Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 27

Project Titles and key words

- Molecular genetic analysis of hypothalamic phenotype

  brain, hypothalamus, neuron, gene, DNA

- On the mechanisms of risk for psychiatric illness

  Schizophrenia, autism, genetics, learning disability, development

- A transgenic approach to control avian influenza in birds

  Transgenic chickens avian influenza

- The immunology of inflammatory disease

  Immunology, Human, Inflammation

- Development of novel strategies for neural repair

  Spinal cord injury, peripheral nerve injury, CNS injury, neural repair, axon growth

- Time-dependent mechanisms in learning and memory

  Learning, memory, habituation, timing, conditioning

- Modelling gastrointestinal inflammation and tumorigenesis in the mouse

  Cancer, intestine, oesophagus, inflammation

- Physiology and pathophysiology of the auditory system

  Hearing, tinnitus, neuroscience

- Immune and vascular responses in inflammation

  Inflammation; leukocyte migration; blood vessels

- Zinc finger gene therapy for Huntington's Disease

  Gene therapy; neurodegeneration; mice; synthetic zinc finger proteins.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Molecular genetic analysis of hypothalamic phenotype</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>brain, hypothalamus, neuron, gene, DNA</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>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>In this project we will generate and study genetically altered rats and mice in order to understand the role of genes in the brain. Our aim is to define genes and associated proteins (gene ‘regulators’) that allow some brain cells to retain ‘immature’ characteristics or ‘phenotype’. This new knowledge could help to develop treatments for some brain diseases.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This work is important because in helping to define the role of specific genes in the brain it will potentially add to the capability of neurologists and psychiatrists to intervene in brain disease progression. More broadly, the type of precisely defined and carefully controlled genetic work conducted in this project will add to our understanding of ‘genome biology’. Following the sequencing of all of the DNA in both the human genome, and that of other mammals, it has become abundantly clear that the ‘annotation’ of these genomes – the details of their functional ouput - is the real key to understanding our genetic makeup. Each original and</td></tr>
</table>

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

	carefully performed experiment that we undertake will add to the bank of publically available 'genome annotation' and allow us to make wiser choices about future scientific and medical requirements.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the entire five years of this project, we expect to use a total of approximately 2500 mice and 4000 rats. Many animals used in these studies will simply be maintained in small groups, used in natural matings, and then humanely killed for brain analysis.
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 many animals used in this study, adverse effects are anticipated to be minimal. A smaller group of animals will be used in experiments where a specific drug is injected (subcutaneous, intraperitoneal or intravenous routes), or implanted within a 'minipump' under the skin in order to modify brain gene expression. Adverse effects to the drugs are anticipated to be minimal but will result in humane killing where required. In other groups of animals where substances must be injected directly into the brain, special cannulae will be surgically implanted for this purpose. For both implantations, adverse effects of surgical and post-surgical pain will be controlled with general anaesthesia and analgesics. Animals used to provide single-cell embryos for genetic modifications will be given hormone injections; extremely rare adverse responses to these injections will result in humane killing. For animals undergoing surgery for genetic modification procedures, adverse effects of surgical and post-surgical pain will also be controlled with general anaesthesia and analgesics. All animals will be humanely killed at the end of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal models are used because we want to learn about genes and gene products within their natural context, i.e. within the complexity of the whole brain and whole animal. Precise

	genetic modifications in rats and mice allow us to study individual genes within the context of the mammalian brain and also within the context of a controlled laboratory environment. The full extent of the complex molecular interactions that individual genes are involved in is not currently understood and so computer simulations cannot replace this animal work. Similarly, the use of individual brain cells or pieces of brain in a 'test tube' environment are not always useful because the 'long distance' nervous pathways in the brain are lost.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our experiments will have a common sequence that is tried and tested in our previous studies. Genetic alterations are introduced into the genome of rats or mice using well-established techniques. The animals are bred to establish stable colonies of identical, genetically-altered animals and when this has been established they are killed at defined times in order to sample specific areas of the brain. The expression of genes and gene products are then measured; results are compared between genetically modified and normal animals. In some cases, pilot studies are undertaken, for example to determine the best time for samples to be taken in the context of a particular gene under study. Sometimes our animal experiments are complemented by 'cell culture' and other 'test tube' analyses when we need to look at particular genes and proteins in isolation. This latter work can both confirm and support aspects of the animal work and, at the same time, reduce the need for some animal work.
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	We choose to use rats for most experiments because the brain of this species is the most completely mapped and defined of any mammalian species. We also use mice, however, because a greater range of genetic modifications is

minimise welfare costs (harms) to the animals.	possible in mice, and we can import established mouse strains, circumventing the need to further animal use in generating our own genetic model. The parallel use of two species in our studies provides a valuable ‘comparative’ approach that strengthens our experimental results and conclusions. Welfare costs in this project are generally only found during surgical procedures when anaesthesia, analgesia and aseptic procedures all conducted with veterinary advice will minimise harm.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>On the mechanisms of risk for psychiatric illness</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Schizophrenia, autism, genetics, learning disability, development</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)[3]</td><td>
<table>
<tr><td>Basic research</td><td><u>Yes</u></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>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[4]</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>Recent findings in genetics have advanced our understanding of psychiatric disorders in two important ways. First they have indicated that disorders like schizophrenia, autism and intellectual disability share genetic risk factors and are therefore likely to result from similar underlying brain abnormalities. Second, they point to an important role in these disorders for abnormalities in synapses, the structures through which brain cells communicate with each other. We will work across these diagnostic groups to understand how genetic risk factors impact on brain function and behaviour. By studying animals, as well as cells and patients, all carrying the same genetic risk factors we will be able to link abnormalities in brain function and behaviour seen in patients to abnormalities in brain circuits.</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 help to pave the way for the development of a new understanding and classification of mental disorders based on biology. Advances in biological understanding of risk for mental disorders will take us beyond current syndromal classification and, we anticipate, will lead to the development of novel approaches for the treatment of these disabling conditions.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>Species: Rats, Mice
Numbers: 5000 rats; 2500 mice
Time period: 5 years</td></tr>
</table>

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

over what period of time?	
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 procedures we will use are not anticipated to produce serious adverse effects in the animals studied. We will use behavioural methods, as well as non-invasive imaging and post-mortem electrophysiological approaches. We will also conduct molecular studies on post-mortem tissues. We will additionally study the interaction of early life events with genetic risk. If any animal shows undue distress during the study, we will consult with veterinary services and where appropriate humanely euthanase the animal.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is necessary to use animal models to study the mechanism through which genetic (and environmental) risk factors for psychiatric illness operate because: (i) we cannot directly access the relevant tissue in patients (ie the brain) AND (ii) cellular models and more basic systems cannot fully recapitulate the complexity and function of brain circuits.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use appropriate statistical and experimental approaches to optimise the number of animals used in each study. We will only generate animals when needed and will avoid excess breeding. The extensive experience of the investigators in the techniques used in this licence will also help minimise the number of animals required.
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.	Rodent species are chosen as they provide a combination of genetic flexibility, a sufficient and established behavioural repertoire and suitability for physiological/molecular investigation. Animal welfare will be maximised by close liaison with animal support and veterinary staff. In addition the experience of the investigators in these approaches will minimise harms. Notably the licence does not involve either recovery surgery or substantial severity procedures, further minimising welfare costs.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">A transgenic approach to control avian influenza in birds</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Transgenic chickens avian influenza</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)[5]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Avian influenza is a major challenge to poultry production. High pathogenicity avian influenza has an extremely high mortality rate in chickens and turkeys, where it is usually controlled by mass culling. It can also transmit to humans, where it has killed several hundred people in South East Asia and Egypt. Scientists believe it arises from low pathogenicity avian flu, which can circulate efficiently and persistently in large flocks of poultry without being detected. Low pathogenicity avian influenza can also have a significant impact on egg production in commercial layers.

Transgenic chickens have already been produced and preliminary tests indicate that they do not transmit high pathogenicity H5N1 influenza as efficiently as standard birds, providing good evidence that this is a worthwhile approach to use. New lines of birds are being produced and the aim is to test them for infection and spread of low pathogenicity avian influenza, which has not been tested before.

The transgenic chickens used in this project are hybrid layers, similar to those used for commercial egg production. The long term aim of this project, together with our collaborators is to develop new breeds of broiler and layer chickens that are resistant to influenza and other respiratory pathogens. The transgenic chickens described here are the first example of genetically modified animals with reduced susceptibility to viral infection bred in the UK. Development of this approach has the</td></tr>
</table>

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

	potential to improve animal welfare, food security and public health both in the UK and Worldwide.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The work described here will develop a project that has the long term potential to reduce the impact of avian influenza on commercial poultry and egg producers Worldwide, thus contributing to improved animal welfare and food security. It also has the potential to reduce transmission of avian influenza to humans.
What species and approximate numbers of animals do you expect to use over what period of time?	The likely maximum number of chickens to be used is 560, over a 5 year period. Animals will be chickens, some of these will be genetically modified to contain inhibitors of avian influenza, some will be standard bred for comparison. Avian influenza is species specific: chickens have been selected as they are a susceptible target species for the infection but also the ultimate target species for commercial development of transgenic livestock. Considerable time, finance and animals are required to generate transgenic lines and ultimately the target species needs to be tested prior to future commercial development of the transgenic technology.
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?	Chickens will be challenged with small volumes of avian influenza by the respiratory route, which is unlikely to cause any lasting harm but may cause temporary stress whilst being handled. Most of the chickens used in this project will be challenged by the 'natural' route, by mixing infected birds with uninfected birds in floor pens. Chickens may experience clinical signs, but low pathogenicity avian influenza usually causes subclinical infection and any signs are therefore expected to be very mild. Chickens will be euthanased by a schedule 1 method at the end of each experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The species selected is the target species for the infectious agent. Avian influenza replicates efficiently in this host and the effectiveness of the transgenic approach needs to be tested in the target species before it can be considered for commercial poultry. Influenza A viruses only infect avian and mammalian species, so this programme of work can only be carried out using one of these species

2. Reduction Explain how you will assure the use of minimum numbers of animals	To keep the number of animals required to a minimum, pilot experiments will be conducted to calculate the infectious dose of the viruses used in both standard and transgenic chickens prior to carrying out full scale challenge or transmission experiments. This will ensure that an adequate dose of virus is used and should eliminate the need for repeat experiments. Power calculations will be used to minimize the number of animals needed whilst maximising the probability of experimental success.
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 protocols involve minimally invasive procedures which are commonly applied to infection flock control in field environments. They will be performed by caring and trained staff who will acclimatise the birds to being handled prior to procedures. Animal Care and Licenced Staff have received extensive practical training from experts knowledgeable in all the procedures and species listed. The NACWO has 20 years expertise working with avian species and will guide and train others in all welfare matters for the chickens.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The immunology of inflammatory disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immunology, Human, Inflammation</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></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</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>An important cellular interaction in immunity is the recognition by white blood cells of protein antigen presented by products of the major histocompatibility complex (MHC). This interaction lies at the heart of immune mediated clinical disease. Examples include the uncontrolled immunity to self-antigens characteristic of autoimmune disease and immunity to infection. A clear understanding of the molecular interactions between the white blood cell receptors, antigen and MHC allows a better understanding of disease mechanisms and will facilitate the design of specific treatments. The central objective of this programme of research is therefore to use models of human disease to study underlying disease mechanisms and develop therapeutics that should ultimately have impact on the clinic.

Objective: (1) to study the impact of white blood cell function and immune regulation in inflammatory disease</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The potential benefits of this project are a greater understanding of the mechanisms that regulate the immune response in inflammatory diseases that will inform the development of new treatments in the clinic.</td></tr>
<tr><td>What species and</td><td>We will be working with mice and would expect to use approximately 3000 mice over 5 years.</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?	
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 mice will be bred and used to study the mechanism of action of immune responses following immunisation and/or disease induction and to attempt to modulate the immune response. Outcomes will be determined by a combination of observation, test bleed, lung function studies, imaging studies, serological and other studies of immune response. The maximal level of severity of this project is moderate. At the end, animals will either be killing by a non-Schedule 1 method at a designated establishment by exsanguination by cardiac puncture and/or removal of organs for perfusion fixation performed under terminal anaesthesia (AC) or killed by a Schedule 1 method at a designated establishment. All animals are monitored on a daily basis by CBS staff. Animals will be inspected on at least a weekly basis or more frequently if clinical signs indicate. Animals undergoing surgical interventions will be weighed at least weekly, or more frequently if clinical signs indicate. Expected adverse effects include transient pain that may occur at an injection site or breathlessness after inhalation. For mice undergoing irradiation, lung function studies, imaging studies and infection studies please refer to Appendix 2, 3, 5 and 6 respectively. For radiation studies there may be weight loss, diarhoea, increased susceptibility to infection, anaemia and bleeding. For infection studies the animals will experience either no symprtoms, mild or moderate illness. The following humane end points will be used. Mice showing breathlessness manifest by an abdominal pattern of breathing for more than 2 hours, weight loss of 20% of pre-procedure body weight, loss of appetite, immobility, or dehydration will be killed using a Schedule 1 method or by exsanguination by cardiac puncture and/or removal of organs for perfusion fixation performed under terminal anaesthesia (AC).
Application of the 3Rs	
3. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Unfortunately there are no real alternatives to the use of animals. We need to be able to study in precise detail the sequence of events in immune regulation and ascertain the pharmokinetics of therapeutics in a complex system. These studies are not possible in isolated human cells because we are studying complicated and overlapping biological pathways of immune regulation. *In vitro*

	cell lines simply don't reflect these processes in a complex biological system. It would not be ethical to carry out these studies in humans. Genetic and environmental influences often complicate human studies and inhibit the possibility of studying the impact of a single intervention. In the long term we need to determine if our therapeutic interventions are harmful, beneficial or have no impact. For all of these reasons the use of *in vivo* models is essential.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We aim to get the maximum amount of information from each experiment. For example, when studying inflammation we try to collect the following data: functional studies; total number of viable cells in the organ being studied; phenotype of infiltrating cells at each site; activation status of cells and which proteins they are making; frequency of process specific cells using tetramers; inflammation severity scores; total and specific serum responses Having extensively validated the models we can judge the impact of an experimental intervention on outcome. For example, these outcome measures can highlight the effect of a therapeutic or vaccination protocol when compared to suitable control groups.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The immune response in mice is very similar to that seen in humans. For example, in inflammation the composition of infiltrating cells, proteins produced, and disease parameters measured closely replicate those observed in man. Using murine models it is possible to study immunity in a genetically and environmentally identical system. Mice breed quickly allowing age/sex matched groups to be used to study experimental interventions and results can easily be reproduced in suitable numbers to attain biologically relevant statistical significance. Multiple disease parameters can be measured following an experimental intervention. Finally, using genetically modified lines we can study the impact of individual genes / proteins on immunity and pathology. The mouse is the lowest species in the evolutionary tree we can use to provide satisfactory and translational to human results. We always seek to minimise welfare costs to animals by doing a carefully planned programme of work with highly trained staff and clearly defined humane endpoints.

Development of novel strategies for neural repair
Spinal cord injury, peripheral nerve injury, CNS injury, neural repair, axon growth

- Summarise your project (1-2 sentences)

The prime goal of the project is to develop new treatment strategies to repair the injured nervous system and determine whether a combination of treatment strategies is more effective than a single strategy

(33 words)

- 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.

Injury to the central nervous system (CNS) results in permanent neurological deficit of affected parts. Although a variety of experimental strategies show a certain degree of effectiveness in the treatment of spinal cord injury in animal models, the results are still far from satisfactory. None of the current strategies have shown convincing improved outcome in patients. Therefore, new treatments still need to be developed, and it is likely that a combinatory treatment strategy will be more effective than any single treatment strategy.

(82 words)

- Outline the general project plan.

Injuries that mimic neural injuries in people will be performed in rodents. This is done under general anesthesia, so the animal does not suffer during the procedure. The Bioactive agents will be tested on cultured neurons to assess their effects on promoting nerve fibre growth before progress to animal studies. Potential new treatments such as the implantation of cells/biomaterials or treatment with new agents, or a combination of treatments will then be tested in vivo. Behavioural assessments will be used to evaluate their effectiveness in promoting recovery of neurological function over a period of up to 9 months. Tissue pathology will be studied to determine how the treatment modifies the injury site.

(111 words)

- 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.

Injuries that mimic neural injuries in people will be caused by cutting part of the spinal cord, part of a spinal nerve, or a nerve in a limb of a rodent. Depending on the individual study, the operation may result in moderate neurological deficits, such as weakness and loss of sensation to the limbs. The extent to which the animals will be allowed to develop impairment of movement or other symptoms following the surgery will be clearly defined and, if the clinical signs shown by the animals reach defined endpoints, they will be killed to prevent unnecessary suffering.

(98 words)

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

The information obtained should have a high value, particularly to pre-clinical scientists and clinicians working on traumatic injury to the nervous system. The programme of work will not directly deliver new treatments to the clinic, because of the limitations of modelling CNS injury in rodents. However, in combination with other studies, the data generated will contribute towards the development of effective treatments for devastating and currently untreatable injuries to the spinal cord, brain, spinal nerves and their roots.

(78 words)

- 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.

About 1000 rats and 600 mice will be used during the 5 year period. Mice and rats are the animals of lowest neurophysiological sensitivity required to achieve the scientific aims. Assessing the responses to treatments for damage to the nervous system in living animals is complex. To reduce the numbers of animals, individual experiments will generally involve factorial designs, rather than a one-thing-at-a-time approach. This will allow us to maximise the information obtained from the minimum resource.

(77 words)

- 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.

We intend to use cultured neurons to assess the neuronal growth property of candidate pro-growth molecules (in vitro methods). However, the limitations of these in vitro methods does not allow them to replace the use of experimental animals.
1) Whole animal behaviour cannot be modelled using cultured cells, so the clinical effects of the experimental treatments for neural injury cannot be assessed using in vitro methods. Only the molecules that show significant effects on nerve fibre growth in culture will be progressed to experimental animals to test their functionality in promoting nerve fibre growth and neural repair. 2) Neural regeneration occurs over a relatively long time period, which rules out the possibility of using decerebrate or terminally anaesthetized animals.
3) The pathophysiology of CNS injury is so complicated that no computer model can mimic the injury process and no such model has been built.

(147 words)

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

We shall use well-established injury models which only cut particular nerve fibre pathways without affecting other pathways of the nervous system. Therefore they cause less severe damage to the animal and less suffering.

(33 words)

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Time-dependent mechanisms in learning and memory</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Learning, memory, habituation, timing, conditioning</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</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>
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<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[10]</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">Links can form between the memories of events that occur in close temporal proximity. These links or associations influence adaptive behaviour. As yet, little is known about the how the short-term activation of memories results in learning and the formation of associations. The project will test different psychological models of adaptive behaviour and will investigate the neural substrates responsible for the behavioural adaptation.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">The project will provide information that will advance our knowledge of how learning is achieved in the brain. This is of fundamental importance for a wide range of academic disciplines such as Psychology, Neuroscience, Psychiatry, Artifical Intelligence, Ethology. In addition identifying the psychological processes and neural substrates responsible for normal cognition will aid our understanding of abnormal cognitive processes that occur in neuropsychiatric diseases.</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">It is anticipated that approximately 3000 mice and 550 rats will be used over a period of five 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 colspan="3">The majority of the work will involve behavioural studies in which the level of severity is mild. For some studies animals will receive drugs by systemic injections or will undergo surgical procedures so that substances can be administered directly into the brain. These procedures will be of a moderate level of severity. The effects of these moderate procedures will be specific to cognition and behavioural performance on learning and</td></tr>
</table>

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

	memory tasks. However, animals will require a period of time to recover from surgery before returning to a level of health at which the behavioural work can be conducted. Animals will be killed humanely at the end of the study. In some circumstances it will be necessary to collect brain tissue for analysis under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to establish the neural substrates that are necessary for learning and memory it is necessary to manipulate neural function in a manner that is not ethical nor practical in humans. Computational models, whilst useful for generating novel predictions, rely on empirical data from experiments. Therefore, although I hope that the work will lead to the development of computational models that will determine future research directions, they will not, ultimately, replace the need for the animal research proposed.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used will be kept to the minimum necessary to achieve the scientific goals by several means. First, where appropriate, with behavioural studies, manipulations of different factors will be conducted within the same animal. This will reduce the total number of animals necessary. Second, counterbalancing of non-crucial factors will rule out potential non-specific explanations of the results. This will reduce the total number of experiments necessary to reach conclusions. Third, statistical analyses have been conducted to calculate the numbers of animals necessary to avoid false negatives. Fourth, procedures will be constantly evaluated with the aim of increasing sensitivity of manipulations and measures. This will ultimately lead to decreasing the numbers of animals necessary for answering specific questions.
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.	Rodents will be used because (i) there are clear structural and functional equivalents between rodent brains and human brains. (ii) Cognitive states can be studied easily in rodents and they are the lowest vertebrate group in which the behavioural tasks have been developed. (iii) Genetically altered rodents provide a means of examining the functions of specific genes, physiological processes, and anatomical systems in cognition. The health of animals throughout all procedures will be monitored daily. Post-surgical malaise will be minimised by the use of appropriate analgesics and animals will be regularly monitored for signs of distress.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Modelling gastrointestinal inflammation and tumorigenesis in the mouse</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, intestine, oesophagus, inflammation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[11]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td></tr>
<tr><td>Regulatory use and routine production</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>No</td></tr>
<tr><td>Preservation of species</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td>yes</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>Our overall goal is to use mouse models to understand how tumours develop in the intestinal tract particularly the intestine and oesophagus. We wish to know how inflammation causes cancer in intestinal tract and how gene mutations drive the tumour growth process. This includes determining how genes interact with each other. We also aim to facilitate the development of novel anti-cancer or anti-inflammatory agents that are designed to prevent the disease occurring. We also propose to reassess existing therapies to improve their effectiveness.</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>Bowel cancer is a very common in the UK population and remains one of the three major causes of cancer related deaths. Some individuals particularly those with inflammatory bowel disease such as ulcerative colitis have a markedly increased risk of bowel cancer. Cancer also occurs in other parts of the intestinal tract such as the oesophagus and patients with oesophageal cancer have very poor survival prospects. Inflammation also plays a part in the development of this cancer. We propose that by combining studies using mice with our work on human disease we can provide major insights into how tumours develop and, importantly, produce an innovative route into the design of better therapies for cancer. We also hope to inform the design of better anti-inflammatory agents to prevent cancer in the first place.</td></tr>
<tr><td>What species and approximate numbers of</td><td>Mouse; approximately 5,000</td></tr>
</table>

animals do you expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mild and moderate severity will be expected. Animals with intestinal tumours and inflammation may show signs of pallor of limbs and ears because of rectal bleeding. If this is observed then animals will be killed immediately. However, if only pallor and no distress are seen then animals will be kept under close observation for signs of bleeding, and then be killed at first sign. Tumours may also cause intestinal obstruction resulting in anorexia and constipation, abdominal swelling and discomfort or illness. Animals will be inspected at least daily and those affected will be killed immediately and undergo post mortem. Otherwise mice will be culled at predetermined time points and tissue harvested for multiple analyses.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not yet possible to study the mechanistic complexities of cancer development in humans, cultured cell lines or reproduce events using computers alone, although these do have parts to play in completing our understanding of cancer. Understanding events in the whole organism is a prerequisite to developing new treatments and this requires experimentation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use good experimental design based on an up to date understanding of what is known. We will investigate disease in mice that best reflect the human condition and use experimental procedures according to best practice. Multiple analyses will be performed on tissues from individual animals to make best use of his resource. In addition different experiments can be run in parallel in order integrate analyses and to make best use of all animals. We will use computer-based analyses wherever possible to reduce experimental need.
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 evolutionarily lowest vertebrate group for which suitable models are available for study is the mouse. A significant amount of information is available to allow an accurate assessment of the strengths and limitations of our mouse experiments. This knowledge, coupled with an urgent medical need to improve survival rates of cancer patients, has paramount in our choice of protocols. Best practise will be at all times, which will be continuously reappraised based on our experimental outcomes and information from the 3R's newsletters, peer-reviewed literature, from animal husbandary staff and Local Ethical Review. Refinement measures also include the use of anaesthetics and pain control. In addition experiments are planned with predetermined

	endpoints that limit distress.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Physiology and pathophysiology of the auditory system</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Hearing, tinnitus, neuroscience</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 yrs</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</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></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project will study the structure and function of the auditory system in normal animals and compare these findings with those from animals in which we have induced tinnitus. We will record the activity of single nerve cells in response to sounds in anaesthetised animals, use anatomical techniques to show the expression of various neuro-chemicals in auditory structures, and map how these change in animals in which tinnitus has been induced by sound exposure or drug treatment. We will use a behavioural assay to assess the presence of tinnitus.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The project will advance our understanding of the brain pathways involved in hearing at the level of single nerve cells. It will help us to explain how sounds are encoded and represented in the brain. It will also investigate the changes that occur in these pathways in an animal model of tinnitus. The work will benefit the development of prosthetic brain implants to alleviate some forms of deafness. By gaining an understanding of the mechanisms that underlie tinnitus it will contribute to the development of treatments for a condition which has a serious impact on the quality of life of about 3% of the population.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Guinea pig 300, rat 250, mouse 100 over the lifetime of the project</td></tr>
<tr><td>In the context of what you</td><td>All animals will ultimately be used in protocols rated</td></tr>
</table>

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

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?	Non-recovery using anaesthesia throughout. Some animals will undergo other protocols first. Where animals are allowed to recover from surgery they will be regularly monitored under the direction of a vet for signs of pain or other adverse reaction. A few protocols without anaesthesia are required for behavioural testing and drug treatment in some animals. These procedures are either non- or minimally-invasive, and no adverse effects are expected. All non-terminal protocols are classified as moderate or mild severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To understand an organ as complex as the brain and its diseases there is no alternative to studying the brain itself. The use of intact animals is essential because we need to know how the brain responds to sounds. Non-invasive alternatives, such as fMRI, applied to humans are informative about where processing occurs, but they do not tell us how it is achieved at the cellular level of single nerve cells. This is essential to understand tinnitus and to identify targets for therapeutic agents. Cell culture doesn't allow access to the working sensory system, and we don't yet have sufficient information to build usefully realistic computer models. The development of animal models for the investigation of tinnitus requires that animals be exposed to the same stimuli that induce the condition in humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will obtain as much information as possible from a single animal including through continued use in different protocols where possible. In the current project we will implement electrophysiological recording of single neurons using a multiple (up to 32) electrode system. This will enable us to make simultaneous recordings from our target brain region. Fewer animals will be needed to obtain sufficient recordings to achieve statistical significance.
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 animal chosen for the majority of these studies is the guinea pig. It is an excellent model for auditory function because its sensitivity to low frequency sounds is similar to that of humans. We also need to use rats and mice for some experiments. Rats have also been used extensively in an animal model of tinnitus. The inclusion of rat and mouse will also allow us to make comparisons with studies from other laboratories that have used them, thus ensuring that our findings make the broadest possible contribution to knowledge. In all experiments the vet will decide the appropriate course of action in the best interests of the animal's welfare; including

	early termination of the experiment if deemed necessary.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Immune and vascular responses in inflammation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Inflammation; leukocyte migration; blood vessels</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>Inflammation is a key defence response to infections and injury. The inflammatory response has numerous components including the movement of white blood cells from the bloodstream into tissues and organs, leakage of proteins from the blood into surrounding tissues and the development of new blood vessels stemming from existing blood vessels.
Despite its crucial role in protecting the host, inappropriate, excessive or prolonged inflammation can also be the underlying cause of many diseases such as asthma, rheumatoid arthritis, stroke and heart disease. These conditions are often associated with the accumulation of white blood cells and inflammatory proteins in the affected tissues eg within the artery wall in heart disease, or within the joints in arthritis.
The overall objectives of this project are to obtain a better understanding of how normal inflammatory responses defend against infection and promote healing and how these processes can go wrong causing ill health. For example the project will aim to investigate how and why white blood cells are inappropriately activated to invade and ultimately damage healthy tissues and organs. Furthermore we will aim to use this knowledge to understand the mechanisms associated with particular diseases with the objective of identifying novel modes of treating patients and/or drug development.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>As the exact processes underlying inflammatory diseases are at present poorly understood current therapies are mostly aimed at alleviating the</td></tr>
</table>

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

advanced or humans or animals could benefit from the project)?	symptoms of the disease (eg steroids) and not at inhibiting the cause of the disease. Through detailed analysis of the causes of inflammatory tissue damage the findings of this project will discover novel ways of preventing detrimental inflammatory responses, eg responses that can cause conditions such as heart disease. Collectively the findings of this work could lead to better management and treatment of patients suffering from inflammatory diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use rodents eg mice and rats. Based on our extensive previous experience of using animal models, over the 5 year period of the project we estimate to use ~ 19,000 mice and ~600 rats.
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?	As blood vessels are complicated structures which cannot be accurately created in the laboratory, it is necessary to use animals to study the biology of blood vessels and how white blood cells cross blood vessel walls. We will investigate this process by applying substances to a tissue such as skin, usually through local injection, to cause an inflammatory response. This response is then analysed (normally by the use of microscopes) for white blood cells presence and changes in the structure of blood vessels in the inflamed tissue. The triggers used to cause inflammation give minimal discomfort to the animal and are often used under full anaesthesia. In order to study the role of particular genes and proteins in our inflammatory reactions, we will sometimes use genetically modified animals that lack a particular gene or protein. Any differences seen in these animals as compared to normal animals will provide information about the functions of specific genes and proteins, information that can be very useful in identifying future drug targets. Of note, none of the genetically modified animals that we use have any on-going ill health or developmental problems due to their genetic alteration. As stated above, much of our procedures are conducted on fully and terminally anaesthetised animals so they do not experience any adverse effects or discomfort. Some procedures do however involve a process (eg injection of a drug) or surgical interventions (eg tying off a large blood vessel) that are conducted under anaesthesia after which the animals are allowed to recover. Such

	procedures would be classified as having mild to moderate level of severity. The former is often carried out to test the effect of a drug locally (eg an anti-inflammatory agent) and the latter is carried out to mimic scenarios analogous to those suffered by patients experiencing a heart attack or trauma. Animals undergoing surgical procedures are potentially susceptible to developing infections. To minimise the chances of this occurring, such procedures are conducted using sterile methods, eg performed in a clean area and with the use of sterile equipment (similar to procedures used when humans are operated on). As a result we rarely note any adverse effects of our procedures, which as stated above overall can be rated as mild to moderate. Where appropriate painkillers are given to the animals post-surgery and their recovery is carefully monitored for any signs of infections. At the end of all experiments animals are humanely killed, often with an overdose of anaesthetic. The animal's blood and tissue samples are then fully examined for markers of inflammation (eg white blood cell levels) using various analytical procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our studies involve many experiments conducted in test-tubes using cultured cells, which help us to understand how individual cells function. However the findings from these studies also need to be evaluated within animal systems. This is because we cannot accurately recreate in the laboratory complex structures that are critical to the occurrence of inflammation. These include blood vessels, tissues and organs (eg lungs). Hence, in order to acquire physiologically and pathologically meaningful findings it is necessary to employ animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	As far as possible and where scientifically sound, first line studies will be performed using non-animal systems. All animal studies will incorporate carefully planned pilot experiments in order to minimise excessive or unnecessary animal use. With the increase in the size of our research group, inevitably more animals are required for the present research programme than we have needed in the past. However, by working as a team, experiments are designed such that maximal information is obtained from every experiment by multiple researchers. We will also seek statistical advice at the experimental planning stage to ensure that

	correct number of animals is used for each experiment. This ensures the use of fewer animals as well as ensuring a more coherent mode of experimentation and collaboration.
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 project seeks permission to conduct experiments on rats and mice, these being the lowest vertebrate group on which well characterised and minimal severity inflammatory response, similar to those seen in man, have been developed. Indeed there is a large body of evidence from both our work and the work of other experts in the field that rat and mouse models of inflammation have similarities to human diseases. Much of our work will be carried out on mice, the reasons being that there are many valuable genetically modified strains of mice available as well as a wide range of inflammation inducing and inhibiting drugs that can be used in mice. In addition there is substantial existing data from mouse studies both from our group and others which will be valuable to the design of future studies (ie will ensure un-necessary need for replication of works in other animals). The majority of our work involves anaesthesia without recovery, under which animals suffer no pain. However where minor procedures have to be performed on conscious animals, or when the animals recover from anaesthesia, we will routinely employ analgesia as required for pain reduction and/or employ sterile procedures to eliminate/minimise the occurrence of infections.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Zinc finger gene therapy for Huntington's Disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Gene therapy; neurodegeneration; mice; synthetic zinc finger proteins.</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)[17]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</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[18]</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>Huntington's disease is a terrible lethal brain disease with no cure. We have developed a way to switch-off the mutant gene that causes the disease. This is based on a small artificial protein, called a zinc finger, that we have designed to stick to the disease gene's DNA. After about 5 years of trials developing the off-switch in cells, we progressed to testing in mice. We were able to inject modified (safe) viruses containing the off-switch into mice that had the Huntington's disease gene. Although we managed to switch off the gene, resulting in some disease symptom improvement, the effects only lasted about two weeks. We are therefore working to improve delivery in the brain for longer times. Unfortunately, this can only be done using mice. Cell culture cannot mimic the factors in the brain which are currently stopping the treatment from working long term, such as the immune system attacking gene-therapy treated cells.</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>We want to develop a safe effective human therapy for a disease that causes a slow and unpleasant death. Huntington's disease is inherited and devastates affected families, with 100% of carriers developing the disease.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We will use two kinds of genetically modified mice that mimic the features of HD in humans. We expect to use less than 2000 mice over a period of 5 years.</td></tr>
</table>

[17] Delete Yes or No as appropriate.
[18] 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?	One of the mouse models used develops neurological symptoms such as tremoring, loss of weight and premature death. We have set a plan of careful monitoring and humane endpoints that will lead to humane killing of the animals when these symptoms reach severe harm to the animals. The other mouse model is asymptomatic. The surgical procedures and experimental approaches cause moderate distress to the animals. All the mice will be humanely killed at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no feasible alternative that would entirely replace the use of a living animal. The main scientific issue we are tackling with this project is to achieve sustained therapeutic effects. This can only be carried out in mice, because cell cultures cannot mimic the complex environment found in a living organism, with an immune system and a multicellular environment affecting the outcome of the gene therapy.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The proposed experimental designs are based in previously validated experiments by others and us, and supported by careful statistical analysis to meet the reduction criterion (a power analysis is used to calculate the minimum number of animals for statistically significant results). Before a new zinc finger design is tested in the animals, we will test it in cell cultures to select only the most promising ones to be applied to mice.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We intend to use two mouse models of HD: a transgenic mouse (carrying a the human sequence in its genome) with early-onset neurodegeneration and short life span and a knock-in mouse (carrying the mutation in the endogenous mouse gene) which mimics the molecular situation found in humans but which is asymptomatic in the early stages. These mice are commercially available, extensively characterized, and have been used successfully used in pre-clinical essays for HD rendering reproducible results which allows minimizing the number of animals required for a given protocol. The careful selection of analgesia and anaesthesia methods under the advice of the veterinaries, the monitoring of the condition of the animals with standardised protocols, the appropriate training of the experimenters and technicians in mouse handling techniques and humane endpoints will minimize the adverse effects.