Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 19

Project Titles and key words

- Origin and regulation of HPA axis rhythms

  HPA axis, stress, pituitary, adrenal gland.

- Development of vectors for gene targeting into skeletal muscles

  Duchenne muscular dystrophy, gene therapy, plasmid vector

- Why do mosquitoes vary in the rate at which they transmit disease?

  Mosquito, gerbil, Aedes, lymphatic filariasis, Brugia malayi

- Investigations into immune recognition and responses in mammals

  Immune, genes, mice, mild, infection

- Immune suppression in Cancer

  Cancer, Immune Suppression, Treatment, Vaccination

- Imaging Neurodegeneration In Rodents

  Imaging, neurodegeneration, stroke, Alzheimer's disease

- Provision of Biological Materials

  Blood, tissues, animals, supply, in vitro

- Preparation of time-mated rabbits using administration of Luteinising Hormone

  Time mating; rabbits; reproductive toxicology; luteinising hormone.

- Memory mechanisms in mice

  Mouse, memory, brain, molecules

- Muscle protein turnover regulators in disease

  Muscle, sarcomere, titin, autophagy, homeostasis

- Signal integration for visual perception.

  Cortex, neurophysiology, decision-making, parietal, occipital.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Origin and regulation of HPA axis rhythms</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>HPA axis, stress, pituitary, adrenal gland.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Glucocorticoid hormones are released in pulses every hour or so from the adrenal glands, and these pulsatile patterns of hormone are critical for organ function. These pulses become disrupted during ageing and in stress-related disease, but we currently have a poor understanding of how and why this happens, and what consequences this has for target organs. The purpose of this project is to understand how the pulsatile release of glucocorticoid hormones is regulated by the hypothalamic-pituitary-adrenal axis, and why the system becomes dysfunctional during ageing and under conditions of stress.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The primary potential benefit relates to new knowledge about the origin and regulation of glucocorticoid pulsatility. The findings of this project will provide a deeper understanding of disorders of the HPA axis associated with changes in CORT pulsatility, including Addison’s disease, congenital adrenal hyperplasia, pituitary deficiency, and stress-related disorders. Furthermore, our findings will also be important for the development and refinement of novel methods of glucocorticoid administration in humans, with improved efficacy and decreased side effects. This is very important, as steroids are one of the most commonly prescribed classes of drug in the UK.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Approximately 870 rats will be used over a period of 5 years.</td></tr>
<tr><td>In the context of what you propose to do to the animals,</td><td>Experiments are designed to minimise animal suffering and maximise animal welfare by utilising a</td></tr>
</table>

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

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	well-established automated blood sampling system. Procedures used in this project include implantation, under general anaesthesia, of vein and subcutaneous catheters, administration of substances, blood sampling, lesions of specific brain regions, exposure to stressors and altered light/dark cycle, and injection of biologically active substances that will change the transcription of genes involved in the HPA axis activity. The number of procedures per rat will be limited and the adverse effects moderate in severity at most.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We aim to elucidate the complex interrelationships between the anterior pituitary and adrenal glands in generating hormone oscillations. As these organs do not exist in isolation but are interacting components within the same system, it is crucial that we study how the whole system integrates its activity. Therefore, *in vivo* work is required to understand the fundamental mechanisms of rhythmicity in both healthy and pathological conditions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Serial blood sampling and multiple measurements of the effects of biological active substances and/or stressors on multiple target organs from each rat will reduce the number of rats used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The rat is the most appropriate animal for studying the HPA axis, as there are many similarities between the HPA axes of rat and man, including a similar pattern of circadian and ultradian rhythms of CORT. Furthermore, the physical size of the rat facilitates the surgical procedures required for such studies. We will use well-established methods for painlessly obtaining small blood samples from a jugular vein cannula. Furthermore, none of the stress models proposed in our project result in any overt signs of distress in the animals.

Title: Development of vectors for gene targeting into skeletal muscles

Key words: Duchenne muscular dystrophy, gene therapy, plasmid vector

- Summarise your project (1-2 sentences)

We propose a novel approach combining the existing and well characterised gene delivery methods with factors triggering specific immunological unresponsiveness. Our ultimate objective is to improve the effectiveness of gene therapy to treat Duchenne muscular dystrophy, the most common and lethal inherited muscle disorder. For this we will use the dystrophic mouse as the most widely accepted pre-clinical model.

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

Duchenne muscular dystrophy (DMD) is the most common and lethal inherited muscle disorder. It results in gradual loss of muscles, muscle weakness, severe disability and ultimately death. No cure is available. Replacement of the mutant gene (gene therapy) could cure this disease and several approaches are currently being tested. However, gene therapy components are seen by the immune system as foreign and the resulting immune responses decrease the effectiveness of such treatments. This project is directed at development of methods that would prevent this.

- Outline the general project plan.

We will target established therapeutic gene constructs under general anaesthesia using a well characterised method of direct injection into skeletal muscle of a mouse model of DMD. We will combine this with factors triggering specific unresponsiveness of the immune system. While all the components have been tested in experiments in cells, the ultimate efficacy of this approach needs to be tested in live animals. There is no method that can mimic the function of the entire immune system. The dystrophic mouse we propose to use is the most widely accepted pre-clinical model. The efficacy of this approach will be known following analyses of blood and muscle samples. Providing that the efficacy is high, we shall apply methods allowing gene targeting into many muscle groups and subsequently test treated mice for improvements in muscle strength and function.

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

The animals used under this protocol have symptoms resembling the human disease but mild and thus not causing suffering. Anaesthesia will be used throughout to eliminate pain from injection. Injections of the therapeutic gene as well as the occasional blood sampling cause transient discomfort only. The optional tests of muscle function are non-invasive. Mice get used to handling, which minimises stress. At all stages mice will be carefully monitored and, in an unlikely event, animals exhibiting any signs of distress will be killed.

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

The successful completion of this project will lead to an improved treatment for otherwise highly debilitating and ultimately lethal disease and can find application in all other gene therapy approaches where immune responses are found to be a problem. Moreover, results of these studies may help us understand some of the mechanisms causing cancer cells escaping the immune system surveillance.

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

The number of animals (300) has been calculated using power analysis and verified in previous studies. This mouse model of DMD is considered the most appropriate for pre-clinical testing and its use is required for comparison of the efficacy of our method to other approaches. The number of animals needed for the results to be conclusive will be

minimised by using tissues from the same animal for a number of tests e.g. complementary molecular and biochemical tests, histological examinations will be run in parallel.

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

Efficacy of individual approaches and has already been tested using cells in culture but use of animals is the only way to assess the very complex and interrelated immune response and immune tolerance processes.

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

All injections of therapeutic constructs will be done under general anaesthesia and generally only once. The desired and expected outcome of such injections is the improvement of muscle health. Local anaesthesia will be used for all other procedures. The optional muscle function tests are voluntary or non-invasive.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Investigations into immune recognition and responses in mammals</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Immune, genes, mice, mild, infection</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The project involves the study of human immune system genes responsible for disease resistance and susceptibility. The genes we study are part of two groups of genes that greatly vary between individuals, the MHC and LRC genes. These groups of genes are genetically linked to more human diseases than any other region of the human genome. The diseases include diabetes, MS and Rheumatoid Arthritis. We are doing this project because it provides insight into the functions of key disease genes, as explained above, encoded in the MHC or LRC regions. The project plan for the immune functions we are studying is to investigate gene knockout and/or transgenic mice; to obtain tissues to study expression and physiology; to raise monoclonal antibodies to proteins of interest. Mice are essential to these experiments and no other animal model is available. We are running many cell-based experiments in parallel with the mouse work. We will use the absolute minimum number of mice as befits the requirements of the experiment. We do not anticipate using large numbers of mice, in general less than 100 per year. The protocols we will use involve limited suffering as they are all mild – moderate treatments. We have not interest in any work that involves animal suffering. The procedures involve obtaining tissues and injecting mice to raise monoclonal antibodies. This basis research should help in</td></tr>
</table>

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

	understanding of human diseases such as infection, autoimmunity and cancer.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Once we have some information on the functions of the important disease-related genes we are studying in the mouse it will inform us about human disease susceptibility. We would endeavour to use the knowledge we gain from the work to develop new therapies against infectious agents as well as autoimmune conditions such as diabetes, arthritis and cancer. The approach here is to ultimately assist in the development of reagents such as peptides and antibodies that would influence the immune response either in a positive or a negative direction If we can produce specific antibodies to the human proteins we are studying they will be of benefit worldwide to the research community. In our work, they will enable us to probe the functions of individual proteins with precision. This fundamental research will help to understand why some people suffer from diseases and others do not.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 650 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?	95% of animals used under this project may experience clinical signs of mild severity. Less than 5% of animals are expected to show signs of moderate severity because of the substances administered. These animals may show clinical signs such as hunched posture, piloerection and if these persist then the animals will be killed. The animals may also lose weight and if they lose up to 20% of their body weight then they will be killed. All animals will be killed at the end and their tissues and organs used in the laboratory.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The functions of human immunoproteins are difficult to study. In order to understand how human Btn-related proteins function we have studied their biochemistry and molecular biology in cell lines. These experiments are limited in the amount of information they can provide, as they do not recapitulate the complex in vivo environment. By comparing the

	immune responses of knockout, transgenic or immune manipulated mice with their proficient littermates we will gain insight into the contribution of the genes and proteins to immune competence and resistance to infection. Ultimately this will have benefits for understanding human health, as some but not all of the mouse genes have direct human homologues. Similarly, the generation of monoclonal antibodies remains an essential part of biology. There are other methods of generating antibodies and we have used these where appropriate. For example, we have made some antibodies with a novel technique in collaboration with other groups. We have also attempted to make human monoclonal antibodies but this has proved fruitless as it depends on finding an individual who has been naturally 'immunised' with the protein of interest by infection.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By far the majority of our experiments require single mice to obtain data. We do not require large cohorts of animals to obtain statistical significance. Our record in the last five years shows that we use the minimum number of mice to obtain significant findings. Mouse experiments are extremely expensive and this is a very strong incentive to keep mouse numbers to an absolute minimum.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the standard model of mammalian immune functions. They are also the smallest and fastest breeding mammals available. In addition, the gene knock-out and transgenic technology has been developed mostly with mice; they provide the most accessible model at present. Many of our experiments require only single mice.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Immune suppression in Cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, Immune Suppression, Treatment, Vaccination</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td>Yes</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>One in three people are affected by cancer at some point in their lives. Further therapies are required to tackle the disease. The immune system is incredibly powerful and designed to detect and kill abnormal cells in the body. However, cancer vaccines used to raise immune responses against established tumours, have failed in the clinic. One reason for this failure is that tumours have developed mechanisms to suppress the immune system, which prevent it from attacking the tumour. We believe we have uncovered an important pathway through which tumours can suppress the immune response. If we can develop therapies, aimed at interfering with this pathway, it is hoped that we can circumvent this immune suppression, which we believe would permit cancer vaccines, and their generated immune responses, to effectively attack and control an established tumour.</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>Should our hypothesis be correct, and we identify a therapy which can alleviate immune suppression in tumours, the therapy would complement the already existing repertoire of human cancer vaccines and permit these vaccines to be efficacious in the clinic. This would result in greatly increased survival rates for the disease, and in the best case, potentially even a cure for some cancers, which would have significant impact on those suffering from the disease.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>We expect this research to require 7,000 animals over a 5 year period. We require the use of mice, as the models of cancer which we wish to use,</td></tr>
</table>

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

over what period of time?	which closely mimic the human disease, have already been established and validated in this species. We have consulted with statisticians who have calculated the minimum number of mice per experimental group we will need, and these are likely to be 8 mice.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will use tumour models in mice which have already been established to be safe, and accurately mimic the human disease. These tumours will be closely monitored and will cause the animal minimal, if any, harm. We then intend to vaccinate the mice to generate an immune response which will target the tumour, there will be minimal side effects and will cause the mouse no more harm than when humans are vaccinated. We will then administer immune modulating agents to the mice which we believe will alleviate immune suppression and permit the vaccine induced immune response to clear the tumour. The primary candidates for these studies, will be therapies with potential use in humans, and have a prior history of safe use, which as such, means that they will have low toxicities and cause no lasting harm to the animal. Mice at the end of the procedure will be humanly killed before the onset of any pain or harmful effects are felt.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Mice have very similar immune systems to our own, and murine models of cancer, which have already been developed, share many characteristics of the human disease. The complexity of the immune response and its suppression in cancer cannot reliably be modelled in the laboratory, and as such the use of mice is an absolute requirement to address these important questions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will conduct a study of human cancer tissue alongside our work in mice, this will be used to inform us of cells of interest and additional targets we may need to consider, which will allow us to conduct informed experiments, and use fewer mice. Further to this, we will ensure that the minimum number of mice will be used for this study, through the implementation of models which have previously been validated, and the implementation of in vitro studies where possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to	The mouse models of cancer which we are using have previously been established and validated. These models have been selected as they mimic the human disease, and with close monitoring, will cause the animals minimal harm. In addition, the therapeutic interventions to be tested are designed to be directly translatable into the clinic. As such,

minimise welfare costs (harms) to the animals.	the therapies to be tested in the mouse models will not cause the animal any harm, as they are being tested for their potential use in humans.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Imaging Neurodegeneration In Rodents</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Imaging, neurodegeneration, stroke, Alzheimer’s disease</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)[7]</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[8]</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Neurodegenerative brain diseases such as stroke, brain injury and Alzheimer’s disease (AD) represent a major medical burden to society. In AD it is thought that the slow decline in brain function may happen decades before any symptoms appear, and upon diagnosis of AD the brain damage may be too advanced for drugs to work. It is our hypothesis that changes that occur in the brain of AD, such as deposition of abnormal proteins amyloid and tau may represent a target for new imaging agents that could detect the presence of the disease years before symptoms appear so that drug treatment could be started before the brain becomes too injured. In contrast to specific markers of AD there are also other changes such as brain inflammation that are common to all neurodegenerative diseases including stroke and brain injury and these may also represent targets for the development of new imaging agents or drug treatments The aim of this project is to identify mechanisms and markers of neurodegeneration in rodents and develop imaging agents that bind to these targets</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>Development of new imaging agents specifically targeted at abnormal proteins in AD e.g amyloid/ tau or more general features of brain injury such as inflammation will be critical for disease diagnosis and development for new therapies in the clinic</td></tr>
<tr><td>What species and</td><td>This project will use rats and mice and it is anticipated that no more than 1000 animals (500</td></tr>
</table>

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

approximate numbers of animals do you expect to use over what period of time?	mice, 500 rats) will be used over the 5 year project duration
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For rodent AD models it is unlikely that the mutation required to produce AD in these animal brains will produce any adverse effects. However chronic drug treatment using surgically implanted devices may mean that there are risks of side effects following surgery such as infection and this is up to moderate severity. In head injury models there is physical brain damage that can lead to behavioural effects such as deficits in manual dexterity on the brain injured side. The expected severity limits for these studies are also moderate. At the end of the experiment the animals will be killed by a humane method such as overdose of an anaesthetic or perfused to fix the tissues for histology
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Since neurodegeneration is an ongoing process that progresses over time in the living brain it is not possible to use non-animal alternatives. Seeing how the brain damage develops over time in AD or brain injury models is essential to the development of new imaging targets and potential new therapies. Wherever possible in vitro tests on diseased animal or human brain will be used in the early stages of the project to select suitable candidates for the development of imaging agents and testing in living animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers used will be reduced by careful selection of the rodent models, the use of powerful imaging technology and careful monitoring of the experimental conditions. For example AD models show large amounts of brain damage that the imaging agents stick to so few animals are needed to see the difference between AD and normal 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.	Imaging studies are conducted in rodents such as mice or rats. Rats have been extensively investigated in brain injury models and the advantage of using mice is that genetic modification may model some aspects of the neurodegenerative process. Recently, genetically modified rat models of AD have been described which could prove a key model for this project. For brain injury studies, animals that have had a severe brain injury will be killed at the end of the imaging session whereas in animals that recover the techniques used have been refined so that the desired brain effects are seen but with no obvious effects on behaviour. Infection following surgery is

	minimised by the use of aseptic techniques and pain is controlled by the use of analgesics that do not interfere with the progression of brain injury are used to provide pain relief following surgery.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Provision of Biological Materials</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Blood, tissues, animals, supply, in vitro</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td><s>No</s></td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td><s>No</s></td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td><s>No</s></td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td><s>No</s></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</td><td><s>Yes</s></td><td><s>No</s></td></tr>
<tr><td></td><td></td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The aim of this licence is to provide high quality tissues to laboratories for in-vitro research and diagnostic work which will aid the discovery and development of new medicines, treatment and prevention of human and animal diseases or any other animal based research to assist essential aspects of scientific research.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The scientific benefits derived from this project are dependent on the progress of the research projects of our clients. Under previous licences the tissues have contributed to the knowledge of disease processes in man, animals and food crops., understanding of the development of the immune system and its regulation and extension of the knowledge of neurobiology and associated neurological disease.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Approximately over 5 years
Mice 130510
Rats 80600
Guinea Pigs 78,100
Rabbits 13,100
Dog – Beagle 350
Birds 1,500</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will</td><td>The majority of procedures will involve a general anaesthetic from which the animals will not recover ie. at the end of the procedures animals will be killed. These animals will experience only the restraint necessary for and any discomfort involved</td></tr>
</table>

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

happen to the animals at the end?	in anaesthetic induction. Some animals will be blood donors so that they will experience several sampling procedures. The adverse effects experienced by the donor animals is limited to the restraint for the duration of the procedure and the discomfort of a single puncture of the skin for each blood sample taken.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The procedures performed under this licence are led by client demand for animal derived products. By providing this service we enable our clients to replace their use of whole animals by using products produced by us for established alternative methods. The use of the tissues in *in vitro* studies in many cases replaces the use of animals on subsequent studies. One procedure involves the provision of neurons from foetal or neonatal rodents. There are no viable alternatives to using animals to provide the neuronal cell cultures. Use of human neurons is not possible and established animal cell lines can change phenotype during each cell division, making them unsuitable.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We provide a service that enables reduction, making maximum use of each animal by taking blood and as many tissues as possible from the same animals, where possible and sharing the product between wide numbers of researchers. Our ability to product share ensures that each animal used for biological materials will be fully utilised and wastage kept to a minimum.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	This is a service licence so the species, models and methods used are based on customer requirement. The use of this centralised service is in itself a refinement because the benefit of this is that multiple products can be supplied from individual animals and provided to clients who do not have appropriate facilities and/or expertise or capacity to keep the animals and/or to produce the products themselves. We have staff with species specific expertise in the care and welfare of the animals. As they regularly perform the procedures to obtain the products they maintain a high level of competence. We therefore have the necessary expertise, equipment, staffing

	and funding in place. The majority of animals used are either used after death or under terminal anaesthetic so experience minimal pain. The animals held as donors are trained to cooperate during the sampling procedures and are kept in enriched group living environments.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Preparation of time-mated rabbits using administration of Luteinising Hormone</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Time mating; rabbits; reproductive toxicology; luteinising hormone.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)[11]</td><td>
<table>
<tr><td>Basic research</td><td>~~Yes~~</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>~~Yes~~</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>~~No~~</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>~~No~~</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>~~No~~</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>~~No~~</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td>~~Yes~~</td><td>~~No~~</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To supply groups of time mated rabbits to users who continue work under their own project licence authority.

To ensure high success rate of pregnancy we administer luteinising hormone (LH) to the doe immediately after mating.

The purpose of giving the Hormone injection (Luteinising Hormone) is to facilitate conception enabling pregnancy. It would be a waste of animal life to undergo scientific procedures only to find that the animal is not pregnant and the data needed cannot be collected.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The animals supplied under this licence will aid the work carried out on safety testing of compounds to prevent and/or treat diseases.

Luteinising hormone aids the onset of ovulation to increase the chances of pregnancy. It helps to ensure that ovulation successfully occurs (the stimulus associated with mating may not be enough) and thus maximises the potential pregnancy rate thus ensuring a successful regulatory acceptable study and avoiding animal wastage.

Regulatory requirements stipulate that pregnant animals are used for testing compounds.</td></tr>
</table>

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

	Animals are only injected with the LH to order, thus ensuring the minimum number of animals are supplied to clients. The LH aids the onset of ovulation to increase the chances of pregnancy.
What species and approximate numbers of animals do you expect to use over what period of time?	Rabbits, approximately 5,000 over five years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	LH will be administered by an injection into an ear vein by a competent technician to maximise the chances of successful pregnancy. No adverse effects are expected from the administration of the LH to the rabbits All technicians giving the injection are trained and competent, this will assist in less stress to the animal. The animals are then placed back into housing with enrichments and monitored until dispatch to client. The animals are supplied for continued use under another project licence to contribute to the assessment of any adverse effects on reproduction of potential new medicines.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The animals supplied under this project licence are generally required. for toxicology studies and occasionally research that currently cannot be replaced by non-animal methods. Certain studies carried out by clients are to assess toxicological effects on pregnant animals including the embryonic and foetal development of the offspring. The studies are a regulatory requirement and therefore part of the development process.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Rabbits will only be time mated and treated with the LH to order ie for specific regulatory studies..
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	The model is as required by regulators as background data over many years is available Information on success rates provided by the customer will allow unsuccessful males to be removed from the breeding colony. We also

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	maintain records on the environmental conditions in the facility, animal technician carrying out the technique so that should unsuccessful pregnancies occur we can investigate the cause.

- Memory mechanisms in mice

  This project advances knowledge of the involvement of molecules in learning and memory in mice.

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

  A fundamental biological principle is that animals and humans can make, store, and retrieve memory. Memory processes occur in the brain and several brain disorders, including Alzheimer's disease, impair memory. Current knowledge is not sufficient to understand the precise nature of memory processes, a lack of information that precludes the development of treatments of memory disorders. Specifically, it is important to know what molecules in the brain are involved in memory processes. Such molecules can be targeted by drugs to treat memory dysfunction.

  The overall objective of this project is to advance the understanding of how memory is formed, stored, and retrieved in health and disease. Specifically, we want 1) To identify new molecules and interaction between molecules that are required for memory formation and retrieval, 2) to test whether these are impaired in mouse models of memory disorders., and 3) to find new approaches to enhance learning and/or to prevent learning deficits in mouse models of disease.

- Outline the general project plan.

  For this project the mouse is chosen as experimental animal, because for this species a large number of techniques have been developed to study the function of particular molecules and in mice human diseases can be modelled. Thus, mice are the ideal species to investigate the nature of memory processes. Our project will use non-transgenic and transgenic mice. The mice will be tested in behavioural memory tasks such as their ability to remember where an obscured platform in a tank of water is located. The behavioural tasks are designed to study memories that rely on brain regions that are impaired in diseases. Some of the mice will also undergo manipulation of molecule function.

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

  Most of the procedures are of mild severity. However, in some cases mice will need to undergo surgery to affect molecules in the brain. These procedures are of moderate severity. The surgery involves the use of anaesthetics. The discomfort will be experienced only during the period of recovery from surgery and will be controlled with the use of pain controlling medicines as advised by a veterinary surgeon. The manipulation of a molecule is expected to specifically impact on learning and memory abilities, and not to be toxic in any way.

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

  The benefits of this project are to advance knowledge about protein mechanisms underlying learning and memory in normal animals and disease models. Protein mechanisms must be known for designing pharmacological treatments. Thus, our studies will be fundamental for developing treatments of memory deficits that are

currently not available.

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

  For this project the mouse is chosen as experimental animal, because for this species a large number of techniques have been developed to study the function of particular proteins and to model human diseases. Various transgenic mouse lines will need to be bred and maintained. This will involve maximally 1,000 mice per annum. Maximally 100 mice per annum will be used for brain analysis after killing. Maximally 500 mice per annum will be tested in behavioural memory tasks.

  For the behavioural memory studies power calculations will be performed to use minimal sample sizes and, if possible, factorial designs will be implemented to further reduce animal numbers.

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

  To advance the understanding of memory processes animal experiments are essential, allowing for sophisticated manipulations, which cannot be done in humans. Memory, which is a property of the intact brain, will need to be studied in animals undergoing behavioural testing. Consequently, in vitro experiments or computer simulations are not suitable replacements for the studies with animals undergoing behavioural testing.

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

  This project aims to study learning and memory mechanisms. Animal suffering impacts on learning and memory abilities in an unspecific manner. Therefore, it is in our interest to avoid animal suffering as much as possible. However, some manipulations will require moderate treatments. These include surgery. These moderate treatments will only be used when absolutely necessary. Only animals that have fully recovered from surgery will be considered for our learning and memory analyses.

Muscle protein turnover regulators in disease

Muscle, sarcomere, titin, autophagy, homeostasis

5 years

Basic research	Yes	
Translational and applied research		No
Regulatory use and routine production		No
Protection of the natural environment in the interests of the health or welfare of humans or animals		No
Preservation of species		No
Higher education or training		No
Forensic enquiries		No
Maintenance of colonies of genetically altered animals[13]	Yes	

Muscle is a complex tissue that remodels itself according to the workload. This can be increased size that accompanies exercise in muscles such as the biceps, or heart muscle due to high blood pressure overload. We plan to understand how the muscle can regulate these changes by understanding how the change in activity is communicated within the muscle and how that leads to changes in protein stability and turnover. We are interested in a cellular process called autophagy and how this regulates the mechanism by which muscle can change its size and function. Autophagy is a mechanism by which tissues can recycle excess or damaged parts for the rebuilding or survival of cells in times of stress. These stresses may be environmental such as reduced food intake, age related build-up of damaged proteins, or misfolded toxic proteins as a result of pathogenic mutations and the failure of their clearance. This damage can all lead to reduced efficiency and activity of the muscle activity, leading to heart and skeletal muscle disease but the mechanisms behind this process are unclear. The objectives of this project are therefore to understand the fundamental process involved and how this relates specifically to heart and skeletal muscle disorders.

We hope to understand how muscle protein turnover is regulated, both by the propagation of signals in muscles by an important muscle protein called titin, but also how these signals lead specifically to the remodelling of muscles under stress. This could identify potential markers or targets for therapeutic intervention in muscle wasting conditions such as geriatric nursing and post-surgical inactivity. We are also looking at a mouse model of a human disease, Vici Syndrome, which is caused by the lack or mutation of a protein involved further downstream in the autophagy process, and is a complex disorder of which one symptom is heart disease. We will also be analysing a mouse with a mutation in a gene commonly found in heart disease, and by analysing these mice in the context of a defective autophagy pathway, to see if this may be a mechanism by which the disease state could be caused, or modulated. This knowledge could be then applied to look for targets to benefit patients pharmacologically.

We will use mice for our studies as we can genetically alter or remove some important proteins and assess what effect this has on the complex mouse muscle size, structure and function. We would use the minimum possible number of mice to provide statistically significant results, and expect to use no more than 1000 mice per year.

The majority of mice we use have no obvious defects. We often only see molecular

[13] At least one additional purpose must be selected with this option.

differences when the muscles of the mice are tested by increased exercise or wasting but the mice show no gross adverse effects. We humanely kill the animals at the end of the tests and analyse the hearts and leg muscles.
Muscles are complex structures, often made of different cell types and fibre types to give different physical properties- this cannot be easily represented in non-animal models.
All experiments have been designed to produce statistically significant results from the minimum numbers of animals possible.
Mice are used as they can be readily genetically modified to produce animals lacking proteins, or with disease-associated mutations and have similar muscle structure and genetics as humans. This will allow us to assess the effect of the loss, or mutation of a single protein in a whole animal model, and allow us to analyse, for example, both cardiac and different types of skeletal muscle in a single animal, and thus to reduce the total number of animals used. We can analyse the total mix of proteins from a single tissue and how this may have changed by increased or reduced physical activity, during which the mice will be closely monitored for distress.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Signal integration for visual perception</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cortex, neurophysiology, decision-making, parietal, occipital.</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)[14]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[15]</td><td>~~Yes~~</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Every day, we have to make countless decisions based on the information we receive from our senses, for instance when to cross the road or what to eat. In order to make even simple perceptual choices about the visual appearance of an object, our brain has to integrated many different types of information. These can be different types of visual information that have to be combined, like motion, colour and depth. It can also include other types of information, e.g. what is the pay-off for a certain choice or what have others decided. While it is clear that all these factors affect decisions behaviourally, it remains unclear how they do this.

We investigate where in the brain and how different types of information are integrated and evaluated in specific brain circuits and how this affects perceptual decisions. We will generate models of these processes that can be applied and tested in different contexts. Altered decisions about sensory information, as studied here, are hallmarks of many mental disorders including schizophrenia and autism.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The results from this study combined with human behaviour and imaging using the same task will allow us to probe and build models of how primates make decisions that can be used by others.

Our findings will generate knowledge and contribute to the larger body of work required to understand</td></tr>
</table>

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

	the brain mechanics of how we make cognitive decisions from input to our senses, through to the behavioural response. How this knowledge is used has relevance to areas as diverse eyewitness accounts, economics and the law. The quantitative models we generate will better explain how the relevant brain processes operate in healthy subjects. They also provide insights into and means of investigating, how visual perception and decision making heuristics might be altered in such disorders as autism and schizophrenia.
What species and approximate numbers of animals do you expect to use over what period of time?	8 Rhesus macaques, of which up to 5 are studied for a period of about 4 years and up to 3 for less than 1 year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	First, animals will undergo up to two magnetic resonance scans under general anaesthesia, with similar risk involved as for human patients. Then, animals will be gradually trained to carry out visual decision tests. They will be introduced to the apparatus and tests slowly and progressively. Initially, 'treats' will be used to motivate animals. Eventually, animals will be trained to earn their daily fluid intake through responding to images on a computer screen. Animals will be carefully monitored for health and well-being throughout the study. The neurophysiological recording and testing require that the animals' heads are restrained to remain still, and the animals will also be gradually accustomed to achieve this. Surgery will be required to implant devices on the skull that allow the neurophysiological recording from the brain. At the end of the experiment, animals are euthanized, so their brain connections will be studied as part of this project.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The study of brain mechanism of perception and decision-making with the relevant spatial and temporal resolution (brain cells and milliseconds) requires to-date invasive experiments, which cannot be carried out in humans. The same applies to linking the circuitry of such neurons to their connections. Human imaging methods, which we use in parallel, measure brain activity either indirectly or not on the right scale to allow the study of the underlying brain computations. Neuronal responses in brain slices cannot be linked directly to behaviour and therefore to perception and cognition. While we use our data to build computational models of perceptual decision-making, we are still at a point where we do not

	know enough to have a ‘definite’ model of perceptual decision-making, rather experiments are needed to test and develop such models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The required number of animals is small, because the experimental unit is not the individual animal but the neuron or recording site. Neurophysiology and electrical stimulation leave the structure and function of the brain intact. By collecting data from a sample of brain sites from each animal, animals provide their own controls while at the same time we gain the necessary understanding of the population response within a brain area. Experiments built around the same behavioural task. Therefore, a minimum of three animals is needed for the neurophysiological experiments. An additional two animals are needed for the histological analysis of the circuitry. Additional animals may be needed for this if an animal cannot be trained in the specified time or if experiments cannot be completed in an animal for welfare reasons. In these cases, although the data collected will be useful, our objectives will require additional animals. Different parts of the protocol are carefully staged in a sequence such that we can achieve the objectives with a minimum of 5 animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The macaque is the only suitable animal model for this project. Lower species are unsuitable because of a lack of cognitive ability and/or because the underlying brain circuitry is not directly relevant to man, and such invasive studies cannot be undertaken in man. There are currently no other, non-invasive methods available to elucidate neuronal mechanisms and functional circuitry at the level of single neurons in real-time. To study the neural basis of cognitive tasks like perception, brain activity and behaviour must be linked statistically robustly. The behavioural task is based on judgements monkeys (and humans) have to make implicitly on a daily basis when they move through their environment. Animals will be socially housed and carefully monitored for well being. They will gradually be trained to carry out a behavioural task for fluid rewards. Training schedules and rewards are tailored to the individual animal. Fluid protocols and monitoring schemes are designed to ensure each animal’s wellbeing. The risks associated with general anaesthesia, magnetic resonance imaging and surgery for skull implants is similar to those for humans; we work to

	the same aseptic standards. All implants are formed from medical-implant grade materials to osteo-integrate. The movement of electrodes within the brain is painless. Eye movements are recorded non-invasively. Animals will be monitored daily by researchers and veterinary staff. Animal care and veterinary staff are closely involved in the care and monitoring of all animals under study and in the development of protocols.