells change their patterns of wiring. Analgesia will be given during the acute recovery period. Weekly testing will be performed to determine whether functional recovery occurs. Histology will also be performed on each rodent to see whether predicted or unexpected changes occur. The principles of refinement, reduction and replacement will be adhered to throughout. Cell culture studies will be conducted before some animal experiments to select effective vectors for gene manipulation. This reduces the numbers of animals required. Effective pain relief and anaesthetics are used rigorously. The number of animals used will be chosen based on prior experience in experiments of this kind, upon pilot studies and with an aim to reducing this number but retaining effective statistical power in all cases. We will also seek to reduce the number of animals studied by careful experimental design, the adoption of sensitive outcome measures with small variation and the study of only the most relevant time points. For all the experiments proposed we will use a group size which is the smallest compatible with achieving statistically meaningful and robust results. I consult statisticians where necessary. However, I have considerable experience in this type of work, and have published extensively in peer-reviewed journals. Thus, I already have a very good working knowledge of the optimal way to design and execute this type of experiment. Animals will be housed communally and in many cases in enriched environments to maximise social / welfare / rehabilitation considerations.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use rats or mice in our experiments. We require a mammalian model as the studies need to correlate to the human brain and these species represent the lowest sentient mammalian species. Furthermore, rats and mice are the lowest species commonly used in experiments of this kind over the last 50 years meaning a great deal is known about their anatomy, neurophysiology, genetics and behaviour. Mice are being used in some of these studies because a powerful approach to studying the role of

	genes is to use genetically altered animals. Animals with an inducible or a tissue specific mutation are likely to be of particular value. Rats are being used in other experiments because they are more easily assessed after nervous system injury using behavioural techniques. Recovery experiments will be conducted under general anaesthesia and the animals' physiology will be closely monitored. We have experience of all the techniques detailed in this project and the experiments we conduct on animals are expected to cause minimal stress. All animals are subject to regular inspections by the scientists, NACWO and veterinary surgeon and mild health problems are dealt with accordingly. In the event of any unexpected adverse reaction during experiments the animal will be humanely killed.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Regulation of Brain Function across the Lifespan</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Stress, glucocorticoids, early-life programming, cognitive ageing, mood disorders</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)[15]</td><td>
<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[16]</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>Stress throughout life is known to be is associated with increased incidence of psychiatric disease. Stress elicits the release of stress hormones, glucocorticoids, which have wide-ranging effects on the brain that consequently affect behaviour. This project seeks to identify the behavioural consequences of altered glucocorticoid action on the brain and the molecular mechanisms that underpin these effects, taking into account the differential effects that occur in development through to ageing. Glucocorticoids exert long-lasting effects in development that alter brain behaviour throughout life and stress and glucocorticoids exacerbate memory decline with age. Understanding and delineating how the glucocorticoids exert these consequences on brain function, will enable the development of novel treatments and/or identification of biomarkers for brain disorders.</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>This research will contribute to our understanding and knowledge of how stress hormones work and are controlled.

Knowledge resulting from this research may contribute to refinements in clinical use of the glucocorticoids already licensed.

This research might identify novel applications of glucocorticoids or could potentially lead to</td></tr>
</table>

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

	development of new drugs. Our research on glucocorticoids has already led to the development of new drugs currently undergoing clinical trials in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years, Rats, 1278 per year; 6388 over 5y Mice, 3088 per year; 15440 over 5y
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the animals will be used in breeding programmes. *For experimental animals:* *The stress paradigms. A number of stressors may be used that include exposure to psychological (e.g. isolation in novel cage) or physical (e.g. exposure to cold environment) stimuli. These stimuli will induce acute stress responses or when exposed to stressors on multiple occasions, chronic stress. The expected severity limit will be from mild to moderate.* *Substances will be administered at doses known to be non-toxic, based on experience and dosages reported in the literature, and at volumes in accordance with Best Practice*. Bodyweight and behaviour (e.g. lack of grooming, coat condition) of animals receiving substances will be routinely monitored. If weight loss exceeds 20% in a 72h period, animals will be humanely killed. Surgical procedures will be necessary to, for example, remove the adrenal gland that produces the stress hormones. This will then allow hormone replacement at the level required. *During surgery, deaths resulting from anaesthesia or surgical complications are uncommon and will be minimised by correct dosing of anaesthetics, by accurate weighing and by maintenance of body temperature during and post surgery e.g. use of heat pads. Pain will be controlled using analgesics. Best Practice for surgery/post-surgical care, anaesthesia and analgesia will be followed at all times.* *Imaging. Most imaging procedures require transient or complete anaesthesia which will be administered according to guidelines, animals will be constantly monitored throughout scanning, and contrast agents, if required, will be administered at doses known to be non-toxic.* *Behavioural testing is not associated with any adverse effects.*

	At the end of experiments, animals will be humanely killed and tissues collected for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The study of the effects of stress on brain function across lifespan means that we need to use whole animals in our experiments. Some effects of corticosteroids on eg neurones can be investigated in vitro but these can only provide information on one small part of the processes that contribute to altered brain function. Animal models of psychiatric disease and inflammation recapitulate key aspects of the disease in humans and allow mechanistic dissection of the contributory processes and factors. They can then provide vital proof of concept data to enable translation of key concepts to humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used in our investigations is based on power calculations to determine optimum group size and statistical power. Where possible, a multi-factorial design is used to increase power and reduce the overall number of animals required. The use of inbred mice reduces experimental variability and thus overall numbers required. Imaging techniques (similar to those used in humans) in live animals allow sequential non-invasive measurements, providing repeated measures within a single animal, increasing statistical power and reducing the number of animals required for experiments. The effects of treatments are based on comparison with appropriate control groups. Study design is based on current best practice and, where necessary, following discussion with statisticians.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	In all our experiments we are mindful of the need for refinement to reduce suffering, and appropriate modifications to protocols are incorporated where possible. In carrying out an experiment, when we identify ways to reduce animal suffering without compromising the scientific integrity of the experiment, we will always seek to incorporate these refinements. We have already done this in several of our protocols. For example, body weight and coat condition are monitored for all rodents e.g. undergoing aging, imaging, drug dosing or extensive behavioural testing etc and are provided with rapid treatment for any health issues that should arise. Where possible in chronic dosing experiments drugs are administered orally to reduce the suffering

	associated with daily injections. When metabolic cages are used, if experimental design allows, animals are housed in pairs to reduce the stress associated with these cages. We seek advice from the named vet and animal technicians to reduce suffering and where further opportunities arise to reduce suffering, whilst maintaining scientific rigour, we endeavour to incorporate them