Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 1

Project Titles and key words

- Perceptual decision-making in the primate brain
  Temporal cortex, frontal cortex, electrophysiology, neuroimaging

- Optogenetic analysis of neural microcircuits
  Nerve cell, neocortex, inhibition, transgenic mouse, optogenetics

- Peripheral nerve interfacing
  Neural interface; Peripheral nerve

- Regulation of inflammatory responses in fibrosis
  Infection, immune response, inflammation, fibrosis

- Gene therapy for neurodegenerative diseases
  Viral vector targeting, neurogeneration

- Test signalling in cell motility and tumourogenesis
  Signalling, tumourogenesis, RhoGTPase

- The impact of co-morbidities on Alzheimer's disease
  Alzheimer's disease, obesity, infection, memory

- Friedreich ataxia mouse models
  Friedreich ataxia, inherited neurodegenerative disease

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Perceptual decision-making in the primate brain</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Temporal cortex, frontal cortex, electrophysiology, neuroimaging</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td>~~Yes~~</td><td>No</td></tr>
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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 ability to recognize complex visual stimuli and to make decisions based on those stimuli (i.e., “perceptual decision-making”) is vital to our ability to successfully interact with our environment. Much of our knowledge of how objects are recognized stems from the recent discovery of a small number of discrete regions in the temporal cortex of humans and monkeys that are selective for various categories of visual stimuli (e.g., faces). These areas form networks with decision-making areas in the frontal cortex to guide perceptual decisions. Damage that includes these regions, such as that which occurs following stroke or traumatic brain injury, can lead to significant visual processing deficits, such as prosopagnosia (“face blindness”), where patients lack the ability to recognize and/or discriminate faces but have an otherwise intact visual system. Deficits in perceptual decision-making are also common to a wide range of developmental disorders, including autism spectrum disorders (ASD).
Despite extensive study into visual processing, little is known about the precise composition of the neural networks involved or the properties of neurons within these networks. The overall objective of this project, therefore, is to map these cortical networks and to characterise the neuronal processes associated with perceptual decision-making in the primate brain.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or</td><td>This programme of work is expected to reveal how brain areas involved in decision-making interact with and influence those involved in visual processing. We will relate the information we obtain from activity patterns of actual neurons in the monkey brain to the more indirect estimates of brain activity that we can obtain from humans (e.g., using</td></tr>
</table>

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

humans or animals could benefit from the project)?	neuroimaging). These data will be used to guide future studies of visual processing in non-human primates, including those planned in our laboratory. Furthermore, our findings are expected to assist in the development of animal models appropriate for the study of neurological disorders that include deficits in perceptual decision-making (e.g., agnosia following stroke).
What species and approximate numbers of animals do you expect to use over what period of time?	This work requires the use of monkeys (rhesus macaques), as they are the only laboratory species with the cognitive abilities and physical characteristics (e.g., brain size) necessary to achieve the desired objectives. We expect to require 16 animals to achieve all objectives. We expect each animal to be on study for between 3-5 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 will collect neuroimaging and neuronal recording data while monkeys perform cognitive tasks. The potential adverse effects of these procedures include the following: The daily experience of the animal on protocol includes prolonged and repetitive restraint, extensive training/testing on complex cognitive tasks, the potential for being on protocol for many years, and the potential for stressful motivational/training techniques including fluid control/restriction and the use of pole-and-collar. As a result, the animals may experience periods of stress related to behavioural testing. Further, there is a moderate risk some animals may experience transient periods of mild dehydration, particularly during the initial stages of training or when tasks suddenly increase in complexity; and transient weight loss when starting on fluid control. There is a moderate risk that their implants may become mildly infected. There is also a small risk that the implant may become damaged/fall off and require repair or replacement, or become damaged. There is a very small risk of seizure, infection, and/or haemorrhage associated with neuronal recordings, temporary inactivations, and surgical implantation of recording devices. At the end of the study, the animals will be enthuanised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Achieving the scientific objectives of this research project requires that we measure neuronal activity in awake, behaving subjects and artificially influence activity within deep brain regions. This work involves invasive techniques, including neuronal recording and temporary lesions, and therefore cannot be performed in humans.
2. Reduction Explain how you will assure the use of minimum	Reduction in this project is achieved by: a) reducing the number of animals necessary; b) reducing the duration of each daily experimental session; and c) reducing the number of experimental sessions (thereby reducing the time an

numbers of animals	individual animal spends on study). Our experiments are based on a "within-subject" design, such that research questions are based on comparisons in one experimental condition vs. another, which can be embedded within the same behavioural task. We design the behavioural tasks to be as simple as possible given the experimental objective. This reduces the duration of individual experimental sessions and ensures that few (if any) animals need to be removed from study for failure to learn/perform the task. We also design the tasks to enhance differences between experimental conditions (wherever possible) to reduce the amount of data necessary to achieve statistical reliability. Finally, we design our experiments such that a single animal/experiment can contribute to multiple objectives (without increasing the cost to the animal), thereby reducing the total number of animals necessary.
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.	Achieving the scientific objectives of this research project requires that we measure neuronal activity in awake, behaving subjects and that we artificially influence activity within deep brain regions using microinjection and microstimulation. Monkeys are the most suitable animal for the following reasons: 1) The brain must be of adequate size to allow for precise sampling/inactivation of restricted cortical regions using both fMRI and recordings; 2) The animal must be able to be trained and subsequently perform complex identification and discrimination tasks, including in the MR scanner; 3) Accurate monitoring of eye position must be possible. We have taken every measure possible and established multiple intervention points to ensure that each individual animal will experience the minimum potential possible for any adverse effects (pain, suffering, distress, or lasting harm) and only when it is absolutely necessary to achieve the scientific objectives; and equally important, that no more animals are used than is absolutely necessary. (1) We have designed the scientific programme in order to maximise the amount of data that can be obtained with each experiment, with many experiments contributing data to more than one objective. The individual experiments are designed to build upon one another to ensure that they are conducted with the most up-to-date information possible and to achieve maximum scientific benefit from each animal. (2) We have incorporated flexibility in terms of recording techniques to ensure that the best available recording technique will be used for each scientific objective (to be determined for each individual animal/target area). This will maximise the quality of data and minimise the amount of time the animal is required to be on protocol. (3) We have established intervention points between each new procedure to ensure that no new procedure will be performed unless the NVS, NACWO, and researchers agree that the animal is not likely to experience an unreasonable increase in risk for adverse effects. This will not however, compromise the utility of the data obtained prior to these intervention points should it be

	deemed inappropriate for the animal to continue. (4) We have developed a progressive method of instituting reinforcement training/fluid control (and monitoring its efficacy through behavioural monitoring) that will serve as the basis for developing individualised programmes for each animal (to be done with the NACWO). This will ensure that no animal will experience any more restriction than necessary to overcome specific hurdles in training. (5) We will use the latest technology/methods to increase the quality of the data (e.g., using contrast-agent enhanced MRI, fMRI-targeted neuronal recordings, recording arrays, etc.) thereby minimising the duration of experiments and the time each animal is required to be on protocol. (6) We have specifically designed (and continue to refine) our restraint systems (both headposts and restraint chairs) in order to minimise the discomfort experienced by the animal during prolonged periods of restraint. (7) Finally, we will continue to maintain close relationships with the NVS/NACWO to continually refine our behavioural and health monitoring procedures.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Optogenetic analysis of neural microcircuits</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Nerve cell, neocortex, inhibition, transgenic mouse, optogenetics</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)[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[4]</td><td>Yes</td><td></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The complexity of the brain is a serious barrier to understanding how neural circuits are configured and how they process information. Recognizing this problem, we have developed an experimental approach in which particular types of nerve cell in the mouse brain are modified genetically so that a flash of light activates them by remote control. We will use this approach on explanted mouse brain tissue to examine which inhibitory nerve cells communicate with which excitatory nerve cells, to measure how inhibition affects the function of the excitatory cells, and to determine how changes in the functional state of the brain (for example, as a result of aging or injury) alter these networks of cell-to-cell communication.</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 principal benefits of our work are new levels of understanding of the operation of the brain and of the effects of pathological processes such as alcohol abuse, epilepsy, and Alzheimer’s disease. We also expect to be able to evaluate potential therapeutic interventions in the physiological but experimentally tractable setting of explanted tissue.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We expect to use approximately 4,000 mice over a period of 5 years.</td></tr>
<tr><td>In the context of what you propose to do to the animals,</td><td>Most of the mice will be used for breeding purposes. A minority of animals will serve as</td></tr>
</table>

[3] Delete Yes or No as appropriate.
[4] 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?	donors of brain tissue, and only if absolutely necessary will live animals be infected with a crippled virus that does not cause symptomatic disease. All genetic modifications are, in themselves, harmless. Thus, the level of stress or discomfort will be minimal or nil.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The function of the brain can be studied only by using the brains of animals or humans. Computer models are inadequate because our knowledge of brain function is too rudimentary to generate realistic models. Cell culture systems are inadequate because they do not preserve the functional architecture of intact brain circuits. Studies in humans are impossible because they would be invasive and require genetic modifications. This leaves the use of animals—specifically, mice—as the sole option.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Care has been taken to minimise animal numbers through streamlined breeding and analysis schemes, and to eliminate suffering by working on explanted tissues rather than intact, living 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 mouse was chosen as the experimental species because its cortex possesses the canonical six-layered architecture of mammals; because germline genetic modifications can be made and propagated with ease; because a wide array of mild methods of sensory deprivation are available (including genetic methods); and because a large body of existing literature provides a valuable context for new scientific findings. The experimental model of choice is brain tissue explants. Acutely harvested brain slices preserve the neuronal microcircuitry in functional form for up to 10 hours and are accessible to targeted microelectrode and/or optical recordings. The overwhelming majority of tissues will be obtained from humanely killed mice carrying the genetic modifications necessary for rendering particular types of neurons responsive to light stably integrated in their genomes. These animals will not undergo any procedures except possibly ear notching or tail tipping, which in some cases will be required for marking and genotyping purposes. No adverse effects of the genetic modifications themselves have been encountered. The expression patterns of some genes are refined greatly during development, so that initially broad expression of the gene gives way to much greater cell-type specificity. To take advantage of this

	increased specificity requires that the DNA recombination event allowing expression of the actuator protein is prevented during early development and only triggered at a later stage. This is achieved through genetic modifications that use drug-inducible versions of DNA recombination enzymes. Only if the desired expression patterns cannot be obtained by crossing driver and responder mouse lines and using timed recombination will we resort to viral transduction to express sensor and/or actuator proteins in circumscribed brain regions

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Peripheral nerve interfacing</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neural interface; Peripheral nerve</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The project is designed to develop a peripheral nerve interface, which is an implanted medical device that connects a peripheral nerve to electronic circuitry, with the aim of allowing recording of signals from motor axons within the nerve. The device will be based on a microchannel array architecture which we have previously shown has several important advantages over other designs. It is eventually intended to be used in human amputees to allow advanced limb prostheses to be connected directly to the nervous system, radically improving control of the prosthetic compared to currently available technology.</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 neural interface that is being developed is the key component that is needed in order to radically improve prosthetic control systems. At present, advanced upper limb prostheses contain highly sophisticated robotics but are controlled by myoelectrics, which is 40 year old technology that provides an extremely basic level of control permitting only one or two movements at a time, without force gradation and with considerable mental effort on the user's part. The interface should offer virtually normal motor control over the prosthetic. By the end of the five year period of this licence we aim to be at the point where human trials can be planned.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Approximately 60 rats per year</td></tr>
</table>

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The severity level is moderate. All animals are expected to have weakness of the right hindlimb but this does not significantly affect their mobility or welfare. Experience with similar procedures in this strain of rat (Lewis) indicates that more serious adverse effects are very rare. At the end of the procedure the animals will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Interface designs will be screened initially by mathematical modelling and then their electrophysiological aspects tested using an *in vitro* model. However *in vivo* studies are needed to (i) verify that the results of the *in vitro* experiments using non-regenerated nerve tissue are valid for regenerated axons in the implanted scenario; (ii) investigate measures to combat fibrosis, which is a complex chronic inflammatory effect that cannot be replicated *in vitro*; and (iii) obtain examples of the complex multi-channel recording data that these devices will produce, to allow development of signal processing and interpretation systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The use of mathematical modelling and *in vitro* experiments will prevent *in vivo* experiments that are unlikely to succeed. Where possible each group of animals will provide data for more than one of the research areas in the project.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats are used because they have peripheral nerves that are biologically and electrophysiologically similar to human nerves, they are the smallest species where nerve implantation is feasible, and we have a lot of prior experience with, and data from, the rat sciatic nerve model. Welfare measures include the routine use of perioperative and postoperative analgesia, prophylactic antibiotics and aseptic techniques.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Regulation of inflammatory responses in fibrosis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Infection, immune response, inflammation, fibrosis</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)[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>Yes</td><td></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>Peritoneal dialysis (PD) is one of the major forms of treatment for kidney failure and is used by up to 30% of the UK dialysis population. The major reason for treatment failure is bacterial infection and the inflammation induced by it. Repeated episodes of infection/inflammation damage the peritoneal membrane and result in this excellent treatment no longer being possible. Despite 30 years of research there is no specific treatment that reduces damage to the peritoneal membrane and the clinical need is constantly increasing as the elderly population and the number of individuals with diabetes (the main cause of kidney failure) are on the rise.
This project aims to increase our understanding of the immune mechanisms involved in normal infection clearance and resolution of inflammation and of how these mechanisms may become progressively dysregulated following repeated infectious episodes. In the context of the peritoneal cavity, our work will use animals with carefully controlled infection and inflammation as models of what occurs in the abdomen of PD patients. These studies will help to establish the link between repeated infections and fibrosis development in PD patients with the aim of developing novel therapeutic strategies to reduce peritoneal membrane damage and improve patient outcomes.</td></tr>
</table>

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

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will define more clearly the role(s) that several components of the immune system play in peritoneal immunity and their contribution to the complex inflammatory processes that lead to the development of PD-associated fibrosis Our data will also improve the overall understanding of the complex process leading to normal clearance of infection and resolution of inflammation as well as of the factors contributing to the development of chronic inflammation. In this manner, the observations that will be made within the peritoneal cavity are likely to have wider implications, since the processes regulating infection and/or inflammation-induced fibrosis, and their prevention, are relevant to all organ systems. In addition, our project will explore several potential therapeutic avenues to control the development of acute and chronic inflammation in general, which could be beneficial to a variety of inflammatory conditions in addition to PD-induced fibrosis.
What species and approximate numbers of animals do you expect to use over what period of time?	About 7500 mice are expected to be used over the 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The main adverse effects induced by the protocols proposed for this project will be linked to the induction of bacterial peritoneal infection (peritonitis). Although high doses of live bacteria or bacterial products may cause severe distress and/or death, our protocols describe well-defined models of transient and mild peritonitis, which use the lowest doses of pathogens (or pathogenic compounds) necessary to produce reliable, consistent and robust scientific measurements to achieve the specific objectives. Experimental protocols are not expected to induce distress, pain or suffering to an extent exceeding the Mild severity limit, although a precautionary Moderate severity limit is in place for some of the proposed protocols to allow for the apparition of moderate adverse effects that cannot be predicted. Such effects may for example be due to the administration of substances that have not yet been tested/titrated *in vivo* and need to be used as a replacement/complement to the substances

	typically used or to genetic alterations leading to a particular susceptibility to the injected substances. Animals will be monitored throughout the course of the infection for the development of potential adverse effects and humane end points are in place based on the expected adverse effects. These include minor changes to appearance (e.g. general lack of grooming and/or slight coat staring) and behaviour (e.g. diminished activity, minor lethargy, reduced alertness). Some body temperature variation is also expected, and will be monitored if deemed necessary. Any animal showing signs of distress, pain or suffering to an extent exceeding the severity limit of the proposed protocol (Mild or Moderate) will be humanely killed. At the end of the experiment, animals will be killed humanely and the peritoneal membranes examined by a variety of appropriate scientific techniques to assess the level of inflammation and membrane damage as well as the impact of treatment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Peritoneal inflammation is a dynamic process involving the resident tissue and cells as well as recruited immune cells from the circulatory system. It is impossible to fully reconstitute this system *in vitro* and investigate complex multi-factorial changes leading from repeated peritoneal inflammation to peritoneal fibrosis, which is why the use of an *in vivo* model of the pathology remains necessary. Much of our experimental hypotheses have been driven by data obtained *in vitro* from human cell culture experiments or by observations made *ex vivo* in peritoneal dialysis patients. **Using various *in vitro* experimental models we have thoroughly characterised several inflammatory mechanisms and indentified novel molecules with the potential to modulate them**. However, the **use of a dynamic *in vivo* model is indispensable to** address the precise role of these mechanisms in the genesis of peritoneal fibrosis **and evaluate the therapeutic** potential of the compounds that were found capable of targeting and modulating these mechanisms.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Due to our significant experience with this infection and inflammation model, optimal experimental time points and doses for many of the inoculums and treatments have already been determined. The aim is to produce clear and unambiguous results, and with this in mind, animal numbers will be kept to a minimum. Where appropriate power calculations will determine the size of experimental groups. **As much as possible, we will attempt to group different experiments, so that the control groups can be used for several experimental conditions, thus reducing the number of animals needed.** **Prior to undertaking novel experiments information will be sought within the literature and used to small scale pilot studies will minimize numbers during early investigative 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 objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	To date there are few *in vivo* models of peritoneal fibrosis. These are either based on the daily instillation of dialysis fluids through an indwelling catheter (predominantly using rats) and are associated with significant complication rates (stress, infection, catheter blockage and mortality) or the daily injection of chlorhexidine (usually in mice) that results in the development of severe fibrosis and bowel encapsulation. Our model is significantly less invasive, involves significantly less trauma than these models and has produced clear experimental endpoints that better parallel the clinical disease scenario in dialysis patients. Experimental protocols follow strictly regulated standard operating procedures and are performed only by experienced and trained staff, ensuring that animal stress and discomfort are kept to an absolute minimum while improving data quality and reproducibility. As part of these protocols, light anaesthesia will be administered prior to injection. This will serve several purposes: it will reduce stress and discomfort for the animals, ensure the accuracy of the injection and minimise the repeated injections-associated damage to the peritoneal membrane and allow precise weighing of the animals.

	Mice will be thoroughly monitored and scored regularly for signs of pain or distress according to a number of well defined criteria. Unfortunately, veterinary treatments (e.g. analgesia) aiming at reducing pain or discomfort will not be used in this experimental protocol, since they may interfere in a unknown manner with the normal immune response of the animal and produce results that are neither reliable nor meaningful.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>GENE THERAPY FOR NEURODEGENERATIVE DISEASES</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>VIRAL VECTOR TARGETING NEURODEGENERATION</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 YEARS</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<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[10]</td><td>Yes</td><td></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The project proposes to research into diseases that affect nerve cells, either in children that were born with a genetic disease such as Spinal Muscular Atrophy or in adults and the elderly who suffer from diseases such as Parkinson’s disease and motor neuron disease. These diseases are common in the elderly and are incurable at present, so there is a strong need to devise new ways of treating them.</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>Some gene therapy has been introduced to patients already, for a limited number of human diseases (i.e. Duchenne Muscular Dystrophy, Parkinson’s disease and Haemophilia B) but it is anticipated that such research as proposed here, might lead to the wider application of gene therapy in human clinical trials particularly for neurodegenerative diseases.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Our research will only use mice and rats. An average of 300 animals are expected to be used in these projects per year.</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 happen to the animals at the end?</td><td>Rats and mice will be injected with gene transfer vectors via different routes to assess how best to target neurons more effectively. Transgenes will be marker genes such as the green fluorescent protein, beta galactosidase (stains blue), or firefly luciferase that allows live imaging, so as to measure the gene transfer. The vectors/route of administration combination that gives the best</td></tr>
</table>

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

	profile in the targeting studies above, will be validated in animal models of disease. In particular these include known models of Parkinson's disease such as the partial or complete 6-OHDA rat or a genetic model models of Huntington's disease and Amyotrophic Lateral Sclerosis such as the SOD1 G93A transgenic mouse, transgenic knockout mouse models for Spinal Muscular Atrophy (type I or III). Transgenes in this case will be capable of inducing or suppressing expression in a therapeutic manner. We do not anticipate any adverse effects to occur in our surgical proceedures, but guidelines in dealing with rare adverse effects will be put in place and followed in case these do occur. We will apply all recommended methods of minimising animal use and reduce suffering. For example appropriate anaesthesia will be utilised in surgical proceedures and appropriate anaelgesia will be used so as to minimise post-operative pain. Level of severity varies according to model used from mild to severe (paralysis models). All animals will be humanely culled at the end of the proceedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Viral gene therapy vectors (disabled viruses that are not pathogenic) are used here to introduce therapeutic genes back in the vulnerable cells thus trying to save them (this approach is known as gene therapy). Such a strategy will be used in cell culture first and then tested in rodent models of the above diseases, so as to determine if the method is effective. The use of animals is important as we study any immune reactions to the vectors and therapeutic genes that cannot be studied in cell cultures.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will apply all recommended methods of minimising animal use and reduce suffering. Mimimum numbers of models will be used according to statistical power calculations to give us a conclusive answer. Also small pilot studies will be done where possible to assess feasibility.
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	Our research will only use mice and rats, for these species are well characterised and are genetically close to humans and bred so as to reduce variability. The animal models chosen are considered the golden standard in the scientific literature and that gives us the ability to compare our results to those of others and only take the most efficient therapies through to the clinic. The

(harms) to the animals.	general measures in place to minimise harm to these animals is to use the best breeding, good surgical proceedures (anaesthesia, aseptic technique, analgesia etc), and to assess the animals frequently after that for possible health issues that might be causing them stress and if unable to relieve them of such (ie by using drugs) to humanely cull them.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Test signalling in cell motility and tumourogenesis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Signalling, tumourogenesis, RhoGTPase</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)[11]</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[12]</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 regulation of cell adhesion and motility is essential to development and continues to be of critical importance throughout the lifetime of multi-cellular organisms. Deregulation of these fundamental cellular processes also frequently occurs during pathological situations such as tumour cell metastasis. By characterising the signalling networks and underlying cellular machinery regulating and mediating both cell adhesion and migration we can attempt to understand disease processes where these key elements of cell behavior are disrupted.</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 project could answer key questions about cancer growth and spread. The project will provide new mechanistic insights into cancer development and metastasis, and the involvement of cytoskeletal and Rho signalling in these processes, and may identify new potential targets for therapeutic intervention.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">We will only use mice in these studies. A maximum of 300 animals will be used /year, 1500 over the life of the licence.</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 happen to the animals at the end?</td><td colspan="3">A very small percentage of these mice may get tumours but their welfare is closely monitored throughout the entire process and study animals will be humanely killed and data collected before pain and distress arises. A very small percentage of animals of the genetically altered lines die within a day of birth without intervention, but we will carry</td></tr>
</table>

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

	out hourly checks on any pups at risk and humanely kill these if they begin to show signs of moderate suffering. Our mice will be housed in groups wherever possible and will be provided with nesting material and if required, refuges. Even those mice not undergoing any procedures are closely monitored by well-trained staff who can deal quickly with any unforeseen problems that arise.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Many aspects of cell motility in normal and cancer cells can be studied *in vitro*; we have considerable expertise in these assays and use them to investigate the role of Tes and other focal adhesion proteins and in place of animals wherever possible. However, **animal use in these studies does not supplant any of the existing strategies we already employ to characterise genes and protein interactions involved in cell migration. Furthermore, it is complementary to our approaches by providing us with cell lines which can be used in further *in vitro* studies.**
2. Reduction Explain how you will assure the use of minimum numbers of animals	Transgenic breeding will be reduced by collaborative access to strains. Experimental design will use no more animals than needed to obtain informative and statistically significant data. We will maximise the data obtained from each animal by studying multiple cell types. As outlined above we can reduce the numbers of mice used by marking proteins of interest with different coloured tags
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 were chosen embryonic development and biology are well described, so we will be able to identify and characterise abnormalities.** Cells can be derived from the animals and extensively analysed in culture, maximising the information from an experiment. In addition, there is a sophisticated understanding of husbandry methods and recognition of symptoms of pain, discomfort and distress, should these arise, so mice can be provided with a comfortable living environment. We will use information gained from *in vitro* analysis to optimise the design of animal studies. Use of tumour cells in which interaction between eg Tes and Rho can be regulated in a controlled manner will allow us to measure directly the dependence of tumour growth and behaviour on this interaction. Our main focus is in the capacity to produce tumours rather than the behavior of the tumour itself. This means that generally we do not have to allow a tumour to develop to the point where it would impact on the health of the animal. To minimise suffering, whenever possible cells will be derived from embryos following timed matings

	rather than pups or older animals.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The impact of co-morbidities on Alzheimer’s disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Alzheimer’s disease, obesity, infection, memory</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Alzheimer’s disease (AD) is the most common cause of dementia in the elderly with over 800,000 people in the UK being affected, and this is expected to double within 20 years. Therefore, AD represents a significant medical problem, especially as there is currently no cure and no way to slow progression of the disease. There is therefore an urgent need to understand in more detail the underlying mechanisms that contribute to AD, so that new treatments can be developed.

Several factors increase the risk of getting AD, including obesity, and infection/inflammation. This project will look at the effect of some of these factors on AD in more detail.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Our work will have relevance to understanding the risk factors involved in AD, and if dietary interventions or anti-inflammatory treatments could be beneficial to those people who are at risk of developing AD in later life.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice (and sometime rats) will be used as there are several available mouse models of AD and some new rat models. Over the period of the licence (5 years) it is estimated that approximately 4700 mice and 800 rats will be used, the majority of which (>50%) for breeding purposes, and therefore will undergo no actual regulated surgical procedures.</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 we will use appear normal and healthy but develop problems in their memory over time. Disease progression will be monitored over several</td></tr>
</table>

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

effects and the likely/expected level of severity? What will happen to the animals at the end?	months by mainly studying pathology and behaviour. Pathology will be tested post-mortem, but we will also scan the brain, which will allow the progression of AD to be studied in the same animal over a long period of time, which will reduce animal numbers. We will also test the memory using a series of well-described tasks that are not harmful to the animals. Much of our analysis will be carried out post-mortem on tissue from animals that undergo no treatment and therefore no adverse effects are expected in these animals. Where there is treatment or an intervention (dietary modification/infection) some mild changes may be observed e.g. infection may cause a fever and sickness-like behaviour in the animals, but these effects are transient. One of the behavioural tests (Morris Water-Maze) involves placing the mice in a pool of water, which may induce mild stress. Animal welfare will be continually monitored and any issues will be discussed with the named veterinary officer. No serious adverse effects are expected and we are very well aware of minor adverse effects that may be seen, and have taken great efforts to reduce them. All animals will be humanely culled at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Studying the risk factors and mechanisms involved in AD is extremely complex and involves understanding the interactions between several systems in the body. It is difficult to mimic such complex interactions in cell culture systems, using computer modelling or other non-animal means. Whole animal *in vivo* experimentation is therefore vital. AD is characterised by deficits in learning and memory, and as such this behaviour is very difficult to model in cells. However, wherever possible we will conduct experiments in cell culture systems before proceeding with whole animal studies. Furthermore, if any relevant non-animal alternatives become available during the course of the project, we will implement these in our studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments are designed on the basis of our own findings or published data. Prior to any new study a full evaluation of previous/pilot data will be carried out and we will also perform calculations to work out the minimum number of animals required to obtain valid data. Statisticians have been, and will be consulted, to help with these calculations.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	Although it is possible to use animals with a lower degree of neurophysiological sensitivity (e.g. the marine snail *Aplysia* and *C.elegans*) to learn more about the processes involved in learning and

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	memory, these organisms do not allow one to mimic fully the genetics and associated brain changes of AD. In contrast the use of rodents (mice/rats) expressing genes associated with AD does allow one to model the genetics and pathology of human AD more closely. We have extensive experience of all techniques to be used in our studies and understand the welfare needs of the animals and the minor adverse effects that may be seen. Animals are always monitored closely and we have measures in place to minimise harm, as stated in the licence.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Friedreich ataxia mouse models</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Friedreich ataxia, inherited neurodegenerative disease</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[15]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></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[16]</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 purpose of the project is to investigate mouse models of the lethal inherited neurological disease Friedreich ataxia to gain further understanding of the disease mechanism and to identify novel therapies.</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 benefits will include further insight into a lethal inherited human disease. Preclinical studies will provide essential information before proceeding to clinical trials in humans. Potential treatments will be identified that may halt or reverse Friedreich ataxia disease progression. Findings may also assist the future treatment of other similar inherited and neurological human diseases.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mouse. Approximately 3,300 mice 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, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>Genetic modifications may produce movement disorders, heart pathology and tumour formation in the mice. Therapeutic treatments of mice may produce injuries from injection needles. There is a moderate level of severity. Mice will be killed at the end.</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
<tr><td>1. Replacement
State why you need to use animals and why you cannot</td><td>Some information relevant to the investigation of Friedreich ataxia has been obtained from studies of bacteria, yeast, worms, fruit flies and human cell-</td></tr>
</table>

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

use non-animal alternatives	lines. However, the study of complex systems, organs and tissues similar to humans is now deemed necessary. Use of animals is now required to provide a more complete understanding of this human disorder and to undertake preclinical testing of novel treatments before progressing to human clinical trials.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Breeding will be kept to the minimum amount necessary. Randomised block and factorial designs will be introduced into experimental strategy in order to minimise the number of animals used. Power calculations will be performed to determine the minimal number of animals needed to obtain statistically significant 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.	Mice are the lowest vertebrate animals in which a representational model of Friedreich ataxia has been developed. Welfare of mice will be maintained throughout the project by daily inspection of mice for general signs of ill health, together with the administration of potential therapeutic agents by the mildest available route of administration.