Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 18

Projects with a primary purpose of Other Basic Research including:

- Sensory Organs (skin, eyes and ears)
- Forensic enquiries
- Other Research

Project Titles and Keywords

1. **The genetics and biology of parasitic nematodes**
    - Parasitic nematodes, rodents

2. **Notch-Wnt interaction in embryonic development**
    - Notch, Wnt, endocytosis, pluripotency, stem cells

3. **Malaria parasite development and drug discovery**
    - Plasmodium, malaria, genetics, antimalarial, mosquito

4. **Centrosome biology in mammalian development, aging and stem cell function**
    - Development, stem cells, aging, cell division

5. **Production and Maintenance of GA Rodents**
    - Production, Maintenance, Genetically Altered Rodents

6. **Generation of transgenic mice for gene manipulation**
    - Transgenic, Gene Targeting, Knockout, Embryonic Stem Cells

7. **Re-derivation of mouse strains**
    - Re-derivation; superovulation; embryo transfer

8. **Identification of immunological targets for developing new agents to prevent corneal transplant rejection**
    - Immunological, agents, corneal, transplant, rejection

9. **Mechanisms of movement generation and inhibition**
    - motor cortex, mirror neurons, movement inhibition, subthalamic nucleus, hyperdirect pathway

10. **Responses to centrifuge-induced hypergravity**
    - Mouse; Biomechanics; Posture; Gait; Locomotion

11. **Pharmacokinetics and metabolism studies**
    - Metabolism, Pharmacokinetics, Regulatory, ADME, Non-clinical

12. **Technical development of preclinical imaging**
    - Imaging, non-invasive, quantitative, longitudinal

13. **Doping/medication control for British horseracing**

- Anti-doping, medication, equine, detection time, control

14. Neuroimmune interactions

- Cytokine, brain, liver, microglia, astrocyte

15. Anti-angiogenics in retinal neovascularisation and neuroprotection

- Retina, angiogenesis, neuroprotection

16. Object recognition by zebrafish visual system

- Zebrafish, vision, in vivo imaging, classical conditioning

17. Understanding and manipulating proteostasis

- Neurodegeneration; chaperone; blindness; virus; stem cell

18. Regulation of inflammation in wound repair

- Chronic wounds, inflammation, diabetes

19. Developing therapies for inner ear disorders

- Hearing, deafness, balance

20. Vocal and social learning of avian calls

- Learning, vocalisation, mobbing, birds

21. Eye lens shape and form

- Eye lens cataract accommodation, radiosensitivity

<table>
<tr><td>PROJECT 1</td><td colspan="3">The genetics and biology of parasitic nematodes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Parasitic nematodes, rodents</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 section 5C(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></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</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">Parasitic nematode worms are very common parasites of humans, domesticated and wild animals.

We want to discover what genes parasitic nematodes use to be successful parasites, and how those genes work to allow nematode worms to be successful parasites.</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 potential benefits of this work are to understand the basic, fundamental biology of parasitic nematode worms. More specifically we will benefit from knowing how these abundant parasites manage to live inside other animals, such as ourselves.

In the longer term this fundamental knowledge could be used by others who are working to discoverer new ways to treat nematode infections</td></tr>
</table>

	of humans or animals.
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 4,500 rats over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	There are no expected adverse effects. The expected severity is mild. At the end, the rats will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Parasitic nematode worms are obligatory parasites of animals; their natural habitat is living inside of another animal. Parasitic nematodes cannot be maintained without using laboratory animals, and so this project cannot be done without the use of laboratory animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We reduce animal use by using the highest safe dose of worm infection in each rat, so that overall fewer rats are used. We also use genetically altered rats which keep their worm infections for longer. While there is some slight harm in these rats being genetically altered, by using genetically altered rats it means that overall fewer rats are used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use rats because they are the natural host species of the parasites we study. This means that we are studying a natural, evolved host – parasite association. Wild rats are naturally infected with these species of parasites. The rats become immune to the infections we give them. We give them infections at doses which do not cause noticeable harm to them.

<table>
<tr><td>PROJECT 2</td><td colspan="3">Notch-Wnt interaction in embryonic development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Notch, Wnt, endocytosis, pluripotency, stem cells</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 section 5C(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></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</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">The ageing nature of the population demands new approaches to the treatment of chronic and degenerative diseases, for which the use of stem cells may prove effective. Although substantial improvement has been done towards obtaining stable stem cell cultures from embryos, the biology of the stem cells is poorly understood. This results in the loss of the capability of stem cells to form any cell type in the body as we culture them on dishes.

The present project seeks to improve the stability of stem cell cultures, taking into consideration how two specific proteins (Notch and Wnt) interact with one another. This interaction depends on basic cellular machinery responsible for recruiting and moving proteins inside the cells.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or</td><td colspan="3">The findings of this project will provide essential information of basic stem cell biology, as well as new methods to obtain and maintain stable stem cells in culture. During this project, new mouse</td></tr>
</table>

animals could benefit from the project)?	transgenic lines will be generated that will benefit other scientists interested in Notch and Wnt (also involved in several types of cancer), and also in how proteins move in the cells. Moreover, the findings obtained could be further used in the field of regenerative medicine.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse (3069)
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals used in this project are not expected to exhibit any harmful phenotype. However it is not possible to fully predict the nature or severity of any potential defect and for all types of mice there will be a careful monitoring of possible side effects. Animals exhibiting any unexpected harmful phenotypes will be killed by a Schedule 1 method. The expected level of severity will be mild to moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Prior to embarking on animal experiments, we will collect as much evidence as possible using stem cell cultures. Only the informative cell lines will be transferred to the mouse model. Whilst work on cultures can be informative to understand stem cell biology, work in whole organisms is necessary to understand how the stem cells are formed in the embryo.
2. Reduction Explain how you will assure the use of minimum numbers of animals	It is not possible to predict the success of novel transgenic mouse line derivation experiments, but only skilled personnel will carry them out to reduce the number of animals needed. Embryos from each new mouse line will be cryopreserved so that when the experiments utilising each line have been completed, the breeding and maintenance maybe terminated. The use of recently developed *in vivo* imaging technology allows pre-screening of animals carrying fluorescently labelled transgenes, easily seen using

	specific light sources. Application of these procedures reduces wastage of animals that may inadvertently killed at an unsuitable time-point. We expect to reduce the number of animals used in such procedures by 75% by this in vivo monitoring approach. To maximise the information from a single animal, tissue samples from relevant transgenic mice used in our experiments will be provided to local scientists interested in Notch, Wnt, or movement of proteins in the cells.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	A vertebrate model system, such as the mouse , will produce results that will be more easily extrapolated to humans. To generate transgenic mice, inducible constructs will be used whenever possible. This means that the mice should not display a phenotype until the transgene expression or deletion is activated. For each procedure, we will use the most refined protocol possible, e. g. -anaesthesia and analgesia will be used when appropriate -will only be carried out by skilled personnel -providing any drug treatment by a palatable oral route rather than injection where possible. We predict from previous studies and publications that some transgenic mice could potentially show severe defects when 2 copies of the transgene is present. To avoid pain and distress in the new GA mouse lines, these will be closely monitored for adverse effects. The monitoring will include food and water consumption, weight loss, what the mouse looks like, changes in behaviour or litter size. Animals exhibiting any unexpected harmful phenotypes will be killed, or in case of individual animals of particular scientific interest, advice will be sought from the local Home Office Inspector.

PROJECT 3	**Malaria parasite development and drug discovery**	
Key Words (max. 5 words)	Plasmodium, malaria, genetics, antimalarial, mosquito	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Over half the world's population is at risk of malaria and hundreds of thousands of people, mainly children under 5 years old, die from the infectious disease every year in developed countries. There is growing concern that many antimalarial compounds are becoming ineffective at treating the disease thus new therapeutic treatments are required before the current medications become completely ineffective. One of the first goals of developing new therapeutic interventions for malaria is to have a clear understanding of parasite biology which will then allow the identification and characterisation of putative drug targets. Therefore, the main objective of this project licence will be to provide a better understanding of parasite cell biology and to continue to identify new drug targets for future intervention strategies. We will continue to study cell division as well as other smaller projects to understand host parasite interaction and molecular motors in the	

	parasite in the lab. This will include looking at the expression and function of a variety of genes which are crucial to parasite survival. The second objective will focus on drug development of novel antimalarial compounds which have been shown to eliminate malaria parasite infection in cell culture. We will continue work with several previously characterised drug targets as well as validate various other drug targets using known and novel inhibitors which may one day lead to the next line of defence against the malaria burden.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Malaria is a major burden in many under developed countries and is responsible for hundreds of thousands of deaths a year, thus a new family of antimalarial drugs is essential to treat the infection. The main benefit of this research is an increased understanding the role of key genes in the malaria parasite, which will help to identify new intervention targets that can be used to reduce the burden of malaria. Research on key projects relating to parasite motility and cell division, will undoubtedly lead to a better understanding of cell biology and parasite development in general. This will result in improved knowledge for the scientific community and identification of crucial drug targets for intervention. Furthermore this data will be used for future grant applications and give some lead drug targets to be developed for clinical trials. Based on this fundamental research, we will then continue the characterisation of lead drugs which we wish to further develop to allow progression to clinical trials. We plan to conduct pre-clinical testing of new drugs and inhibitors to find new treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use approximately 9,000 mice and 200 rats over the 5 year duration of the project licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	A level of moderate severity has been applied to this work, although this limit will rarely be reached. However, if the limit is reached or when the end-point of the experiment is achieved, the mice will

level of severity? What will happen to the animals at the end?	immediately be terminated by a schedule 1 technique at the end of the experiment. Only minor adverse effects are generally seen when studying gene function of malaria parasites (objective 1), however occasionally a high parasitemia can result symptoms of anaemia, at which point the animal will be terminated. The animals will be monitored for parasitemia on a regular basis (usually once a day from day 2). Parasitemia will not be allowed to exceed 30% before the animal is terminated to minimise the likelihood of adverse effects occurring. . Drug treatment and screening (objective 2) with novel antimalarials may not always be well tolerated by mice however observable effects will be well below the moderate severity limit and will last no longer than 4 hours. All agents will be used at appropriate, low concentrations that are known to be well tolerated by mice (based on a preliminary pilot study). Clinical signs include mice temporally becoming docile, lethargic and passive but still responsive to stimuli, all well below the moderate threshold of severity. These limits and expected adverse effects are also based on extensive past experience from our previous project licence and experience.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Work on the human malaria parasite, is largely limited to the asexual stages of parasite development in the blood and the species is harder to genetically alter compared with rodent malaria. Rodent malaria allows the investigation of particular genes/proteins throughout the whole life cycle, which is crucial when studying topics such as motility and cell division, as these also occur in the mosquito vector as well as the blood of the mammalian host. However, the rodent malaria line can only be cultured in rodents. Where possible, cell cultures will be used, but these assays cannot adequately model the complete array of molecular, cellular, physiological and behavioural interactions necessary to fully understand how the parasite interacts with the host. Moreover cell culture assays cannot predict adverse effects or the role of

	metabolism when conducting drug screening.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will maximise the use of any material collected from the mice. The smallest number of mice will be used for each experiment whilst still remaining statistically significant, for example drug experiments will use triplicate samples for statistical power.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The malaria parasite, can only be cultured in rodents and, as we normally only a small volume of infected blood, we use mice. Mice are easier to handle and, with regards to malaria parasite breeding, far more information is known about mice. The method used to generate mutant parasite lines reduces the number of mice required to produce these lines significantly.. We have further refined this method during the duration of our previous project licence by reducing the number of mice further by checking parasite viability with more accurate and faster detection methods. The most appropriate method of administration of parasites or drugs will be selected that is least invasive but still provides the most statistically accurate results. All of the animal work will have a moderate severity limit mice, mainly due to the possible effect of high parasitemia, although this is unlikely to be reached. If the animals do reach (or are likely to) these limits, the experiment will be terminated immediately. In most experiments the parasitemia will not be required to reach 30%, which will reduce the likelihood of malaria related symptoms. This will be monitored by smearing tail blood every day (2 days post infection) as well as monitoring the general health of the animals

<table>
<tr><td>PROJECT 4</td><td>Centrosome biology in mammalian development, aging and stem cell function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Development, stem cells, aging, cell division</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)</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</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 aim of our study is to understand why mutations in certain genes cause dwarfism in the human population.</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>Certain mutations in our genes lead to developmental disorders such as dwarfism, small brain or bone and heart problems, but we do not understand why these conditions arise. We propose to use animals to model these disorders in order to improve our understanding of these diseases. Our study has direct relevance not only to the developmental disorders, but also to cancer treatments, since similar mutations are found in many human tumours. We hope that our study will also open up potential strategies for killing cancer cells.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>Mice (Mus musculus)</td></tr>
</table>

over what period of time?	Numbers: 6500 Period : 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?	Majority of the substances administered are not expected to cause any lasting adverse effects. Of itself, injection of substances will cause no more than transient discomfort and no lasting harm. Cross breeding of some of the animals that carry particular mutations will produce offspring with unknown characteristics, which will be interesting to this project and we will analyse them in greater detail. Tumour burden, if developed, will be limited to the minimum required for a valid scientific outcome. Certain proposed procedures such as abrogation of the animal's own immune system and injection of foreign cells in to such animals might result in moderate severity, but such animals will be kept in a clean and controlled environment and will be closely observed to minimize any suffering. In all cases, the general health and condition of an animal will remain the overriding determinant. Mice will be killed if they show signs of ill health, such as piloerection, hunched posture, inactivity or inappetence, which cannot be alleviated by minor veterinary intervention.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Prior to embarking on animal experiments we will collect as much evidence as possible from cell culture work. There are two reasons why we cannot exclusively use cell culture to obtain results. First and foremost, our study aims to understand mutations that interfere with development, stem cell function and cancer. Since all these are complex physiological processes and due to lack of cell culture systems that mimic this complexity, our work necessitates animal models. Second, for *in vitro* experiments cells must be removed from their natural environment. Interaction with this environment may affect their capacity to divide, differentiate, survive or die, and thus isolated cells may not reflect the process that takes place in intact tissues/organs. The transgenic mice selected for this work are valid models for human

	developmental diseases, as indicated by their shared characteristics.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When designing the experiments we will perform statistical analyses to ensure that we use the minimum number of mice per group that will be informative, including carrying out pilot experiments. Cryopreservation of embryos from lines that are no longer in active use will decrease the number of animals needed for the experiments. In order to reduce the number of breeding pairs, mice will be bred as homozygous provided they are fertile and have no harmful phenotypes. We will minimise use of animals by teaming up with other research groups interested in surplus tissues from same animals that are not used for this study. To maximise the information from a single animal, we will aim to collect samples from most organs. These samples may be shared with other scientists to minimise the breeding of further animals.
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 already demonstrated that transgenic mice strains can mimic a range of clinical features present in patients with primordial dwarfism. We therefore believe that despite their small size, mice are a good model system to study growth retardation. When similar genes are mutated in lower vertebrates such as zebrafish, the phenotypes are very crude (ie kinky tail) and as such are less informative. We aim to understand a human developmental disease caused by homozygous mutations in a single gene, meaning that all tissues of patients carry only the faulty copy of this gene. To best mimic this scenario, we need to breed the mice as straight knockouts. To avoid unexpected pain and suffering, mice will be bred and analysed as heterozygous animals first. In case of tissue-specific or conditional deletion of genes, we will only use well-established reagents and protocols for their induction.

<table>
<tr><td>PROJECT 5</td><td colspan="3">Production and Maintenance of GA Rodents</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Production, Maintenance, Genetically Altered Rodents</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 Article 5)</td><td colspan="3"><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</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">To produce unique strains of genetically altered Laboratory mice as required by various research programmes within the facility To rederive or “clean up” animals of a lower health status thereby improving their health status.

To cryopreserve genetically altered mice for future use

On a temporary basis to breed and maintain strains of genetically altered Laboratory mice for use within other research programmes within the facility.</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">This programme aims to provide a core of highly skilled technicians that will provide a service to allow production of new lines/strains without the need for continuous training of new staff members. By utilising a core team the procedures involved will be carried out to the highest possible standards. It will allow unique genetically altered strains of mice to be produced and go on to be used in applied</td></tr>
</table>

	human medicine research programmes. It will allow strains to be cryopreserved for future use or transportation reducing the number of animals or the need to ship live animals. It will allow new colleagues to work without interruption whilst research licence applications are being assessed.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice approx. 12,000 over 5 years – However this is on the assumption that this service licence will be used continually throughout its lifespan – in reality numbers will be considerably lower and will be dictated by user needs.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Hormone injections involve injections of a small volume. The mice will suffer only very mild transient discomfort. Vasectomy surgery is a short surgical procedure. The animals are given pain relief and are able to mate after a two week recovery period. If not used for a long period of time the males may be paired with a female companion animal to prevent long term isolation. Embryo transfer surgery is a short surgical procedure in which fertilised embryos are transplanted into the oviducts through incisions in the flank. The animals are given pain relief seem to behave normally within a day or so. If successful the mice are allowed to give birth and kept with the offspring until weaning. The vast majority of GA mice held on temporary basis for breeding and maintenance for other research programmes, the genetic alterations themselves are overtly normal and will show no changes in behaviour, have no health implications and show no noticeable adverse effects. A small number of mice are expected to mimic a disease and may have variable signs according to the disease but will be limited to moderate.
Application of the 3Rs	
1. Replacement	As this is a service licence purely to maintain and

State why you need to use animals and why you cannot use non-animal alternatives	produce unique strains of laboratory animals then there is no way that animals cannot be used for these purposes
2. Reduction Explain how you will assure the use of minimum numbers of animals	Minimum numbers will be used by ensuring that lines maintained are kept at the lowest possible size to maintain line integrity. Careful line maintenance will be followed to ensure no excess animals are produced. Where possible the most up to date production techniques will be employed to ensure that the maximum number of embryos etc. is harvested from the minimum number of mice.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Animal models used in this licence are essentially dictated by needs and requirements of scientific colleagues who will request use of this service licence. Animal welfare costs will be minimised by the use of experience and skill staff, appropriate anaesthesia and analgesia will be used in surgical procedures and in all other regulated procedures with the exception of the those in which the administration of these substances would result in more distress and suffering than the distress and suffering likely to be caused by carrying out the regulated procedure without the use of these substances. The facility is a modern purpose built facility run as per the Home Office Code of Practice. All equipment is regularly serviced and maintained. There is a veterinary surgeon on site and available for advice The most updated and refined techniques available for all protocols contained within this programme of work will be used

PROJECT 6	**Generation of transgenic mice for gene manipulation**	
Key Words (max. 5 words)	Transgenic, Gene Targeting, Knockout, Embryonic Stem Cells	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This service licence will permit the generation and maintenance of genetically altered (GA) mice at this establishment. The project plan objectives are: 1. Nucleic acid microinj ection to generate new strains of transgenic mice 2. Targeting Embryonic stem cells to generate “knockout” or “knock-in” mice 3. Generation of new cell lines for targeting experiments 4. Applying new technologies to improve the efficiency of targeting and genome modifications	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or	GA mice allow specific gene or genomic elements to bemanipulated and examined in the context of the intact organism. Thus, this provides information on the function of particular genes or genornic regulatory	

animals could benefit from the project)?	sequences in physiological processes and in the development of disease. Many research groups on this site use such mouse models to study the genetic basis for cancer, immunity, epigenetics and inflammation. However, generating GA mice is costly and time consuming and requires expertise and specialised equipment. A centralized service will reduce animal usage because of the availability of trained staff for technically difficult procedures, centralised quality control and quality assurance procedures (including standard operating procedures), because mouse resources may be shared across projects, and because no individual lab would normally be able to devote the amount of time to increasing efficiency, implementation of new technology and troubleshooting that a centralized service can. The potential benefits are new mouse models that are indispensable resources to addressing the biological questions in the search for and development of treatments for human diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	This project may use up to 3000 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The protocols involve injection of embryos with DNA or ES cells, superovulation, surgical implantation of embryos into recipients, surgical generation of sterile mates, in vitro fertilisation (IVF), standard breeding of mice in individually ventilated cages, and tissue collection from such mice for genotyping. Adverse effects might include infertility and mild or moderate phenotype. Any adverse effects will be carefully monitored.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Laboratory mice are well-established and widely used by the scientific community as a model for a wide variety of human diseases and physiological processes. They have the followings advantages: (i) similar anatomy and organ function to humans, (ii) similar genome, (iii) generally used for genome manipulation including gene targeting, (iv) animals can be easily produced.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We will be able to use strain specific embryonic stem cells (ESC) to enable direct production of co-isogenic mice. This will significantly reduce the number of animals used since no backcrossing will be needed. Donor embryos will be collected from mice with the optimal age and weight. The number of embryo transfers performed will be reduced by implanting optimal numbers of embryos, maximising the number of healthy offspring and using recipient females in an outbred strain where larger litters are the norm and maternal behaviour well developed. Generation of novel and currently not available genetically altered animals will be performed by competent staff to reduce animal usage.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is an appropriate experimental model for defining human gene function because of its anatomic, physiologic, and genetic similarity to humans. The mouse is the model of choice to study physiology, ageing and disease progression, where the study of these processes is most advanced and for which most existing genetically altered strains are available Surgery i.e. embryo transfers and vasectomies is performed by skilled technicians with excellent success rates. Appropriate analgesia and anaesthesia is always given to animals undergoing surgical procedures. When appropriate to the stage of embryo non-surgical embryo transfers are used as a refinement over surgery. Numbers of embryos implanted are optimised for maximum numbers of healthy pups from fewest procedures. Vasectomies are performed using the least invasive technique possible. However close health monitoring, provision of appropriate treatment under the guidance of a veterinary surgeon and adapting husbandry routines to the needs of the animal will be used to ameliorate any adverse effects if arise.

<table>
<tr><td>PROJECT 7</td><td>Re-derivation of mouse strains</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Re-derivation; superovulation; embryo transfer</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)</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</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>This project licence enables strain storage by cryopreservation techniques, rederivation and also provides for limited, continued breeding.

The mouse enables gene function to be investigated in an animal whose genetics and physiology is highly relevant to that of a human. An important goal for the study of cancer is the development of sophisticated disease models, something that can only come about by building on an understanding of function of individual gene products and the pathways in which they are involved in an entire mammalian organism.</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</td><td>The animals and their tissues will subsequently be used by scientists and researchers to obtain data relating to the cause and cures of a variety of cancers and other major diseases. The products of this will be published, where appropriate, in the scientific literature as part of their own studies and</td></tr>
</table>

project)?	will enable better understanding of these processes.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice only in this project. Our estimate is that we will need to use up to 10,000 female animals for the provision of eggs or embryos; produce up to 1,200 vasectomised male animals to induce pseudopregnancy in foster mothers; and up to 5,400 female animals to act as the foster mothers. The number of wild type or genetically altered mice expected to be born or bred as a result is expected to be approximately 27,400.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	There are a number of techniques employed to introduce and propagate mutations in the mouse germline which can be addressed in three stages – embryo donation, embryo manipulation and embryo transfer. For embryo donation 'superovulation' is often employed whereby hormone injections lead to the stimulation of the ovary to produce and release unfertilized eggs in great number. Superovulation involves two hormone injections into young mice, most often in the age range 3-5 weeks, followed by a mating to a 'stud' male mouse. The female mice are then culled. Typically 30 – 50 early stage embryos per mouse can be collected following superovulation. There are specific situations where superovulation cannot be used and so natural mating is then required, but embryo numbers are then far lower (up to 10 per mouse). Embryo transfer (surrogacy) is the final stage whereby selected embryos (which can equally as well stem from a thaw, collection from mice with pathogens, from IVF or after an embryo manipulation) are implanted into a 'pseudo pregnant' foster mother, (a mouse mated to a vasectomised or genetically sterile male.) This might require that the foster mouse is anaesthetised and embryos are introduced into the exposed oviduct through a laparotomy. Later

	stage pre implantation embryos can be implanted by a non-surgical technique that introduces embryos directly into the uterus by means of simple injection through the cervix. The embryo number implanted is predetermined so that the most likely result is a small to average sized litter (5-7). CD-1 or hybrid (F1) mice are normally used based on their excellent abilities to care for the resulting litters. In considering adverse affects we can note that procedures are either 'mild' or moderate' in classification. We do not expect adverse effects from the procedures that are mild in classification. In generating transgenic mice there is scope for unpredictable effects to result but this is far more likely to become apparent in later stage breeding which will always be done after transfer from this project licence to one which authorises such work. The scope for adverse effects is greater from the procedures that are classified as moderate. These are the surgical techniques of (surgical) embryo transfer, vasectomy and ovary transplantation. Ovary transplantation is now a rarely required technique. Vasectomy and embryo transfer are routine procedures where experience and skill ensures that adverse effects are also very rare. Surgery is performed by skilled staff who will apply aseptic techniques. Complications are extremely rare.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Clearly our work is based on the use of mice. However to put this work in context, the generation of mutant lines of mice is an important step in defining gene function in mammals. Candidate genes are selected through extensive research that might take place in-house or may be building on work completed elsewhere. Previous work in a plethora of organisms, bacteria, yeast, mammalian cells, fruit flies, zebra fish and toads may provide the basis for an experiment involving the generation

	of mutant mice. As the services that we provide are based on the mouse, alternatives in this case are not appropriate for our consideration but we would require that all laboratories who ask for our services have considered alternative approaches.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Central to the provision of a service for the production of transgenic mice, is the need to reduce and refine the numbers of mice used in both production and breeding phases. Through our techniques and skills and through working closely with colleagues, we do achieve an efficient service of a high standard that demonstrably serves to minimize the numbers of mice employed in such experiments.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are universally used for work involving genetic alterations. The standard protocols, methods and reagents have been optimised for this species and there are acknowledged benefits from their use. The methods chosen are all standard for this type of work. Current guidelines for best practice will be followed, including: GA mouse welfare assessment working group http://www.nc3rs.org.uk/page.asp?id=231 The research facility works in collaboration with other facilities in the UK and abroad. We ensure the techniques are done in the best, most up to date way through, for example, interactions with the International Society for Transgenic Research (ISTT), the Laboratory Science Association (LASA). The large body of scientists we work with also generates collaborations and ideas to further develop the techniques we provide. Such close collaboration creates what I feel should be an essential and on going process of optimization. Although some work can be carried out in-vitro it is necessary to use in-vivo models in order to better understand normal physiological processes and abnormal disease processes. Rodents can be used to study a wide range of morphogenetic processes

	and they provide good models for human pathologies. Mice are the only mammal in which transgene and gene targeting technologies work reliably and their 21 day gestation and weaning means that the gene(s) of interest can be tracked quickly and appropriate breeding programmes established. Genetically altered mouse strains have been bred with assistance from Animal Facilities for many years. These have included strains of both mild and moderate severity limits but the vast majority are animals with no significant adverse phenotypes. The generation of mice requires some skills, some techniques and specialised equipment that could be provided to each laboratory, but given the greater applicability of these techniques now in research it becomes essential that a centralised resource be provided. This can reduce the numbers of mice employed and through experience and endeavour, techniques can be refined such that animal use in what could be an inefficient, even wasteful procedure, is minimised.

PROJECT 8	**Identification of immunological targets for developing new agents to prevent corneal transplant rejection**	
Key Words (max. 5 words)	Immunological, agents, corneal, transplant, rejection	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The purpose of the research described in this licence is to identify factors which cause the normally avascular cornea to vacularise and the immune mechanisms of recognition of and injury to transplanted donor cornea. This will reveal novel targets for therapeutic interventions which ultimately will facilitate longer survival of transplanted corneas and other tissues. Corneal transplantation is the only available treatment for restoration of vision in many patients with such diseases causing loss of corneal transparency. About 4,000 corneal transplants are undertaken in the UK annually. However, the two major problems which limit the impact of transplantation on corneal blindness are (i) blood vessel growth in the cornea and 01.) immunological rejection of donor corneal transplants leading to graft failure. Immune rejection is the commonest reason for corneal transplant failure; this project will investigate the mechanisms of graft recognition and rejection, the rational basis for development of more effective medicines to prevent	

	rejection from occurring. A typical experiment will begin with transplantation of a donor cornea, using the same surgical technique as in patients. Transplanted corneas will be examined on a frequent and regular basis until there are signs of rejection of donor cornea. At selected intervals prior to or following transplant rejection, the recipient animal will be euthanised so that the cornea and lymphoid tissues can be studied for immune changes. Most of the animals used will be mice and rats as their immune system is comparable to that in man. Rabbits will be used in selected experiments in which a species with an eye of larger size is necessary to address research questions. Facilities, husbandry and animal care procedures are based on best practice, regularly monitored and conducted by highly trained staff.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The overall benefits arising from this licence will be greater understanding of the components of a donor cornea that induce vascularisation and rejection. and the identification of new medicines to prevent this. The most promising treatments will be taken forward to trials in patients.
What species and approximate numbers of animals do you expect to use over what period of time?	Rabbit 80 over 5 years Rat 100 over 5 years Mouse 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?	• Insertion of corneal vascularising sutures May rarely lead to corneal infection. In the event of infection, sutures will be removed and antibacterial ointment administered. In the event that infection does not resolve, the animal will be killed by a Schedule I method. • Corneal transplantation 1. The two most frequently encountered causes of surgical technical failure are (i) wound leak (ii) cataract formation. Infection rarely, if ever, occurs. These complications are usually diagnosed on day I or 2 following grafting. Animals are not distressed by these complications, but such recipients, or any animal showing undue distress, will be

	excluded from further study and killed by a Schedule 1 method. 2. Onset of graft rejection may cause minimal distress to the animal. Signs of rejection can only be detected using a microscope. In the event of any distress evident in the animal's behaviour, it will be killed without delay by a Schedule I method. In our experience of this protocol in the term of the previous licences, no mice have had to be sacrificed for this reason. • Removal of aqueous humour samples from the anterior chamber [2 (i) above] can be safely undertaken under general anaesthesia. Cataract or hyphaema (anterior chamber haemorrhage) might result from needle trauma to lens or iris vessels. In that event, the animal would be killed by a Schedule I method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Investigation of the mechanisms of graft rejection can only be undertaken in animal models. While in vitro correlates give some information, they cannot predict the outcome of a graft. Vt is understanding these mechanisms which will improve the outcome of corneal transplantation in patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In the transplantation experiments, experimental groups will comprise approximately 10 recipients. Previous data from the laboratory has shown that in the high responder donor-recipient strain combinations which we use, the interval to rejection onset is very predictable. C3H donor cornea survives for 11-13 days in BALB/c recipient mouse and Brown Norway donor cornea survives for 11-12 days in Lewis recipient rat. A biologically significant difference between two experimental groups would be 33% (equivalent to extending survival by —4days). Data will be analysed by suitable statistical methods. In some cases the numbers of animals in the experimental group will need to be adjusted because of differences in the variables or sizes of the expected biological effect. Thus if we see or (based on previous experiments) expect large differences in survival, we will reduce the sample size. I am aware of the

	importance of the principle of reducing the number of animals to the minimum needed to obtain statistically valid results.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	of corneal transplant rejection are best addressed using the transplant model that I have established in our laboratory. Most studies investigating immunological mechanisms in corneal allograft rejection require for rigorous scientific validity inbred donor and recipient strain animals and these must be undertaken in rodent species. We have in the past and propose in the future to use the rabbit corneal transplant model to address research questions in which a longitudinal sequence of specimen examinations is necessary — the small dimensions of the mouse and rat eye make such techniques as aqueous humour sample withdrawal technically impossible and this justifies the use of rabbit in these selected circumstances. The procedure of orthotopic corneal transplantation is standardised in all animals and is categorised as ‘moderate in severity. Surgical technique is almost identical to that used in humans and will be undertaken by personal licensees who have trained in human corneal transplantation. The aseptic surgery set-up, instruments, sutures and operating microscope are as used in our operating theatres in Moorfields Eye Hospital. There are no variations in the surgical procedure which would alter severity in the different specified species. There are no evident signs of pain following uncomplicated corneal transplantation, with correct attention to surgical technique in order to prevent post-operative graft wound leakage or infection. Graft recipient animals will be maintained for the shortest necessary time following surgery that allows data acquisition for the research question.

<table>
<tr><td>PROJECT 9</td><td>Mechanisms of movement generation and inhibition</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>motor cortex, mirror neurons, movement inhibition, subthalamic nucleus, hyperdirect pathway</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)</td><td>
<table>
<tr><td>Basic research</td><td><u>Yes</u></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td><u>No</u></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><u>No</u></td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td><u>No</u></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The observation of the actions of others is central to our social lives. Action observation rarely triggers movements in ourselves, despite the presence of significant activity within the brain motor network. Moreover, these signals clearly survive in paralysed patients and therefore can be harvested to help them to control external devices by means of brain-machine interfaces.

We propose that studying the mirror neuron system, which is active both during action-observation and action-execution, could offer novel insights into both the command signals that do characterise motor execution, and the mechanisms for suppression of unwanted movements, a key feature of human behaviour. We will search for the signals within motor system which are specifically</td></tr>
</table>

	associated with movement generation and movement inhibition. We will look for different brain sources of movement inhibition that occurs during action-observation. Suppression is exaggerated in Parkinson patients leading to the pathological slowing of movement. Therefore, I will investigate cortical neurons which are directly connected to the subthalamic nucleus (STN), a brain structure belonging to the basal ganglia, and heavily implicated in Parkinson's disease. Stimulation of this structure, known as deep brain stimulation (DBS), profoundly ameliorates the symptoms of Parkinson's disease but it is still not known how exactly this therapy works. Research in NHPs has been fundamental to the development of DBS for clinical treatment of movement disorders.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	My research will advance understanding of the brain activity evoked by action-observation as useful signals for brain-machine interfaces, and the mechanisms underlying the therapeutic effects of DBS for motor symptoms in PD.
What species and approximate numbers of animals do you expect to use over what period of time?	6 NHPs over 5 year period
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All animals are pair-housed and are provided with an enriched environment and have large home cages, exercise pens and forage areas. They interact regularly during the day with investigators. The procedure involves a number of stages for preparing NHPs for long term recording in the awake state. This includes a number of separate and well-spaced surgeries under general anaesthesia. These are carried out under full aseptic conditions and involve a full regime of pre- and post-operative analgesia. All anaesthetic procedures are carried out by our NVS and all

	surgeries are performed under his supervision. Training and recording sessions involve head and body restraint while recordings are taken from multiple microelectrodes advanced into the cortex. Neuronal activity is recorded while the NHP performs its trained task. Recordings are usually taken from pairs of cortical sites. During the course of these studies, which typically last for 2-3 years, both cerebral hemispheres are investigated. The expected level of severity is severe. At the end of this procedure, the NHPs are humanly killed by an overdose of anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To understand the human mirror neuron system and its role in suppression of unwanted movements, we need some invasive work that will allow us to interpret correctly and to calibrate the results of human non-invasive studies. It is not yet possible to sample activity of single neurons in the healthy human brain, and recordings from small populations of such neurons in a non-human primate model are essential for our understanding and interpretation of non-invasive methods such as fMRI and TMS.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use advanced experimental techniques which allow us to record more data simultaneously from different brain areas in a shorter time from a single subject. This directly leads to smaller number of animals being needed before enough data has been collected to allow thorough statistical testing of the scientific hypotheses. .
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	The use of an NHP model is essential for this project. Mirror neurons were first discovered in NHPs. The macaque's motor system, including its mirror neuron component, closely resembles that of the human. Rodent models could not be used for this research due to substantial anatomical differences and their inability to perform the complex tasks required to achieve the aims of the

(harms) to the animals.	project. Optimisation of animal welfare is achieved by a variety of approaches, which include: • Positive Reinforcement Training (PRT) of animals to co-operate with the procedure so as to reduce stress • Giving animals a small dose of oral sedative for the first few days after new procedures are first introduced • Use of appropriate pre- and post-operative analgesic and antibiotic regimes • Use of additional NC3Rs approved refinements to improve the outcome of the experiment and improve animal welfare.

PROJECT 10	**Responses to centrifuge-induced hypergravity**	
Key Words (max. 5 words)	Mouse; Biomechanics; Posture; Gait; Locomotion	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	Y	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To investigate the physical and physiological influences on posture and gait selection: why small animals are crouched; why limb forces go up with speed; and why animals use so much energy when locomoting. Our current understanding of how animals – including humans – select their postures and gaits is poor. Similarly, there remains no fundamental account for why running is more costly than cycling. Current models accounting for these issues make contrasting predictions concerning adaptive responses to altered gravity; these can be tested using centrifuge-induced hypergravity.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	A better understanding of the physical and physiological rules underlying animal – including human – gaits is required if interventions are to be performed within a sound theoretical framework. For instance, should surgery, rehabilitation physiotherapy, prosthetics and exoskeletal gait-aids	

	aim only to replicate normal adult human locomotion? What aspects of posture and gait – and even anatomy – are important; what should be preserved, what systems might show redundancy, and what might benefit from change given unusual constraints imposed by injury or disease?
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 66 Mice: 60 laboratory bred and 6 wild-caught.
In the context of what you propose to do to 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' home cage will be placed into a pivoted gondola attached to the end of a rotating arm. When the arm rotates, the gondola will pivot so that the force applied will remain "downwards" – effectively increasing the force of gravity the mice experience. The mice will be exposed to increased gravity gradually – with incremental increases in both the magnitude of the force and the duration of exposure. Experimental success relies on the animals freely and voluntarily using a wheel within the home cage. This wheel is designed to capture information about how the animal runs. There is also detailed video surveillance. Potential adverse outcomes include loss of appetite due to motion sickness. Excess loading would prohibit all locomotion, or prevent sufficient movement to maintain appropriate levels of feeding and preening. Both issues would be identifiable through direct video and activity monitoring, and through daily handling, weighing and condition checking. Animals will either be killed or housed at the Establishment after their use in these experiments. They will only be kept alive if a veterinary surgeon is satisfied that there have been and will be no residual ill effects from the procedures. They may then be used for non-regulated research, teaching, demonstration or equipment development (e.g.

	sensing rodent wheels).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This study approaches the fundamental physiology and mechanics of animal posture and gait. While computer models can provide insight, they are based on empirically observed 'cost functions'. However, the appropriate nature these 'cost functions' remain controversial. Centrifuge-induced hypergravity allows direct tests of competing hypotheses for dominating cost functions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The studies proposed are novel in many aspects, so signal strengths can only be estimated. However, the 'dose' (multiples of normogravity), and the disparity in predicted outcomes (e.g. more or less 'crouched') mean that simple statistics (in many cases binomial) are expected to be adequate; N=6 is commonly accepted as reasonable for similar studies in comparative biomechanics. Experimental design – including animal numbers – will be adapted after each iteration of experiments, as more information is acquired about relevant signal strengths.
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 will be used as the initial study species because of its past as a model organism (its metabolics, muscle physiology and genetics is generally well understood) and its future potential in genetic/pharmacological studies using hypergravity as a locomotor challenge. Further, the development of instrumented rodent wheels allows monitoring of aspects of gait kinetics during hypergravity. Welfare costs are likely to be minimal: in general, only minor adaptations to standard husbandry procedures are required. Damaging levels of hypergravity are likely to exceed those that prohibit locomotion (the upper limit of these studies) by some margin. Animals will be exposed to hypergravity for up to 24 hours at a time, with a minimum rest of 48 hours between exposures. An individual animal will repeat this exposure-rest cycle no more than 3 times under any gravity loading that reduces activity levels

	(defined as duration of rodent wheel use over a 12 hour ‘night’) below 50% observed under normo-gravity; and once at the maximal loading allowing any rodent wheel use. No animal will remain on this experimental protocol over the age of 10 months, and none will be exposed to hypergravity under the age of 6 weeks.

PROJECT 11	**Pharmacokinetics and metabolism studies**	
Key Words (max. 5 words)	Metabolism, Pharmacokinetics, Regulatory, ADME, Non-clinical	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of this project is to undertake non-clinical pharmacokinetic and metabolism studies and supporting validation/investigative studies in rodent species and non-rodent species, (dogs and rabbits) to enable and support pre-clinical and clinical safety testing programmes. The data will be used to review substances under development or satisfy governmental requirements necessary for approval of clinical trials or bringing products to market. Studies conducted in this project form part of a framework designed to investigate effects of pharmaceuticals, chemicals, agrochemicals or food additives/substances, to facilitate a review of substances under development or satisfy a regulatory requirement. Studies are designed to determine specific metabolic or pharmacokinetic endpoints, ranging from: - Simple administration of a dose followed by sampling of blood and/or excreta to measure dose disposition. -	

	Studies to enhance background data on drug action, dose response or mechanism of action, - Mass balance studies involving intermittent periods of confinement to cages where complete quantitative collection of excreta can be assured - Bile sampling using the least invasive method we can, where a tube inserted under anaesthesia to collect bile - Tissue distribution, where test substance in organs and tissues are quantified from samples or whole-body sections taken from an animal after death. Studies vary in duration from a single dose to daily dosing for up to 13 weeks, depending on the intended use or likely exposure to each substance under study. Additionally where a sponsor has multiple compounds each with a similar mode of action or therapeutic theme, these maybe administered in a series of experiments within a single study lasting up to 2 years. On study completion, animals that have been assessed as fit and healthy by a vet may either be used in another procedure or study, to reduce the use of new animals, or humanely killed under anaesthesia to allow examination of the organs/tissues.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Governments require (and the public expects) that substances we are exposed to are safe or their hazards are well understood. It is an internationally mandated legal requirement. Regulatory approval is required to allow drugs to be tested in human or veterinary trials, or for chemicals, agrochemicals to be marketed. The principal benefit of the project is the provision of data to facilitate sound development or regulatory decisions on e.g. refinement of the development strategy, clinical trial approval or marketing authorisation for new medicines or other substances or articles to which humans, animals or the environment will be exposed, thus contributing to their protection and safety.
What species and approximate numbers of animals do you expect to use over what period of time?	The species and anticipated usage over the lifetime of the Licence (5 years) are below: Rat: 16000 Mouse: 5000

	Dog: 1000 Rabbit: 250 Hamster: 200 Guinea pig: 150 Ferret: 50
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Early studies are performed on the basis of limited information and there may be uncertainty regarding the severity of the response. Most animals are expected to experience no more than mild transient effects such as weight loss or changes in demeanour. A small percentage of animals may show more significant adverse effects indicating moderate severity and a very small number of animals may potentially experience severe adverse effects were it not for humane end-points (early intervention or humane euthanasia) to prevent unnecessary suffering. Animals in surgical studies may experience some adverse post-operative effects similar to those experienced by human patients, however, supportive treatments are given to eliminate or minimise these and appropriate humane endpoints are again applied. All surgical procedures are performed under anaesthesia, with pain relief and/or antibiotic cover provided during and after, as appropriate. On study completion, some animals may be reused in other studies, but most animals are humanely killed using an appropriate method. However, under appropriate circumstances, rehoming is also considered following careful assessment for suitability/compatibility of both the animal and the carer.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although non-animal (lab bench or computer based) studies can provide useful supporting data to limit and decrease the number animal studies, meaningful and reliable evaluation of whole body exposure and distribution of a compound within the body, where it is

	converted in the body and to what, and how quickly its passage and/or conversion through the body occurs (metabolic disposition and pharmacokinetics/dynamics) can only be comprehensively achieved in studies using intact animals where all the organs and systems are intact, interacting with each other and interacting with the compound, yielding a naturally complex interdependent system. For this reason, in vitro and ex-vivo test systems in isolation remain inadequate alone. Use of in-vivo animal models remains a mandatory legal requirement; currently, for many of the study types in this project, there is no scientifically, ethically or legally acceptable non-animal alternative available.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A logical tiered sequential approach is generally adopted. Information is reviewed to decide whether testing is appropriate and ethically acceptable and the studies in a program are designed to achieve the desired scientific endpoints with the least risk of pain, suffering, distress or lasting harm to the animals. The numbers of animals used are kept to the minimum commensurate with meeting study objectives and regulatory requirements and further input from statisticians used where appropriate, to ensure robustness and relevance of the scientific data produced.
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. (Part 1)	Regulatory evaluation of drugs, agrochemicals or chemicals generally requires investigation in two relevant mammalian species; usually one rodent and one non-rodent. This project predominately uses rats, mice and rarely hamsters or guinea pig as rodent test species. For the non-rodent species, rabbits, dogs and very rarely, ferrets are used in this project. Selection of which is most suitable is considered according to the physical, physiological and behavioural requirements of the study, biochemical or metabolic similarities with target species (such as man), similarities of action and response to the compound of study,

	temperament and robustness in response to blood sampling. The rabbit shares many characteristics with rodents but are considered a non-rodent choice when it comes to selection of a second test species to meet regulatory requirements. Where the rabbit is not appropriate, dogs are predominantly selected. All animals are monitored for signs of any adverse effects on their health or wellbeing, and to prevent unnecessary suffering, early humane end-points are applied under appropriate veterinary guidance (e.g. modification/withdrawal of treatment with the test substance, provision of palliative or therapeutic treatments, or humane killing of affected animals).
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. (Part 2)	Where appropriate, positive reinforcement training (treat rewards) is used to encourage co-operation in (and minimise any stress of) handling/procedures. Environmental enrichments appropriate to the species are used within the animal facilities, such as play areas/toys, chew-items etc and except where the scientific objectives of a procedure or animal welfare considerations prevent it, all animals are group housed, so that they can interact and behave socially wherever possible. Study designs are reviewed and new methods considered as technology best practice and standards improve and advances become adopted and approved by international regulatory agencies. Wherever possible, experimental samples are collected under anaesthesia or post mortem to minimise any potential suffering. In some circumstances safety markers will also be collected from the animals maximizing the data from individual studies. Maximising data decreases use of further animals and collecting samples post mortem or from terminally anaesthetised animals, minimises suffering.

PROJECT 12	**Technical development of preclinical imaging**	
Key Words (max. 5 words)	Imaging, non-invasive, quantitative, longitudinal	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project license will allow technical development and optimisation to be carried out on advanced imaging methods at the Preclinical Imaging Facility, University of Leicester. The key objective is to build a library of fully optimised imaging methods that can subsequently be applied to individual imaging studies using the state-of-the art imaging equipment at the facility (magnetic resonance imaging, X-Ray CT, ultrasound and fluorescence imaging).	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This work will ultimately benefit all studies involving small-animal imaging. By performing development and optimisation of imaging methods prior to commencing large scale studies, we will ensure high quality imaging data is acquired. This will allow researchers to use fewer animals in their studies. Preclinical imaging has clear advantages over traditional laboratory methods for assessing physiology, disease progression and response to treatment. Imaging is generally non-invasive and can provide an assessment of organ structure and function in the living animal (i.e. without requiring sacrifice). Furthermore, as the anaesthesia required for imaging is considered minimally invasive, repeat	

	imaging of the same animals is possible. This allows disease progression to be assessed within the same group of animals over time and therefore imaging can provide a much better representation of disease monitoring in patient groups. Already, studies aimed at answering some of the key medical research questions are planning to avail of the Preclinical Imaging Facility at University of Leicester (e.g. potential treatments to reduce the spread of cancer in the body, treatment strategies to reduce the impact of stroke, methods to detect age-related disease). By developing and optimising advanced imaging methods, we will ensure that the relevance, quality and novelty of the imaging data from these studies is maximised.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats will be used, It is estimated that up to 150 animals will be used in total over the 5 year duration of the project. The demand for new imaging methods is expected to be highest in the short term, when techniques will need to be established at the Preclinical Imaging Facility for the first time. Following this phase, it is estimated that demand will decrease, as we will be able to draw upon a growing library of fully developed and optimised imaging protocols.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All imaging to be performed is non-invasive. The animals will be anaesthetised for the duration of the imaging experiments In certain cases it will be necessary to inject dyes into the blood supply of the animals. At the end of each imaging session, the animals will be sacrificed by terminal anaesthesia. There will therefore be not potential for adverse effects or lasting harm arising from this work.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Whenever possible, development and optimisation of imaging methods will be performed using imaging phantoms and/or ex-vivo samples. Computational simulation of the imaging signals will also be used to minimise the need for in-vivo imaging. However, in-vivo imaging will be unavoidable in cases where a live animal with normal physiological signals is required for development and optimisation of a given imaging technique.
2. Reduction	Imaging will only be performed on an anaesthetised,

Explain how you will assure the use of minimum numbers of animals	living animal when it is absolutely necessary for development and optimisation of an imaging method. Following each invivo imaging session, data quality will be assessed offline using well-established image quality tests, in order to determine whether or not further developmental in-vivo imaging is required. Whenever possible, in-vivo imaging data will be shared with researchers, application scientists from the imaging companies and/or colleagues from the preclinical imaging field to assist with data quality assessment. This work will not require breeding or housing of any animals specifically for the purposes outlined herein. Instead, existing surplus mice and rats already housed at the Central Research Facility will be used. Increasing the sensitivity of the imaging techniques prior to starting acquisition of data for a given study, will ultimately result in smaller group sizes being required to detect a required effect. It will also reduce the need for repeat imaging (and consequently repeat anaesthesia) due to acquisition of sub-optimal imaging data. The work carried out under this license can therefore be considered advantageous in terms of reducing the numbers of animals used downstream from the initial piloting phase.
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 work to be performed under this license will result in refinement of imaging techniques for all subsequent mouse and rat imaging studies at the University of Leicester. This will lead to improved data quality, smaller group sizes and fewer repeat imaging sessions. All work will be performed under terminal anaesthesia, and therefore animal suffering will be minimal.

PROJECT 13	Doping/medication control for British horseracing	
Key Words (max. 5 words)	Anti-doping, medication, equine, detection time, control	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
	✓	Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project's objectives are to determine how therapeutic substances used in racehorses are metabolised and excreted by the horse in order to advise on their appropriate use; to understand better how non-therapeutic ('doping') substances which are a potential threat to racehorse welfare and the integrity of horseracing are metabolised and excreted by the horse; and to use (new) laboratory based methods to replace and reduce the use of horses for the above.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	• Veterinary surgeons and trainers will be able to make informed decisions about what therapeutic medication to use and when • The use of non-therapeutic ('doping') drugs will be detected and deterred • Alternative *in vitro* methods will be developed wherever possible	
What species and	The horses in this work are regularly re-used with a	

approximate numbers of animals do you expect to use over what period of time?	maximum of 50 individual horses expected to be involved in the studies over 5 years. The re-use is advantageous in reducing the overall numbers of animals used but also to the individuals as they are able to be re-trained whilst they are at the CRS without any compromise to their welfare being caused by the re-use.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The level of severity expected in these studies is mild. It is possible that a horse may react adversely to the drug being given. This would most likely be at the injection site, with localised swelling and pain which would resolve with time, and if necessary, anti-inflammatory treatment. A more significant (anaphylactic) reaction could involve widespread (systemic) signs, such as fever or filling of the legs. Anti-inflammatory medication and a specific protocol of intensive monitoring and care would be used to deal with this. The other main possible adverse effect is inflammation of the jugular vein from sampling. To reduce the number of times a needle is used to take a blood sample, intravenous catheters will be used wherever the number of samples to be taken within 24 hours is more than six. These catheters may occasionally cause inflammation of the vein, in which case they will be immediately removed. Horses will be rehomed at the end wherever possible but if it is not, they will be humanely euthanased.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The purpose of this work is to produce information about how drugs are handled by Thoroughbred horses. At this time, whilst limited information can be acquired by *in vitro* methods, most has to derive from giving substances to Thoroughbred horses.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Extensive research to use whatever information is already available, either published or unpublished, will be carried out to ensure that only the minimum numbers of horses needed to give scientifically valid results are used, usually two in the first instance. Re-

	use of the horses significantly reduces animal use.
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 aim of this project is absolutely specific to Thoroughbred racehorses; therefore it is deemed appropriate to use this breed of horse to obtain relevant, comparable data and valid results. The study methods are refined for each and every study carried out. Drug dose will be based on that in routine clinical use or the very minimum that is believed from all available information to have an effect. Sampling points will be as few as possible to fulfill the purpose of the study, and based on any knowledge about similar substances or routes of administration. Urine collection will usually be by free flow, not catheterisation. Hair samples will be cut wherever possible rather than pulled to minimise discomfort. Sample collection periods depend upon the drug; they may be limited to as little as a day and would not usually extend beyond 6 weeks. The frequency of sampling will almost always decrease as the study proceeds because it is usually only in the first part of the study that a detailed understanding of what happens to the drug with frequent sampling points is needed. Whilst on study, to minimise the impact of the study and maintain the horses as appropriate models, their husbandry will be much as when not on study, being given normal feed and housed in individual stables with exercise and/or turnout as appropriate to their stage of fitness and presence/absence of an intravenous catheter. When urine is to be collected from horses on study, they will generally stay in their usual stable. Before, during and at the end of each study, horses are examined to ensure that any adverse effects are noted and where necessary acted upon.

<table>
<tr><td>PROJECT 14</td><td>Neuroimmune interactions</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cytokine, brain, liver, microglia, astrocyte</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)</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</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>It has become clear that events throughout the body play a significant role in controlling the response of the brain to injury and disease. The principal goal of the work described here is now to discover how inflammation both in the brain and in the rest of the body can affect brain integrity and nerve cell function. We will build on our discovery using rats and mice that the injection of immune molecules into the brain resulted in a rapid response by the liver and we were surprised to find that that molecules made by the liver coordinate and control the body’s response to brain injury. In our previous experiments inhibition of the liver response reversed brain injury. Thus targeting events in organs distant from the site of injury can change the outcome of brain disease. Under this licence we will principally use rats and mice to continue to explore how, infection, diet, affect brain disease and how brain disease impact on the</td></tr>
</table>

	body's ability to cope with infection.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The results from our preceding in vivo work have been published in at least 30 peer-reviewed articles. Our collaborators, also working under our previous licence, have also published widely. We expect the results obtained to translate to new therapies, and we expect our work to lead to the development of novel contrast agents for use in the clinic. Indeed, we have been awarded a grant application to take our work on novel contrast agents into the clinic.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats and over the 5-year period. 10 scientists working on this project will expect to use approximately 1500 rats and 2000 mice. This is based on our ongoing experience of these experiments.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	It should be noted that we will use pain relief for all surgical procedures and that the use of imaging techniques reduces the overall number of animals required. We have developed a number of models that are clinically silent and we use these wherever possible. However, some animals might exhibit transient hemiplegia after stroke, but otherwise we expect no clinical signs. The animals will all be culled at the end of our experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In the past we have established features of the host response to brain injury and disease in whole animals that could not have been predicted from existing knowledge or have been achieved in cell culture experiments. By the nature of this project it is important not to confound our data by using animals that are stressed or ill. Experienced licensees will gently handle animals and behavioural experiments will be performed in conditions where sound, light and heating are controlled. Many of the molecules that we know are important in the brain-immune system communication pathways are not expressed in fish

	or flies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use state-of-the-art imaging techniques, molecular biological that require less tissue and behavioural experiments to examine the effects of the inflammatory response in the whole body and in the brain on the evolution of pathology in the brain. Wherever possible we will try to sample tissue or image in a serial manner to reduce the number of animals required. We use power analysis to ensure that the most appropriate number of animals are used in our experiments and we use archival material or surplus animals wherever possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The complex regulation of the immune system involves a neural component, which will we also be studying. Rats and mice can be used in our experiments to closely model the human immune responses to brain injury and disease, which is not possible in non-mammalian species. All animals undergoing surgical procedure will receive pain relief and we will use power analysis to ensure that we use no more animals than necessary. Wherever possible, tissue will be retained for further experiments and we will also employ behavioural outcomes and molecular outcomes to ensure the each animals generates multiple outcome measures in situations where we have previously shown that there are no confounds associated with this approach.

<table>
<tr><td>PROJECT 15</td><td colspan="3">Anti-angiogenics in retinal neovascularisation and neuroprotection</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Retina, angiogenesis, neuroprotection</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)</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</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">To determine whether controlling expression of vascular growth factors can prevent or reverse blood vessel growth and neurodegeneration underlying blindness in animal models of :

A Neovascular eye disease, such as wet age related macular degeneration

B. Type I diabetic retinopathy

C. Oxygen induced retinopathy

D. Type II diabetic retinopathy

E. Ischemia induced retinopathy and neuroprotection</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td colspan="3">New treatments for blindness</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	
What species and approximate numbers of animals do you expect to use over what period of time?	Rats (1700) Mice, (4500), Rabbits (50) over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We intend to induce models of disease in the eye by: A) firing a laser into the back of the eye to make a tiny hole (<0.1mm), which stimulates blood vessel growth. B) Using animals with diabetes, C) exposing new-born rodents to varying oxygen levels and D) temporarily stopping blood flow in the retina. We will measure the function of the eye by imaging the retina with a microscope in anaesthetised animals, non-invasively (similar to a visit to the optician), and in some experiments inject a dye into the area of the brain that the optic nerves go to investigate whether they are working properly. Most of these experiments have a very low likelihood of adverse effects and they are mild to moderate in severity. Most animals will not undergo surgery.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We do test the approaches in cells in culture first, but this cannot tell us whether blood vessels (which cannot be grown in culture) can alter their behaviours, and their effect on vision (which cannot yet be sufficiently modelled in cultured cells).
2. Reduction Explain how you will assure the use of minimum numbers of animals	To enable the minimum number of animals to be used we used advanced statistical modelling. The least number of animals necessary to definitively show the properties (or lack of them) of test substances will be used,
3. Refinement Explain the choice of species	Mice and rats will be used because of the extensive characterisation of these models of retinopathy. They are the species with the lowest degree of

and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	neurophysiological sensitivity in which retinal vasculopathy and neovascularization with similar properties to human disease has been characterised. The imaging and recording techniques we use are both non-invasive procedures that do not result in suffering of the animals other than the injection for the anaesthetic. Diabetes can be associated with significant morbidity in humans, but the mouse and rat models we use are relatively mild for the first 16 weeks of diabetes, during which these experiments will be undertaken. Surgery for the intracranial injections will be accompanied by painkillers and will be performed using sterile techniques.

<table>
<tr><td>PROJECT 16</td><td colspan="3">Object recognition by zebrafish visual system</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Zebrafish, vision, in vivo imaging, classical conditioning</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 section 5C(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></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</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">How animals recognise and memorise other animals and different objects is not well understood. We know that the retina and other brain areas (each involved in early processing of visual information) extract certain features from the visual images and that these features are later analysed by higher brain areas during process of object recognition. We also know, that changes in the connections between neurons leading to “long-term potentiation” and “long-term depression” underpin memory formation but we do not know how these changes give rise to memory.

The current goal in the field is to understand the circuit mechanisms of object recognition and to understand how changes in those circuits form visual memory. The proposed project will study this using a combination of imaging techniques</td></tr>
</table>

	performed on young zebrafish larvae and using simple behavioural tests. Our specific objectives are to: 1) Describe zebrafish neuronal circuits, processing spatial information, 2) Describe zebrafish neuronal circuits responsible for simple object recognition tasks, and to 3) Understand how changes in neuronal circuits and connections between synapses lead to formation of visual memory in zebrafish
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our results will contribute to several important areas of scientific knowledge. Firstly, we will study general principles of visual information processing in zebrafish; this is important because zebrafish are an increasingly important model in neuroscience and a better understanding of visual information processing in this species will make it possible to study vision more productively. Secondly, we hope to understand how zebrafish visual system achieves object recognition. Identifying object recognition circuits in simply organised brain, such as that in zebrafish, will be important in understanding how object recognition works in more complex brains, such as that of humans. Finally, this project proposes to study how visual memory in the zebrafish is achieved through changes of connections between neurons since this may help to understand how memory is formed in the human brain.
What species and approximate numbers of animals do you expect to use over what period of time?	For our experiments we will use zebrafish larvae beyond the age of independent feeding. We chose zebrafish for several reasons. First, they are transparent which allows us to study neuronal activity in a non-invasive manner. We will not use any surgical preparation of the animals so we do not expect to cause much harm to the animal. Second, zebrafish genetics is very powerful and allow us to quickly express reporters of neuronal activity in the brain. Finally, zebrafish nervous system is relatively simple, which will simplify our task to study the way it works. We will use around

	15000 fish in the whole project, 3000 fish for line production and 3000 larva for experiments.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We propose methods that will limit harm caused to the animals. In contrast to established and widely used electrophysiology techniques *in vivo*, we will use imaging techniques and will avoid performing any surgical operations on live animals. Our behavioural experiments will not cause pain to the animals, although some degree of stress may be expected. After the experiments the animals will be humanely killed and their tissues used for further analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Unfortunately, we have no choice but to use live animals for our research. This is because theoretical methods can only *propose* how the visual system might work; they cannot address the problem *directly*. Neither can we generally use cultured cells or brain slices because their use frustrates the use and assessment sensory stimulation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will carefully examine the number of animals required for each experiment and experiments will be first proposed using mathematical modelling, done either by us or other theoretical neuroscientists and statisticians.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have chosen to work using a lower order vertebrate, the zebrafish, which has a relatively simple nervous system, and has less complex emotional and behavioural reactions compared with mammals. We will use in vivo imaging of live animals, which is a non-invasive technique and does not require any surgical operation. During our procedures we will carefully monitor the condition of the animals and will apply early endpoints should any notable pain, suffering or distress become apparent.

<table>
<tr><td>PROJECT 17</td><td>Understanding and manipulating proteostasis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neurodegeneration; chaperone; blindness; virus; stem cell</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 yrs</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</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</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>The aim of this project is to understand why nerve cells in the brain, eye and spinal cord die and find new ways to prevent this. Inherited genetic mutations or cellular stress can lead to proteins, the building blocks of cells, to lose their function and aggregate into clumps. This can lead to the proteins losing function, which means the cell loses function, and the clumps can also be harmful in themselves. We do not fully understand why damaged proteins cause nerve cells to die and here we want to understand that process better. We also want to understand how the body’s natural protective machinery works to combat this normally and if we can use this to protect against diseases like Retinitis Pigmentosa, Huntington’s disease, Motor Neuron Disease and Alzheimer’s disease.</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>Through this research we hope to gain a better understanding of how nerve cells function and why they die and discover ways to prolong their function and survival. The understanding and discovery might highlight ways to prevent or slow the progression of these currently untreatable diseases.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We anticipate using approximately 6-7000 mice and 1500 rats over the 5 years period of this project licence. The animals will be of a variety of ages.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse</td><td>The animals will be genetically modified to model human disease. Most of these will cause a mild phenotype, such as blindness, which does not</td></tr>
</table>

effects and the likely/expected level of severity? What will happen to the animals at the end?	affect a rodent very much, because laboratory rodents do not use their eyes as much as their other senses and it is not painful. When the disease is potentially discomforting for the animal, such as causing problems walking, we will use humane end points to minimise suffering and keep this to moderate severity. We will use a range of treatments to try and ameliorate disease. This will be mainly drug treatments, which involve injections or oral feeding. Sometimes we will use surgery to introduce genes, viruses or cells into their eyes to modify their gene expression. We will reduce the adverse effects of these treatments wherever possible, and any suffering, with appropriate analgesia and anaesthetic. We will assess the vision of the animals and their ability to walk using behavioural tests that do not require anaesthetic and are not traumatic. We will also use non-invasive physiological tests of their vision and look into the backs of their eyes to monitor disease, and for this they will need to be immobilised by anaesthetic. Animals will be humanely killed at the end of the protocols and their tissues used to understand the disease processes and how our interventions have affected this.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex mammalian nervous system, and in particular the light sensitive retina, cannot be modelled in the lab and there is no alternative but to use animals to studying these vital organs. We use cell models to do as much background work as possible before considering animal use and to minimise our use of animals. Only when these studies suggest animal experiments are justified (or if we cannot study this in cells at all) do we go ahead and use animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use our experience of these models to ensure that the minimum number of animals is used to get reproducible and meaningful data. We will also use sensitive measurements for changes in function and nerve cell survival that also allow us to follow a single animal over a period of time rather than using multiple animals. We will also techniques to directly modify gene expression and reduce the number of animals that might be required by other approaches, such as transgenics that requires more animals.
3. Refinement Explain the choice of species and why the animal model(s)	We have chosen rodents (rats and mice) because they are the most easily genetically tractable mammals that share a similar eye and brain with

you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	man. We will mainly use mice, but in some instances transgenic rats offer a better model and their larger size makes some manipulations more refined and more likely to succeed. We can also refine our experiments by only targeting the tissues in question and reducing undesirable side effects in other organs. We will also use humane end points to minimise any harm to the animals.

<table>
<tr><td>PROJECT 18</td><td>Regulation of inflammation in wound repair</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Chronic wounds, inflammation, diabetes.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</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</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>Tissue repair and regeneration require dramatic and coordinated changes in cell behaviour in both wound-resident cells at the site of injury and in distant cells that respond to and are recruited to the injured tissue. In the last decade, the influence of inflammatory cells on wound healing has been shown to be highly significant, as they can function to promote or inhibit wound healing. Discovering the underlying mechanisms controlling the behaviour of inflammatory cells is key to controlling these cells for therapeutic benefit. It is often very instructive to compare normal processes with diseased processes in order to understand how that process is regulated.

Diabetic patients and animal models have severely impaired wound healing and often develop chronic wounds. By comparing factors in diabetic wounds with normal wounds, we can begin to</td></tr>
</table>

	understand what is important for efficient wound healing and how to promote impaired wound healing. Inflammatory cells from diabetic patients and animal models are dysfunctional and inhibit wound healing. However, this process is poorly understood and the key mediators that control these cells are not known. Many pro-inflammatory factors are over-expressed in diabetic chronic wounds compared to normal wounds, but whether they are causative or a consequence of the dysfunctional inflammatory cells is not known. Our objectives are to (1) determine differences in how genes are controlled between diabetic and healthy inflammatory cells, (2) identify the key factors controlling these genes (3) test whether we can alter these factors and reverse the dysregulation of inflammatory cells in diabetic wounds. The results of this study will be important in future therapeutic development.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The results of this project are intended to: 1. Identify the underlying molecular mechanisms that contribute to dysregulated inflammation. 2. Contribute to scientific knowledge related to chronic wounds. 3. Identify potential new therapeutic strategies to promote healing in diabetic humans and animals. The potential benefits of this study include the development of potential gene and cell based therapies to aid patients with chronic wounds and reduce the need for limb amputation. In addition, this study would benefit animals with diabetes, particularly pet dogs and cats, which like human patients, develop complications associated with this condition.
What species and approximate numbers of animals do you expect to use over what period of time?	Over a 5 year period: 4500 mice (approximately 2500 for breeding purposes and 2000 for experimental procedures) 800 rats (approximately 400 for breeding purposes

	and 400 for experimental procedures)
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	This study is designed to understand how inflammation is controlled in a normal wound environment and what might be different in a diabetic wound environment. Anaesthetised mice (non-diabetic and diabetic) will receive small (10 mm diameter or less) wounds on their skin so that we can compare the processes involved in wound healing in diabetic mice with the normal situation. We will apply different factors to the wounds that we believe will enhance wound healing, including growth factors and stem cells, in order to find the best treatment for diabetic wounds. After surgery, mice will be provided with pain relief and monitored closely for any signs of distress. Distress in mice after this type of surgery is very rare, however, if there is any indication of suffering we will seek veterinary advice. In some studies we may need to exchange bone marrow from one mouse/rat to another mouse/rat in order to determine the effects of the diabetic environment on how bone marrow cells develop and behave. To do this we condition a recipient with a dose of radiation that will allow for the donor's bone marrow to replace the original. The animals do not feel anything during the radiation treatment and they are given the replacement bone marrow right away following their treatment so they should not feel any ill effects. Four to six weeks later we will be able to track their bone marrow cells using blood sampling and biopsies. In addition, we are developing methods of live imaging in order to reduce the number of animals needed for each study. Strategies to minimise adverse effects due to our treatment, as well as minimise the number of animals needed for these studies include testing the effects of the factors we are putting on the wounds in cell culture first. In this way we will be able to identify the most promising candidate factors without using animals. This will reduce

	the chances of inducing an adverse effect, and reduce the number of animals needed to accomplish the objective. Animals will be humanely killed at the end of each study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have to use animals in this study because understanding how inflammatory cells interact with wound healing in a diabetic environment must be studied in the complete physiological setting in order to get an accurate picture of this process. Mice and rats are the least sensitive animals that accurately mimic the disease in humans. We use mouse and rat models of Type I and Type II diabetes combined with transgenic animals that expresses green fluorescent protein in all of their cells. We can put these cells into wounds and find them again because of the green fluorescent protein marker. This allows us to keep track of how they might be interacting with the wound to enhance healing. We cannot use humans for these experiments because we would not be able to modify their genes nor track the cells from the bone marrow.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By reading the scientific literature, we will avoid repeating anything that has already been done. By consulting with colleagues that have expertise in our area, we will refine our experimental design. By conducting experiments in cell culture (in vitro) first, we will identify many of the factors that may regulate inflammatory cells. We will also test potential therapeutic treatments in cell culture models of wounds first. To plan for our animal work, we have consulted a statistician to establish the minimum number of animals required for each study. Also, where possible, we will use two wounds per animal to reduce the number of animals required.
3. Refinement Explain the choice of species and why the animal model(s)	The species and models we have chosen are based on how well they mimic diabetes in humans, their sensitivity (they are the least sensitive models we can use for our study), how well-characterised

you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	they are, and our expertise. The animals will be given anaesthesia so they will not feel anything when they undergo wounding. They will also be given pain killers so when they wake up they will not have any discomfort. They will be watched closely to make sure they do not show any signs of being in pain or becoming ill. If they appear to be in pain or appear unwell, veterinary advice will be sought.

PROJECT 19	**Developing therapies for inner ear disorders**	
Key Words (max. 5 words)	Hearing, deafness, balance	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall purpose of the project is to identify means to ameliorate hearing impairment and balance dysfunction. It aims to: 1. Characterise the progress of inner ear pathologies in relation to functional deficits (deafness and/or balance disequilibrium) resulting from ageing, ototoxic damage and noise, as well as with mouse mutants or transgenic animals in which the inner ear is affected by the mutation, in order to identify tissue, cellular and molecular targets for pharmaceutical intervention to prevent the dysfunction or death of sensory cells. 2. Breed and maintain mutant and transgenic animals with specific genetic defects that result in hearing loss and/or balance dysfunction. 3. Identify biochemical pathways involved in regulating the formation of sensory tissues in the inner ear during development to identify potential targets for pharmaceutical intervention to enhance regeneration of the sensory “hair”cells. 4. Deliver to the inner ear agents identified as candidates for preventing hair cell dysfunction or loss or enhancing regeneration to assess their activity in *in vivo* models of inner ear disorders. 5. Use our established animal models of cochlear	

	implantation to determine i) the origin and progression of inflammatory responses and fibrosis that following implantation and ii) the mechanism which lead to loss of residual hearing.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Hearing loss affects more than 10 million people world-wide suffer disabling hearing impairment. More than 70% of the over 70's have hearing loss and that number is expected to grow. Balance dysfunction results in dizziness and vertigo. Dizziness is the most common reason for visits to a GP in those over 60. Vestibular dysfunction is also a major contributory factor in falls in the elderly, which cost the NHS more than *£1* billion annually. However, there are almost no therapies for hearing loss or balance disequilibrium. This project will help identify possibilities for therapeutic interventions to prevent or ameliorate hearing impairment and balance dysfunction. It will provide information crucial for assessing the potential for cell regeneration therapies in the mammalian inner ear and characterise the environment of the damaged inner ear upon which any such therapy is intended to act. It will also identify means to improve the use of cochlear implants, which are normally provided only to those with profound deafness, and broaden their candidature to those with some residual hearing especially those with age-related hearing loss. The project will contribute to reducing the incidence of hearing loss and balance dysfunction and the handicaps they cause.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years: 2700 mice 500 guinea pigs 400 gerbils
In the context of what you propose to do to 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 major adverse side effects will come from the induction of deafness or balance dysfunction. In general for animals maintained under controlled conditions in an animal unit, these disorders do not cause significant discomfort. The other source of adverse effects is from the surgical procedures but these are performed under tightly controlled conditions to minimise suffering. At the end of procedures, animals will be culled by approved, humane methods.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Identification of the mechanisms, and of agents that influence the processes under study will be made in *in vitro* studies using explants in organotypic cultures, but findings need to be translated into studies of living animals to confirm their relevance. It is also necessary to develop and assess relevant clinical procedures, most notably in this programme, the use of cochlear implants. There is no model system for the complex specialised sensory organs of the inner ear. Especially in the mammalian cochlea, auditory function and hair cell survival is dependent upon the physiological environment in which the hair cells work. That environment is maintained by the intact functioning non- sensory tissues in the cochlea. The appropriate conditions cannot be modelled in vitro and can only be examined in the intact cochlea in living animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will explore the use of explant cultures as far as possible before experimental studies in animals are undertaken. In animal studies we will use procedures that allow for the maximum number of different analyses and assessments from individual animals and from individual inner ears.
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 proposed studies will be performed with mice, guinea pigs, gerbils and embryonic chickens. The mouse has become the preferred model for many studies of the cellular and molecular biology of the auditory system. The embryonic stages of inner ear development have been well characterised in the mouse, and it is an atricial species in which the animals are born deaf with the latter stages of cochlear maturation and the onset of auditory function occurring postnatally. Furthermore the numerous mutant strains available, and the ability to create genetically manipulated animals provide opportunities to examine the role of particular gene products in the systems under test. Guinea pigs are also a standard model species for studies of the auditory and vestibular systems. The inner ear in this species is comparatively large making tissue dissection relatively easy, and it is relatively more accessible than in other species. Gerbils are of value because their hearing range is similar to that of humans and their cochleae are relatively large and easy to dissect. They provide a valuable small mammal alternative to mice. Chicks are a standard model for studies of development generally, offering opportunities for

	genetic manipulation *in ovo* not easily applicable in mammals. They are also a standard model for investigating hair cell regeneration. The inner ear is relatively inaccessible being encased in bone at the base the skull. In addition, there is a barrier to entry of some agents to the tissues and fluids of the inner ear from the blood stream. These factors mean that surgical procedures are required to provide access in order to make certain measure of cochlear function and for delivery of certain agents such as putative hair cell survival factors as well as for cochlear implantation. From previous experience none of the procedures to be used are expected to cause anything more than a moderate level of suffering. Animals upon which procedures have been performed will be monitored constantly for a period after the procedure and any showing symptoms of distress will either be culled immediately or advice of the named veterinary surgeon will be sought.

<table>
<tr><td>PROJECT 20</td><td>Vocal and social learning of avian calls</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Learning, vocalisation, mobbing, birds</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)</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</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>Language is one of the most impressive outcomes of evolution and culture. Although language learning is pervasive in humans, no other primate learns its vocalizations and vocal learning is also relatively rare in other mammals. In contrast, thousands of species of birds learn to produce their vocalizations. Birds have thus become one of the most important model systems for studying the phenomenon of vocal learning as well as other types of questions in animal communication. Nearly all of this research has focused on one type of vocalisation—sexual signals, or ‘songs’—but we know little about how other types of vocalisations (‘calls’) develop.

This research will examine the development of call learning in birds with a special emphasis on the signals birds use to avoid predators. Anti-predator signals of birds can be very sophisticated,</td></tr>
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	transmitting information about the type of predator, its behaviour, or its threat level to other individuals. Individuals of other species are also known to eavesdrop on the types of calls. Yet we know very little about how birds learn to produce these vocalizations or use the information encoded in them to avoid predators. In this project, young birds will be hand-raised in controlled auditory and social environments to examine how they learn about predators. The objectives of this project are to understand how songbirds learn to produce variations in the acoustic structure of their calls, learn to identify novel predatory threats, and use their calls to communicate about predators with animals of the same and other species.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The results from this research will provide a number of important insights into our understanding about the way animals communicate, including some of the first experimental studies of mobbing call learning in songbirds. In addition to the academic benefits, these results could help inform our understanding of human language learning as avian vocal learning has become a major model system for human language. For example, avian song learning has been used as a model for understanding human diseases, such as Autism spectrum disorder. Information about how birds learn to identify and communicate about novel predators could also be used to help ensure the survival and quality of life when releasing captive-bred endangered or threatened species back into the wild.
What species and approximate numbers of animals do you expect to use over what period of time?	The species studied in this project will be birds. Much of the work will be done on common resident British songbirds (e.g. tits). Up to 600 birds will be used throughout the 5 year period of the licence, though the involvement for most individuals will be shorter (some for months, many for just minutes).
In the context of what you propose to do to the animals, what are the expected adverse	The proposed research is primarily behavioural. The licenced procedures that are necessary for this project are exposing birds to model predators to

effects and the likely/expected level of severity? What will happen to the animals at the end?	record their anti-predator vocalisations and behaviour, collecting blood and feather samples to determine the sex of different individuals and estimate stress hormone levels, and raising some birds in acoustic chambers to control the types of sounds they hear during development. These procedures may produce mild and transient discomfort (blood sampling) or stress (predator presentations) and potentially mild psychological adverse effects (social isolation), but are not expected to have long-term adverse effects. Humane endpoints will be followed for any birds experiencing unexpected suffering. Following the experiments, birds will be released at the site of capture as appropriate after consultation with a veterinarian.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The goal of this project is to understand how animals learn to communicate. Animals must be used in these studies to achieve the goals of understanding the behaviour and vocalizations of animals, thus non-animal alternatives are not available for this work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All proposed experimental protocols are based on those used successfully in previous research. Some individual birds will be used in multiple experiments (e.g. as tutee in one and then as demonstrator in the next) to reduce the total number of animals required. Efficient and powerful experimental designs and statistical analyses (including initial Power calculations to determine necessary sample sizes) will also be used to reduce the overall sample size needed in each experiment to reach satisfactory statistical power.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general	Birds are the chosen model system for this work. They are the ideal system to use to explore questions of vocal learning since, unlike many other species (e.g. most mammals), they must learn to produce their vocalizations. More is known about the behaviour and neuroscience of vocal learning in birds than most other types of animals and thus

measures you will take to minimise welfare costs (harms) to the animals.	there is the potential to greatly increase our understanding of this model system through the proposed research. Songbirds and tits in particular have sophisticated anti-predator communication systems and as such pose the greatest potential for achieving our objectives. Because the work focuses on vocal behaviour, lower vertebrate or invertebrate models are not available. Potential stress will be reduced both in the housing and care and in experimental manipulations. Birds will be singly housed in an isolated but enriched environment that includes auditory playback of conspecific and environmental sounds during the developmental studies. Presentations of model predators will be brief to minimise any potential stress associated with these encounters. Blood and feather sampling will be performed rapidly and efficiently so as to avoid any unnecessary suffering, with the location swabbed with ethanol before sampling to avoid introducing any infection and any bleeding controlled with gentle pressure. All individuals will be carefully monitored throughout the studies to rapidly determine and minimise any signs of stress. All wild birds will undergo reacclimatization procedures prior to release, which should also help ensure the quality of life of any released birds following the experiments.

PROJECT 21	**Eye lens shape and form**	
Key Words (max. 5 words)	Eye lens cataract accommodation, radiosensitivity	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	New knowledge of the biological principles that Lead to eye lens formation arid maintain its optical properties over a lifetime To determine the relative sensitivity of the eye lens to low dose ionising radiation, to understand the process of postertor sub-capsular cataract formation and 5upport radiation safety legislation • To understand the biological mechanisms that benefits influence the responses of the lens cells after cataract surgery so that health and wellbeing of cataract patients can be improved.	
What 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 expected benefits are; L A better understanding of lens cataract and Its causes, 2 New perspective on conventional cataract surgery to Improve Its efficacy and reduce complications	

	Scientific support for the recommended annual and lifetime reduction in exposure to ionising radiation. The national incidence of cataract was 330,000 and a prediction that could increase to O5-18m cases In the UK due to the fact that cataract Incidence Increases with the population age profile. In this country cataract surgery costs approx £0.5b per year. There is therefore a very large soclo-economic Incentive to address the growing problem of cataract in the UK, to improve its treatment and to understand how the disease Is caused. Cataract i the major cause of blindness worldwide (World Health Organisation, 2012), A better understanding of how each indMdual lens cell contributes to the shape and form of the whole lens will help unlock the secrets of the optical properties of the lens. It will also allow new and innovative therapies for the treatment of cataract to be developed because the knowledge base will be more complete and secure. Another very practical benefit concerns medical Imaging that utilise lonising radiation sources. Cardiologists in particular, use X-ray imaging to perform routine procedures such as anglography to fit stents and pacemakers in the treatment of cardiac disease, The International Corn mission for Radiation Protection (ICRP) Is the International professional body that advises governments on the safe occupational dose limits for ionising radiation The new recommendations have reduced these levels by five fold, which could have a sIgnificant impact on the capacity for health care provision involving medical imaging techniques. The recommendations are based upon epidemiological studies of A-bomb survivors, The eye lens is believed to be particularly sensitive to ionising radiation damage, but animal studies are needed to test that hypothesis and also to provide the mechanistic detail to understand the reasons behind this sensitivity. This is an essential part of providing the new knowledge needed to support the

	recommended changes to dose limits.
What species and approximate numbers of animals do you expect to use over what period of time?	MIce (800), rats (800), rabbIts (60) and zebrafish {S000) over 5 years. These are maximum numbers Experience shows that our projects never reach this limit because of judicious experimentation to reduce, refine and replace where possible animal use.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Our project contributes to the Improved health and wellbeing In the UK by investigating cataract surgery arid the effects of ionising radiation on the eye lens. cataract surgery has the potential to cause moderate adverse effects. All other procedures could potentially cause mild adverse effects, nimals are monitored for such problems and the advice of the named veterinary surgeon sought in such cases. Where anaesthesia Is required, then standard p:erioperative and animal care measures, as per the National Veterinary Surgeons (NVS) advice will be followed. Anaesthetlcs Will be administered and maintained by experienced personnel and will be suitable for the species and the duration of the procedures,
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Lens regeneration is a recently recognised property of the lens. It can only currently be studied in viva as It can not be replicated ex vivo or in vitro. Lens cell cultures and lens explants are the available alternatives to using animals. Human lens explants has several obvious problems. This requires donors and these are limited nationally. So experiments using this alternative are restricted, Lens cells can be cultured and where possible that are used to study the cell biological events and to refine the proposed animal experiments. These alternatives are unable to mimic all the complications that can result after cataract surgery eg Soemrnerrins ring, and therefore Inevitably cataract surgery on animals has to be performed. The study of radiation damage to the lens involves not only how lens epithelial cells respond immediately but also over many months to the effects of this

	damage. The response Is not uniform across the lens. Rather it Is determined by the position within the lens epithelium. Any explant system disturbs this order. No cell culture system can mimic this. Therefore there is no alternative at this moment in time We also study the development of the lens in a living animal — the zebrafish. The eye lens starts to form after 16 hours and is complete and functional after 4 days in this animal. Transparent zebrafish mean that we can monitor non-Invasively b high resolution light microscopy all the changes in cell shape, position and division.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We publish and publicise our work at international meetings and in pee-reviewed journals so the scientific community is aware of our research We coordinate our research programmes with the scientific community in general and also our collaborators so that experiments using animals are not repeated unnecessarily. We have refined our procedures so that the most efficient protocols are used in our studies. We also make available our resources to the international community of scientists. Biostatistical advice is sought from experts to best determine the most appropriate sample size to deliver sound experimental data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse and zebrafish benefit from world-wide investment In making available genetically-altered resources to the scientific community Lens regeneration was developed in rabbit lenses and adapted to rat lenses and is possible in mouse lenses. These different animal models have different advantages (mouse — genetics; rat — biomass; rabbits — lens size and the model of choice for ophthalmological studies). Therefore a range of animal models provides the flexibility to refine our experiments and so reduce both animal use and/or to increase health/wellbeing impact of our research. None of the procedures proposed is anticipated t cause severe animal suffering but if any deviation

	from normal behaviour is noted, then the animal will be monitored closely, and if no improvement Is seen within a period of up to 48 hours1 the animal will be humanely dispatched.