Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 12

Project Titles and key words

- New mouse models of human disease

  ENU, Mutagenesis, phenotyping, Age-related

- Mechanisms of Ischaemia Reperfusion Injury in the Kidney

  Ischaemia , Reperfusion, Injury, Kidney

- The pathogenesis of autoimmune peripheral neuropathy

  Antibody, neuropathy, ganglioside

- Ecology of Norway rats in UK island habitats

  Rattus norvegicus, Manx shearwaters, radio-tracking, PIT tags, behaviouralecology.

- Mitochondria and ageing intestinal

  Ageing, mitochondria, cancer, intestine

- Cell senescence and life span in mice

  Ageing, senescence, dietary restriction

- Immune-modulators in autoimmunity and infection

  Autoimmunity, B cells, apoptotic, inflammation

- Understanding and preventing neurodegeneration

  Neurodegeneration, disease, motor neuron disease

- Function and therapeutic targeting of tumour antigens

  Cancer, Passive Immunotherapy, Active Immunotherapy

- The generalisation of high alert states

  Chickens, alarm calls, high alert states

- Regulation of V(D)J Recombination & Transcription

  Antibody genes; Leukaemia; Recombination

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Mechanisms of Ischaemia Reperfusion Injury in the Kidney</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Ischaemia Reperfusion Injury
Kidney</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>3 years</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>Ischaemia Reperfusion Injury (IRI) is a common and serious problem following kidney transplantation. There are multiple complex processed involved at the cellular and molecular level with lots of unknown interactions. Understanding these mechanisms with a view to identifying novel targets for manipulation would help to ameliorate IRI.</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>Identifying biomarkers of the extent of IRI and identifying novel molecular targets for manipulation will allow us to develop therapies that can reduce the deleterious effects of IRI. We hope that such research would be translated into clinical practice in humans.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Adult Lewis Rats.
88 number over the 3-year period.</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>The animals will undergo an operation to create the Ischaemia Reperfusion Injury to the kidneys, from which one of the adverse effects is postoperative pain. This may be of moderate severity and will be controlled with effective analgesia. The animals will be observed twice daily for signs of any distress.

After a period of up to 72 hours of observation, the rats will be terminally anaesthetised and relevant</td></tr>
</table>

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

	post mortem tissue samples will be taken.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Ischaemia Reperfusion injury involves multiple complex biological processes and unknown interactions. To date there is not a suitable non-animal model and therefore the use of animals is the only current method available for the experimental assessment of these interactions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power calculation based on previous literature and results.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The rat is a reliable subject for surgical work and its use as a model of renal IRI is well known and of widespread use. We are experienced with the surgery involved and the facilities are well established within the university to facilitate both the procedures and husbandry of the rats.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The pathogenesis of autoimmune peripheral neuropathy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Antibody, neuropathy, ganglioside</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</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[4]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project licence aims to investigate how the immune system makes antibodies to sugars, and how these antibodies damage the peripheral nervous system, leading to paralysis.</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>By first studying the mechanisms in mouse models of the disease, information can be gathered to design and test novel therapeutics and diagnostic tests for the human disease, Guillain-Barré syndrome (GBS).</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice, approx 10,000. 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>The vast majority of animals will experience few or no adverse effects. A proportion of animals will be affected by neurological dysfunction. In most cases this will be mild or moderate, and in a very small number of animals (less than 100) will be severe. Animals are all 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 use non-animal alternatives</td><td>Use of live animals is essential for studies on modelling a complex disease in which the nervous system and the immune system operate in a integrally cooperative manner. Mouse studies are needed to demonstrate and confirm</td></tr>
</table>

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

	pharmacological activity of drugs to be tested in these studies. At some stages, in order to minimise the number of live experiments, cell cultures and organ bath preparations on harvested tissues can be used. However these cannot be a complete substitute for a whole animal model which remain essential prior to introducing novel therapies into human clinical trials.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimise the number of animals used, a wide range of immunological, physiological, behavioural, and morphological measurements will be recorded throughout all studies using quantitative protocols and statistical analyses appropriate for the measurements. Because of the variable nature of the pathological response between individual animals, even within a single strain and species, animals will be studied in 2-4 groups of 3-5 animals per group, and control animals will also be set up. All quantifiable responses will be subject to statistical analysis using basic measures of mean, standard deviations and errors, and inter-group comparisons using Student t testing or other relevant statistics.
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 group in which well characterised neuropathy models are available, and in which the transgenic manipulations required can be readily conducted. To minimise animal suffering, unrestrained whole body plethysmography (breathing chambers), using non-invasive techniques will be used to record respiratory function, as we are aware that respiratory muscle weakness is an important feature of both mouse and human autoimmune neuropathy and we have recently observed altered breathing patterns in affected mice. Guidelines on current good practice will be followed. We will make use of a new transgenic mouse system that has been developed that expresses fluorescent proteins from the bioluminescent jellyfish, *Aequorea victoria* in a cell specific manner within its nervous system. This allows repeated imaging of nerves without injuring the cells by immunostaining. By using existing techniques that allow repetitive imaging of individual nerves of the mouse, under recovery anaesthesia, we can study the effects of antibody mediated injury over several days, and consider how injury influences nerve function and recovery, plus interventions.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Ecology of Norway rats in UK island habitats.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Rattus norvegicus, Manx shearwaters, radio-tracking, PIT tags, behavioural ecology.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Up to 5 years</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>~~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[6]</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>The primary aim of this research project is to gain a better understanding of the ecology of the Norway rat Rattus norvegicus in island habitats within the United Kingdom, particularly in relation to the potential impact of rats on burrow-nesting seabirds, including the Manx shearwater Puffinus puffinus.</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>Data from this project will help to inform future policy regarding rodent control on UK islands in the context of wildlife conservation, and help to refine or reduce rodent control operations on UK islands and other insular environments, potentially leading to lower risks to non-target wildlife, and better use of resources.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Norway rat; 450 – 900 animals over 3-5 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>Wild Norway (common) rats will be captured in approved cage traps, tagged (with appropriate identification or tracking devices) and released. No regular adverse effects are expected; trapped animals could occasionally be exposed to adverse weather conditions (temporary, mild suffering). Rats may occasionally die during anaesthesia (likely to be less than 1 in 100 animals), although this is not expected to cause significant suffering. At the end of the study animals will be released or humanely killed if not fit for release.</td></tr>
</table>

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

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	**There is currently no alternative method for studying the movement patterns and population ecology of wild animals other than direct or indirect observation of tagged individuals.**
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals will be determined by statistical techniques (e.g. power analysis). This will ensure that sufficient data is collected to answer the research questions without unnecessary use of 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.	T**he specific goal of this study is to investigate the ecology of introduced invasive Norway rats in relation to their impact on native ecosystems. The methods used have been developed during previous projects and have been refined to minimise adverse welfare impacts. Appropriate anaesthesia techniques will be used in order to reduce tissue irritation and reduce recovery time. Trapping protocols will aim to reduce suffering by minimising the length of time that animals are restrained, and limit exposure to adverse weather conditions as far as possible.**

Mitochondria and ageing intestinal
Ageing, mitochondria, cancer, intestine

- Summarise your project (1-2 sentences)

This project aims to analyse the molecular mechanisms underlying cellular ageing and the development and progression of colorectal cancer.

- 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.

Colorectal cancer is the second leading cause of death from cancer in the UK. Age is the biggest risk factor for colorectal cancer development. In order to develop new treatments it is important that the mechanisms underlying colorectal cancer initiation and progression are understood. A common feature of a number of types of cancer cells is that they do not use the normal pathway for energy production which is carried out within the mitochondria, small structures present in most human cell types. Cancer cells use a less efficient system called glycolysis. As we age an increasing number of normal human colon cells also utilise glycolysis as their mitochondria cannot function properly. The project aims to look at the effects of altered energy metabolism on stem cell function and to see if these non-cancer cells with altered energy production are selectively vulnerable to turning into cancer cells.

- Outline the general project plan.

We will generate a mouse which is prone to defects in energy production and in which we can genetically induce tumours at a defined time point. These animals will be allowed to age to 9 months, during this time they will suffer no adverse effects of the genetic alteration. At 9 months we will induce tumours by injection of an agent which will activate the transgene into the abdomen. Two groups of mice will be humanely killed 8 and 30 days post-injection, and one group will be humanely killed once they begin to show symptoms of disease, most commonly weight loss and anaemia. Tissues will be collected at all three time points and the number and size of the tumours quantified and compared between control and experimental animals.

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

The mice will be given up to 4 injections into the abdomen; all injection routes may cause momentary needle-stick pain. Wherever possible injection sites will be rotated, to avoid tissue damage. The animals which go on to develop disease will be humanely killed as soon as they begin to show clinical signs of disease, most commonly weight loss (the animals will be weighed at least once per week until they show any clinical symptoms whereupon they will be weighed and checked daily), and anaemia which can be readily identified as paling of the animal's paws.

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

The mouse is an essential mammalian system to study changes in the intestine which occur with age and lead to the development of colorectal cancer. We aim to generate important insights into the early stages of this disease which may lead to the identification of new targets for treatment.

- 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.

In order to obtain the control and experimental animals with the correct genotype we will have to generate approximately 1200 animals We will use 80 mice in this study, 40 experimental animals and 40 control mice. We have carried out statistical analysis to ensure the least number of animals are used to obtain meaningful results. Where animals have been bred but (unavoidably) are not the correct genotype, they will be humanely culled and wherever possible their tissues will be used for optimisation of techniques or shared with other researchers.

- 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.

In humans we do not know that a cancer is developing until it is reasonably advanced, therefore gaining understanding of the initiation event and what controls initial tumour growth is difficult in human tissues. In addition there are no robust human colon organ systems that can be grown in the laboratory which reliably model what is happening in the entire organ. In mice we can genetically induce a tumour at a defined time point and examine the cells which have turned into cancer cells at very early stages. We can also investigate rates of tumour growth by looking at animals at selected time points after the cancer has been initiated. Alongside the animal model we will use samples of normal human colon and colon tumours to look at the age-related metabolic changes which may affect stem cell function and increase their chance of becoming cancerous. We will also establish a murine organ growth system in the laboratory, which can be propagated for a number of months and stored frozen for future experiments. This will reduce the number of animal experiments in future studies.

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

As we are interested in the very early stages of cancer development, we anticipate that the majority of animals will not suffer adverse effects during the experiment. All animals will be examined comprehensively throughout their life and any mice exhibiting detrimental effects, most commonly weight loss and anaemia, will be humanely killed.

Cell senescence and life span in mice
Keywords: Ageing, senescence, dietary restriction

- Summarise your project (1-2 sentences)

We want to understand how we can slow down ageing and prolong healthy lifespan in mammals. We will test whether this is possible by limiting cellular ageing (senescence).

- 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.

When cells age, they accumulate DNA damage. This in turn switches on signals that completely change the way cells look, function and communicate with their neighbours – they become senescent. We believe this limits the ability of tissues to regenerate and to function properly such that tissues, organs and individuals become more prone to multiple diseases and death with increasing age. We want to see whether we can suppress the 'wrong' signals from senescent cells and whether this will enable mice not only to live longer but to live longer in good health with good physical capabilities and good memory function.

- Outline the general project plan.

We have already identified a number of interventions to block these 'wrong' signals from senescent cells in cell culture experiments. We will test their efficacy in mice. For many experiments we will use mice that accumulate senescent cells faster than normal (and thus also age prematurely), because this will improve the sensitivity of our analyses and shorten the time necessary to perform them. Faster accumulation of senescent cells will be achieved by either implantation of cells made senescent in culture (which will enable us to examine the communication between senescent and non-senescent cells, even within different tissues) or by genetic premature induction of senescence.

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

Most of the interventions we will try involve changing composition and/or amount of food the mice will be allowed to eat. In fact, food restriction by not more than 50% (normally 40% but allowing for variation between animals) will be used as a 'gold standard' for nutritional intervention. As this will not be started before 10 weeks of age, it will not interfere with normal development of the mice. Mice will retain their bodyweights from 10-12 weeks (or go back to values near that, if food restriction is started at a later age) as long as the treatment lasts, but will not lose further weight. While food restriction causes mild to moderate discomfort and changes behaviour (mice become more active when they expect food, but compensate that by sleeping more deeply afterwards), it is beneficial for long-term health. For instance, mice do not get obese, maintain good insulin sensitivity, get less tumours later and live longer in good general health.

We will perform surgical operations to assess the regenerative capacity of organs, especially the liver. These will be performed by trained personal. Animals will anaesthetised under the operation and will receive painkillers after the operation as long as needed. They will be humanely killed within days after the operation to assess the tissues for analysis. We will also implant senescent cells into various tissues by injection. Again, this will be performed by trained personal and animals will receive anaesthetics and/or painkillers as needed.

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

Because of the ongoing dramatic increase in human life expectancy, there are ever more older individuals around with an ever increasing risk of spending more of their later years in ill-health. Ageing is the number one common risk factor for all major diseases in the developed world, including cardiovascular disease, diabetes, dementia and cancer, because it increases the susceptibility to all and any one of these. Understanding the biological basis of ageing and the underlying principles of successful intervention are in the long run the most promising approach to get people grow older with fewer of these devastating diseases.

Recent research has made it highly probable that senescent cells contribute causally to an acceleration of the ageing process. However, it is not at all clear how this works and whether and how it will be possible to delay ageing by interfering with signals that are generated in senescent cells. We hope to answer these questions. Ideally, our experiments might identify lead targets for drugs that could in the future improve human healthspan and postpone the incidence of multiple age-related degenerative diseases.

- 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.

We use mice because they are the lowest sentient animal model suitable for the study of ageing in mammals and because the existence of gene knockouts and transgenics enables very powerful hypothesis testing. Extensive experience with the variance of the parameters we are measuring and the size of effects that we expect allows precise mathematical estimation of the minimum number of animals needed to test our hypotheses. For instance, we need to use 6 mice per experimental group to have an 80% power to detect whether an intervention changes the frequency of senescent cells by 20% (with 95% confidence).

- 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 have done extensive experiments with human and mouse cells in culture and greatly contributed to the understanding of the mechanisms of cell senescence and their possible relevance. However, ageing is immensely complex and the impact of senescent cells on ageing depends on their interaction with the host cells in a tissue and in the wider organism. We will continue to address mechanisms by using cells, which will typically be generated from tissue obtained during ear marking without additional discomfort to the animal. In a parallel project in the lab we have begun to analyse ageing in a 3D skin model, made from two different types of human cells grown in a collagen or plastic matrix. Similar models for other tissues are being developed, but it will still be a long way before these models are stable enough to allow realistic studies of ageing processes. We will continuously review these developments and use them to replace animals in our research if possible.

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

Protocols were designed to minimize suffering. All people involved are carefully trained in all procedures and are fully aware of all possibilities to minimize suffering. All relevant guidelines are being followed. When surgery is performed, mice will be under

anaesthetics and will receive painkillers afterwards. Ageing research frequently requires to keep mice until old age, when they develop age-associated diseases and syndromes (blindness, various cancers, symptoms of frailty including muscle weakness, weight loss and low physical activity). We carefully monitor the mice daily for indications that such diseases and syndromes occur. Mice will be humanely killed if we see signs of suffering even from the normal ageing process.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Immune-modulators in autoimmunity and infection</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Autoimmunity, B cells, apoptotic, 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)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td>Yes</td><td></td></tr>
</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 aim is to understand the regulatory networks of the immune system that prevents autoimmunity whilst also allowing a normal response to injury and infection.
Our major focus over the next 5 years will be to improve the understanding of the pathways controlled by these immune cells with special reference to the way in which they control both the ancient and the more recent adaptive immune system.</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>Ultimately we wish to understand why people develop a condition where the immune system starts to attack otherwise healthy tissue (called autoimmune diseases), so that we can develop better targets for treatments and ultimately a cure.
As well as understanding the way in which the immune system regulates itself, we will also be studying new candidate molecules that control inflammation. These molecules have been shown in preliminary studies to prevent arthritis. We have also discovered that a simple peptide acts as as both an antibiotic and an anti inflammatory agent. We will be studying this in more detail to see if we can develop this for use in human disease (related to both infection and inflammation).</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The human and mouse immune system is very similar and the studies that we propose cannot be replicated in simple cell based experiments in the laboratory. We expect to need to use about 14,000 mice and 100 rats over the next 5 years. The use of rats will allow us the generate antibodies that can be used in subsequent studies in mice.</td></tr>
<tr><td>In the context of what you</td><td>To understand human disease we need to study it</td></tr>
</table>

[7] Delete Yes or No as appropriate.
[8] 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?	in mouse models, which have been very well described over the years. Some animals will develop joint swelling and some may develop weakness or paralysis of their limbs. They will be very closely monitored for any signs of them approaching the expected moderate (for arthritis) or severe (for paralysis) disease severity. In addition mice that have an infection may develop shortness of breath, ruffled fur, weakness, dehydration or a hunched appearance. We will closely monitor for these adverse effects and if this happens they will be humanely put down.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Within our group the extensive use of human cells taken from volunteers has allowed us to make key observations about inflammation and infection. Throughout this programme of work that emphasis will remain. It is not feasible to produce an adequate model of the immune system and the body's response to infection in a dish in the laboratory; thus in this regard there is no substitute to live animal studies to determine immune and treatment responses in infection and autoimmunity.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Wherever possible we will conduct our experiments using immune cells isolated from the whole blood of healthy volunteers and relevant patient groups. These studies will not only guide the number and experimental design of the animal work but will replace mouse usage where possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We wish to dissect how the immune system is regulated and the responses of novel treatments (discovered in our lab following simple experiments on human cells) when autoimmunity develops. In addition we have discovered a new means to treat infection. For the reasons mentioned above, whole animal experiments are the only rational way to address our chosen questions. The mouse immune system is close to man in the vast majority of immune system features. The models of autoimmune disease we have chosen are extremely widely used and have undergone years of refinement. Neither is a perfect model for the human disease (multiple sclerosis or rheumatoid arthritis), but this does not negate their value in dissecting issues surrounding chronic inflammation and its resolution. The mouse will also allow us to study in detail the effect of single cell types in autoimmunity and the techniques that we will use are not available in any other species. We will strive to minimize the adverse effects and all mice will be carefully monitored. The models used have clear scoring systems to allow identification of early, intermediate and late stages of disease and unambiguous endpoints. Lastly it is hoped that if

	the novel treatments for infection that we are studying help the mouse it can also be used to treat human disease too.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Understanding and preventing neuro-degeneration</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neuro-degeneration, disease, motor neuron disease</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</td><td>
<table>
<tr><td>Basic research</td><td><u>Yes</u></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td><u>No</u></td></tr>
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<tr><td>Higher education or training</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</td><td><u>Yes</u></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>Diseases affecting the nervous system such as Alzheimer’s disease and motor neuron disease are some of the most debilitating and devastating conditions to affect the human population. For the vast majority of these conditions, there is no cure. This project will perform fundamental biological research to understand the processes that regulate the breakdown of the nervous system during these diseases. It will then use this information to develop new therapies ultimately designed for use in human patients.</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 ability to slow or halt the decline of patients with a wide range of neurodegenerative conditions would be of significant benefit not only to those individuals and their families, but also society in general (reducing the massive emotional and financial costs associated with these conditions).</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The project will use rodents (mainly mice, but also rats). The total number of animals to be used over 5 years is around 10,000, although the vast majority of these animals will not be subject to any procedures or develop disease symptoms (they are required to generate and maintain breeding colonies of genetically-modified animals).</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>All animals are extremely well treated, being cared for in modern, state-of-the-art housing specifically designed to provide excellent care.

The mice that develop disease symptoms, or are subjected to surgical procedures that model local injury to the nervous system, are monitored daily to</td></tr>
</table>

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

	ensure that they do not become too poorly so as to cause excessive suffering. For example, the animals will always be in a condition where they are mobile enough to have access to food and water. Any mice showing excessive signs of poor health are immediately put down via a humane procedure. All of the new treatments we are developing are designed to improve the health of the animals. All of the procedures we perform are carefully monitored and undertaken by skilled, trained individuals to ensure the minimum of suffering or distress. At the end of a study, animals are sacrificed to allow us to remove parts of the nervous system and examine the progress of disease or injury.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to study diseases processes internal to the nervous system in human patients at the cellular and molecular level. However, we need to examine neurodegenerative diseases in the context of the mammalian nervous system *in vivo*, but not in humans or higher primates. The use of mice and rats that closely mimic a human disease allows us to study all aspects of these diseases (including early events occurring before symptoms appear), and test new treatments, in a controlled environment.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our use of established animal models with known disease symptoms, alongside our experience of performing similar experiments previously, allows us to use the minimum number of animals in order to obtain robust, biologically-relevant experimental data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The welfare of animals is our top priority and we ensure that all our experiments are performed in a way that minimises the amount of disruption and distress caused to each animal. The animal models (mice and rats) we use are selected because they possess a mammalian nervous system and have been shown to accurately mimic human disease. This gives us the best possible chance of treatments developed in animals being similarly successful in human patients. For the surgical procedures we perform, we have considerable experience of minimising the suffering for all animals. For example, we routinely use the

	best possible anaesthetics and analgesics. All behavioural tests to be used are designed to minimise stress for the animals, and have been proven to generate clear data giving an accurate readout of the severity of the disease. There are no protocols with a substantial severity limit.

Function and therapeutic targeting of tumour antigens

Non-technical summary

Key words: Cancer, Passive Immunotherapy, Active Immunotherapy

Objectives
Although recent developments in cancer therapies have improved the survival rate of many cancer patients, cancer is still set to overtake heart disease as the biggest killer of adults in the Western world. Thus there is an overwhelming need for new and more effective means of treatment.
The overall aims of this project are:
1.To develop cancer immunotherapeutics, that is to develop agents that rely on the patient's immune system to control the tumour.
2. To understand the function of various components expressed by the cancer cells that may lead to the development of novel cancer therapies.

Benefits
In the short term we will be able to optimise experimental cancer immunotherapies and obtain safety data required for initiating clinical trials in cancer patients.
In the longer term we plan to develop novel therapeutic therapies that may form the basis for future clinical trials. An understanding of the function of proteins expressed by cancer cells will in the short term increase scientific knowledge and may in the longer term identify novel types of therapies.

Species
We have confined our experiments to mice, including genetically modified mice. It is estimated that we will use 5,500 mice during the 5 years if the project licence.

Outline of experiments
After testing of various antigens to determine if they induce an immune response in the mice, we plan to induce tumours usually by the transplantation of tumour cells. The aim is to see if our therapeutic approaches can control the growth of the tumours. The most severe adverse effect is defined as moderate and the experiments have been planned so that whenever possible, the mice will be humanely killed before clinical symptoms become apparent.
Studies investigating the function of proteins associated with cancer are never expected to be more than moderate in severity and the mice will be humanely killed if they show signs of distress.
At the end of the experiments the mice will be humanely killed.

Replacement: Whenever possible experiments are performed using tumour cells grown in the laboratory and immune cells taken from healthy volunteers. However, the immune system is very complex and it is impossible to reproduce this complexity in dishes in the laboratory so we need to use animal models. Moreover, testing experimental therapies in animals is an essential pre-requisite prior to initiating early stage trials in patients. Although a considerable amount of information can be obtained on the function of proteins from experiments in the laboratory, the contribution of proteins to the function of the whole organism can only be defined in animals.

Reduction: To minimise the number of mice, pilot experiments will be carried out to determine the minimal numbers of mice required to give statistically valid results, thereby minimising the number of repeat experiments required.

Whenever possible tumour growth will be monitored using technology applied to imaging patients' tumours, thus obviating the need to kill mice at defined time-points resulting in a reduction in mouse numbers.

Refinement: Mice are the only animal species used in this programme of work. Mice are less sentient than higher mammals but are still relevant to human biology. Moreover, there is a considerable amount of information in the literature that can be used as baselines and mutated and transgenic strains are available.
Pilot experiments and in vivo imaging should ensure that whenever possible mice will be humanely killed before clinical symptoms are evident. Mice will be regularly monitored and humanely killed immediately if they develop signs of distress.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">The generalisation of high alert states.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Chickens, alarm calls, high alert states</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">1</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>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[12]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">This project aims to test a hypothesis (1) that explains the existence of different forms of ‘high alert’ states. In doing so it will also test a hypothesis (2) about the different types of alarm call in chickens.

Hypothesis 1
A high alert state is one in which an animal is primed to recognise and react to a particular type of threatening stimulus (e.g. a predator). This high alert state could be very ‘specific’ – the priming is in relation to one type of predator - or ‘generalised’ – the animal is primed for any and all types of predators. Theoretical work suggests that the key factor determining a specific or generalised state is whether the response to threats is specific to each threat type or there is one generally suitable threat response. Our aim is to experimentally test this prediction.

Hypothesis 2
A significant question in the evolution of language is how particular sounds come to represent particular objects in the world. Much interest has focused on species that produce different types of alarm call in response to different kinds of predator (termed ‘functionally referential’ calls). On hearing a particular type of call, individuals in these species react appropriately for the type of predator that the alarm ‘represents’. So it’s possible that each particular call-type genuinely represents a particular type of predator. However, further work is needed to confirm this.</td></tr>
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[11] Delete Yes or No as appropriate.
[12] At least one additional purpose must be selected with this option.

	Domestic chickens produce functionally referential alarm calls: they have terrestrial predator and aerial predator alarm calls; they respond differently and appropriately depending on which type of call they hear. We want to know whether if they are alarmed by one type of predator (e.g. a fox) they subsequently worry about being attacked by other predator types (e.g. a hawk), or increase only their readiness for attack by a terrestrial predator.
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 existence of generalised vs. specific high alert states is an important field of study for several reasons: 1. It is important for understanding the behaviour and physiology of prey animals that are faced with multiple types of predators. 2. Understanding the breadth of high alert states has implications for the impact of human disturbance on protected species; prey animals may differ in how human disturbance alters their response to their 'natural' predators. 3. High alert states in non-human animals may be analogous to the emotion state of anxiety as experienced by humans. As per these high alert states, anxiety can have broad effects (e.g. phobias) or very generalised effects (generalised anxiety disorder). This project will test the basic framework that predicts that specific and generalised high alert states should exist under particular circumstances. The extensions of this framework will begin to tackle the questions above at a later date. However, this project will form a direct test of hypothesis 2, providing evidence supporting the fact that functionally referential alarm calls represent a particular class of predator.
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using 60 Sebright bantams (domestic chickens) in our experiment. These are the breed that have been used almost exclusively in experiments that look at alarm call responses in chickens.
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?	In the short-term the chickens will experience some distress from being tested in social isolation; from being exposed to videos of predators; from being exposed to a stuffed predator (for half the chickens); and from hearing other chickens' alarm calls. However, we know from previous experiments that this stress is only transitory since the chickens quickly return to normal behaviour (foraging again within 5 minutes) and show no effects of the experience in the longer term. Therefore, in the medium to long-term there should

	be absolutely no adverse effects. After this experiment the chickens will either be rehomed at another university for further behavioural studies or we will try to rehome them with hobby poultry keepers. Any chickens that cannot be rehomed will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Unfortunately we have to use animals to test our hypothesis since it relates directly to them: we are testing an idea about why animals behave in particular ways and the states that might exist in these animals. Therefore it is not possible to use a non-animal alternative in this case.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experiment has been designed to use the minimum number of required birds: we have chosen to test the birds in isolation (doing so in pairs would be less stressful for individual birds but would double the number of chickens required); we have also used a 'repeated measures' design, repeatedly using the same chickens to examine the response to each alarm call type; we have matched the sample size from previous experiments that have found similar effects.
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.	Different responses matched to different alarm call types (and hence predators) have only been documented in vertebrates so far. It is therefore not possible to use an alternative, invertebrate (or 'simpler') species. The experiment needs to be conducted in a laboratory setting, therefore domestic chickens are the best choice of available vertebrates, since they will not be unduly distressed by daily husbandry and handling. The experiments are based on previous similar experiments, so many aspects of this experiment must follow the established procotols. However, we have included a number of refinements aimed at reducing any distress caused: 1. Exposure to each stressful stimulus will always be brief; 2. Exposure to each stressful stimulus will always occur in a 'test' room, while they will be housed in a separate part of the laboratory in large aviaries. Therefore for the majority of the experiment the chickens will spend their time in a stress-free aviary environment that has no connection with the potentially distressing stimuli; 3. We will minimise the amount of time they spend in the 'test' room in general, and in particular after they are exposed to potentially stress-inducing stimuli;

	4. During all test procedures each chicken will be remotely monitored. In the unlikely event that a chicken shows signs of undue stress the experimenter will end the experiment and return the chicken to the flock in the holding aviaries.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Regulation of V(D)J Recombination & Transcription</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Antibody genes; Leukaemia; Recombination</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)[13]</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>
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<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 colspan="3">Lymphoid cancers are the fourth most common cancer with over 15,000 new cases each year in the UK alone. A frequent cause of these cancers is mistakes in the production of antibody genes. During the production of these genes, one part of the gene is selected from a large pool of gene segments and is joined to a second part of the gene from a separate large pool. The vast number of different combinations of gene segments that become joined generates genes that encode millions of different antibodies. However, because the reaction involves the breakage and rejoining of DNA, the wrong pieces of DNA can become joined and can lead to the activation of cancer-causing genes. Hence, it is important to understand what regulates the breaking and re-joining of these gene segments to then understand how the wrong pieces of DNA are selected for joining.
This project specifically aims to address:
a) how the antibody genes become activated to undergo the breakage and rejoining reaction,
b) how the parts of the genes are brought together for cutting and joining and
c) how the broken DNA becomes rejoined.
Mistakes at any of these points can lead to leukaemias and lymphomas.

In a separate reaction, by-products of the cutting and rejoining reactions have been shown to become reinserted into the genome. This can also lead to cancer by the activation of cancer-causing genes adjacent to the sites of re-</td></tr>
</table>

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

	integration. Importantly, part of the protein, RAG2, has been shown to inhibit this re-integration reaction. A second objective of this project is to map the inhibitory region of RAG2 with the longer term goal of mimicking this inhibition to develop inhibitors of the dangerous re-integration reaction.
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)?	Forty per cent of leukaemias and lymphomas appear to have arisen from mistakes in the production of antibody genes. Thus, better understanding of how the cutting and joining reaction is regulated under normal circumstances will then allow us to determine how mistakes lead to selection of the wrong pieces of DNA and therefore enable us to gain an insight into how leukaemias and lymphomas are triggered. In addition, mapping the region of RAG2 that inhibits the re-integration reaction will facilitate longer term studies to develop inhibitors of this leukaemogenic reaction.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice were chosen for these experiments since they are the only animal into which short pieces of extra DNA can be reliably introduced to perform the genetic experiments we propose. In addition, the cells that produce antibody genes have been extensively characterised in mice and thus these cells can be reliably isolated for study and we can build on extensive previous knowledge. These experiments are likely to use between 500 and 800 mice per year over a 5-year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All of these experiments involve the permanent introduction of short pieces of DNA into the genome of mice. These sequences already occur naturally in mice and the experiments only involve the introduction of extra copies of, or removal of, tiny lengths of DNA. Very similar experiments to those proposed are already underway and the mice have not been observed to suffer any adverse effects whatsoever. The mice will be bred and housed under optimal conditions until they are humanely killed prior to the use of their cells in experiments. The expected level of severity is mild since the procedures primarily involve breeding genetically modified mice where the genetic modification has not been shown to have any adverse effects on the mice.
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 some animals in this work since tissue culture systems do not exist that faithfully recapitulate the regulation of the production of antibody genes: The production of

	these genes take place in a number of sequential cell stages and in vitro systems do not exist that properly progress from one cell type to the next. Also, it is only possible to achieve the genetic modifications we need for some of these experiments by breeding differently genetically modified mice. Although we need to use mice for some experiments, we are trying to replace the use of animals in other experiments. Specifically, we have generated genetically modified mice where a protein is over-expressed in one cell type. This activates the cutting and joining reactions of one antibody locus. We aim to make a cell line from these animals to test if this will enable us to replace mice in these specific experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise the numbers of animals used by expanding cells from the animals in vitro prior to use in experiments and/or by using highly sensitive assays to detect the changes we are examining.
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 only animal with which this type of work can be routinely performed is mouse since this is the species where the technologies for insertion and deletion of small pieces of DNA are well established. Moreover, a large number of studies have characterised the production of antibody genes in the mouse. This allows us to build on existing knowledge and use existing reagents. In particular, our studies make use of a number of existing genetically modified mice. A further advantage of mice is that the cell stages where antibody genes are assembled are well characterised, thus allowing the required cells to be reproducibly isolated. None of the proposed procedures are likely to cause adverse effects to the animals; they are a continuation of existing experiments where the animals have not shown any signs of distress.