Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 36

Project Titles and key words

- Genetic etiology of cardiovascular disease

  Endothelium, inflammation, cardiovascular, genetic

- Examining the control of female meiosis

  Oocyte, Aneuploidy, Women's Health

- Perivascular drainage in Alzheimer's disease

  Alzheimer's disease, amyloid, cholesterol, apolipoprotein, aetiology

- Regulation of normal and leukemic blood cell development

  Leukaemia, Stem cells, Transplantation

- Genetics of embryonic cell differentiation in mice

  Stem cells, embryo development, placenta development

- Genetic control of antigen presentation in mouse

  Autoimmunity, diabetes, T-cells, MHC class II

- Radiopharmaceuticals for Cancer Therapy and Imaging

  Cancer, Radiotherapy, Radiopharmaceuticals, Imaging

- Characterization and inhibition of norovirus infection

  Norovirus, gastroenteritis,

- Cognition and behaviour in the normal and abnormal brain: understanding and treatment

  Cognition, behaviour, neurological disorders

- Understanding the role of signalling networks in the immune system.

  Immunology, autoimmunity, signalling

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Genetic etiology of cardiovascular disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Endothelium, inflammation, cardiovascular, genetic</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)[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>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The primary goal of the project is to investigate the genetic etiology of cardiovascular disease focusing on genetic alterations which modulate inflammation and endothelial cell biology. Cardiovascular disease is the largest cause of mortality and morbidity in Western societies, and is also emerging as a major health burden in the developing countries. New strategies to prevent or regress cardiovascular disease and reduce treatment failure must be based on rational studies of the biology of cardiovascular disease, since empirical drug trials have failed to provide solutions. In the last 20 years, much has been learnt about the mechanisms that regulate endothelial function and inflammation in vascular disease. However, there remains a pressing need to identify the key factors in cardiovascular disease for future therapeutic intervention. Furthermore, new therapeutic targets need to be identified to improve the long term outcomes of bypass graft and angioplasty/stenting procedures; as, the success of this procedures is limited by re-stenosis.

The major aim of this project is to identify new and better targets for the treatment of atherosclerosis and related cardiovascular diseases. Specifically, the objectives of the licence are:

1) To characterise the role of candidate genes in the development of cardiovascular disease e.g. mediated by inflammation, cholesterol and endothelial function
2) To identify therapeutic targets for cardiovascular disease e.g. hypertension, diabetes and atherosclerosis</td></tr>
</table>

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

	3) To characterise the interplay between known cardiovascular risk factors such as diabetes with candidate gene and novel treatments of cardiovascular disease. 4) To characterise the role of candidate genes and novel therapies in the development of pathology associated with surgical and intervention treatments of cardiovascular disease. The project builds on a national and international track-record from the previous Project that now provides the scientific foundation of the current Project application. The previous Project led to publication of more than 80 peer-reviewed scientific papers in leading international journals in the field of cardiovascular medicine. In particular, the Project identified how systemic and local changes in endothelial cell function can alter atherosclerosis progression and regression.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will advance our knowledge of factors which contribute to the initiation, progression and regression of cardiovascular diseases, and has the potential to identify new therapeutic targets for future prevention and treatment. Specifically this Project will address: (1) In the short/medium term, it will provide the necessary molecular and functional basis to determine how changes in endothelial, inflammation and cardiac function are related to disease progression in cardiovascular disease. (2) In the longer term, it will pave the way for novel pharmacologic, genetic or molecular approaches to prevent or reducing cardiovascular disease (3) The roles of endothelial dysfunction and inflammation are also central in other disease states such as transplant vasculopathy, therapeutic angiogenesis and ischaemia-reperfusion, so the results of this project should have wide application and potential benefit in increasing knowledge in other fields of cardiovascular biology, in both the short and long term. A continued clinical need for prevention and treatment of cardiovascular disease may be addressed in future by identifying new therapeutic targets through better understanding of known biological pathways, and by identifying entirely novel pathways that were not hitherto recognised to contribute to cardiovascular disease pathogeneses.
What species and approximate numbers of animals do you expect to use over what period of time?	All work will be carried out in mice. In order to ensure that the minimum numbers of animals are used in each experiments power calculations will be carried out prior to the start of the experiment to establish appropriate sample sizes to be set. For the majority of experiments a significance level of 5% with 80% power will be used

	to establish statistical significance. When possible experiments will have a factorial design to allow maximum information to be obtained for minimum input and good laboratory practice will be introduced to avoid bias such as randomisation of treatment and blinded assessment of outcomes. It is estimated that approximately 40000 animals will be used in the life time of this licence.
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 protocols on the licence are of a mild or moderate severity. It is anticipated that the vast majority of the animals on this licence will experience mild procedures, with a small number undergoing more invasive procedures. We expect adverse effects to be minimal based on our extensive experience and our continuing commitment to animal welfare and application of the 3Rs. To achieve a more detailed understanding of the genetic etiology of cardiovascular disease and to help elucidate the key cell types involved in the regulation of cardiovascular disease we will need to use genetic technologies to generate transgenic models of cardiovascular disease. A large proportion of the animals used will be used to generate models of cardiovascular disease such as atherosclerosis and hypertension. As is typical with humans it is expected that this will be asymptomatic. Disease progression may be monitored using non-invasive imaging and measurements of cardiac function which may be conducted under anaesthesia; it is expected that these procedures will result in minimal adverse effects. At the end of these experiments the animals will be humanely killed and tissues collect for biochemical and histological analysis. In a small number of animals we will use cardiovascular surgical intervention models such as bypass grafting and angioplasty to look at how genetic interventions alter the outcomes of current surgical treatments for cardiovascular disease. Some discomfort is associated with the surgical interventions. Animals will be monitored regularly and surgical discomfort will be alleviated by analgesia. At the end of these experiments all of the animals will be humanely killed and tissue collected for biochemical and histological analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cardiovascular disease is a complex interplay between metabolic and inflammatory mechanisms acting in numerous systems such as the vasculature, nervous system and the heart. Despite advancements in computer modelling, *In vitro* cell based system and the use of clinical studies in patients with cardiovascular

	disease these methods are still unable to fully model the complex biological processes in cardiovascular disease. Hence the use of animals is unavoidable if important biological questions about this condition are to be addressed. Where possible we have established cell based assays to test the role of genes implicated in cardiovascular disease and potential therapeutic strategies in place of in vivo models. We have created cell based models that have been stable or transiently transfected with our genes of interest and we routinely utilize siRNA as a method to investigate consequence of loss of function of our genes of interest. Cell lines have been useful in establishing mechanism of action e.g. assays to establish interactions between inflammatory cells and endothelial cells. However, cell-based studies cannot address the impact of our manipulations on In vivo disease initiation, progression or regression.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The majority of animals are used for breeding or in mild procedures; approximately 85% of animals. We will manage animal breeding carefully to reduce animal numbers to the minimum required for our phenotyping experiments. We hold weekly lab meeting where we critically review animal usage including the estimated need for animals in the coming months, this enables us to ensure that animal over breeding is kept to a minimum. We work as a team to ensure that maximum use of all available tissue is made from each animal. Power calculations will be carried out prior to the start of the experiment to establish appropriate sample sizes to be set
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 disease models in this licence are already well established in our laboratory and we have worked hard to optimise animal welfare per- and post-operatively. Detailed protocols have been written in collaboration with other groups who use these techniques in order to ensure best practice. We constantly look for refinements and replacements that we can adopt in our studies. This includes literature searches to check for refinements and possible replacements. Frequent communications with collaborators and other scientists to establish if they have any refinements that would be applicable in our models. We continue to develop new imaging techniques in rodents to increase sensitivity and decrease variability in our models. We are currently optimising μCT to image atherosclerosis in order to decrease the high variability associated with current quantification techniques.

Examining the control of female meisos
Oocyte, Aneuploidy, Women's Health

- Summarise your project (1-2 sentences)

  Investigation into how a healthy mature egg is created with the correct number of chromosomes and the potential to be fertilized by a sperm.

- 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.

  We want to understand how a healthy egg is produced. This has a number of facets but the most prevalent to go wrong is having the right number of chromosomes. Eggs that are made with the wrong number of chromosomes are described as being aneuploid. Such aneuploid eggs go on to form aneuploid embryos, and these are mostly non-viable and die on or before the time of implantation. It is estimated that up to 60% of ovulated eggs from women are aneuploid. This leads to infertility, early pregnancy loss and birth defects – because a few aneuploidies can result in live births. The most prominent type of aneuploidy is trisomy 21 (Down Syndrome).

  Currently we do not know why eggs should end up being aneuploid at such a high a rate. So we need to investigate this phenomenon if we are to move forward and to either develop ways of reducing aneuploidy or screening for it more effectively. Especially intriguing is how the rate of aneuploidy increases with maternal age. There is an increasing trend to have children later in life, hence the relevance of aneuploidy to human fertility is on the rise.

  Mice also show a high rise in aneuploidy as they age and are therefore an appropriate tractable system to study this area- without the ethical issues of using human oocytes. Put simply there are also too few human oocytes available for research to produce much scientific progress. Therefore in order to understand why aneuploidy happens and how a healthy egg is produced this project will investigate the process in mice.

- Outline the general project plan.

Oocytes at different stages of their maturation will be removed from euthanized mice. These mice have previously been hormonally treated in order to recover the most oocytes, and so reducing the numbers of animals needed. We will in principle investigate how normal healthy viable embryos are produced by examining the maturation and fertilization, and early embryo development of these oocytes. Mostly we will be concerned with examining for proteins and any other factors that control the segregation of chromosomes in oocytes. When this segregation is not faithful it leads to aneuploidy. There are a number of protein candidates we want to explore to determine their relevance to the process, and in particular one protein, FZR1, appears especially important in preventing aneuploidy. Although at present we don't understand why or how. We use a series of imaging techniques to examine the process of chromosome segregation and such imaging allows us to determine precisely what is going wrong and when, for all manipulations of the system we make.

- 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.

Female mice are used as a source of eggs. The protocol is very mild, and involves either 1 or two intraperitoneal injections of hormones. These hormones mimic endogenous hormones, and help follicle growth and ovulation. This simple and quick procedure ensures that we can reduce animal numbers by obtaining the most useable numbers of oocytes per mouse. The only feasible adverse effect is infection from injection, however this is minimised by only using sterile equipment and solutions.

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

Women and couples: the main benefactors in the long term are going to be women who will have an increasing control on their reproductive health. Knowledge from this project will help underpin future strategies to develop ways of improving egg health during the 40 years of reproductive life women have. Especially relevant is finding ways of reducing the effects of ageing on aneuploidy. However any strategy to do this has to be based on scientific knowledge of how aneuploidy comes about. Such knowledge will then aid in developing appropriate methods to circumvent or reverse the deleterious effects of ageing.

- 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 will use ~500 mice per year. This equates to 10 per week, or two mice per day. This equates to around 50 oocytes, which is the number of oocytes we can image at any one time. Numbers are reduced by using only hormonally treated animals, a process that increases the numbers recovered.

- 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.

Reduction: we only use mice that have been hormonally treated, so reducing the numbers we need to a minimum. Hormonally treated mice will produce more eggs than non-treated.

Refinement: we are using a priming and superovulation hormonal treatment that has been refined over the past 40 years. One of the great advantages of using mice is that this procedure is so common, it has been refined over decades to be used with the utmost effect.

Replacement: the long term goal would be to replace the use of mice. However, there are no in silico models or cell cultures that can be used. The only available source of oocytes is from the ovary.

- Explain why the protocols and the way they are carried out should involve the least suffering.
- All staff carrying out the procedure are fully trained and have personal licences that allow the procedure to be performed.
- Intraperitoneal injection is not a painful location or a time consuming location to perform injection. It is complete within less than 5seconds
- The volumes to be injected are kept very low
- All fluids to be injected are sterilised, and all equipment used is sterilised. There is minimal chance of infection
- All animals are appropriately monitored post procedure for any signs of infection. No animal is allowed to suffer from any infection should it arise.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Perivascular drainage in Alzheimer’s disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Alzheimer’s disease, amyloid, cholesterol, apolipoprotein, aetiology</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>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></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[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>Alzheimer’s disease (AD) affects more than 800,000 people in Britain and 35 million people worldwide. Old age, genetic factors and having high levels of cholesterol are risk factors for the development of AD, but it is still not understood why.

One of the pathological characteristics of AD is the build-up of β-amyloid (Aβ), a toxic protein that kills brain cells. This build-up occurs as a result of the failure of the brain’s capacity to remove Aβ. Aβ is normally removed from the brain by drainage along basement membranes that are present in the walls of blood vessels. Therefore, increased deposition of Aβ may result from changes in the health and/or structure of the basement membrane.

The objectives of this project are to understand how Aβ drains from the brain normally, how risk factors for AD affect the basement membrane and Aβ removal and to explore some new ways in which to improve Aβ removal from the brain.</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>Old age is the strongest risk factor for the development of AD. As the proportion of people over 60 years old is growing faster than any other age group, it is predicted that over 115 million people will have AD by 2050. Current therapies do not stop or reverse the disease progression.

Understanding how Aβ is removed from the brain under normal and pathological conditions is</td></tr>
</table>

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

	essential to understanding how AD develops. The findings from this project will give a better understanding of how factors such as age and cholesterol affect the efficiency of Aβ clearance from the brain. This will provide a new direction for effective preventative and therapeutic treatments for AD to be developed.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 5000 mice will be used over the 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	*1. Breeding procedures.* Tg2576 mice have increased mortality (~20%) when bred on a pure C57Bl/6 background. Therefore, Tg2576 mice will be bred onto a mixed C57SJL background, which has proved successful in preventing premature death *2. DNA genotyping.* Small samples of tissues will be used for genotyping. Ear biopsy should cause only transient discomfort and no lasting pain. *3. Feeding a modified diet.* This may result in weight loss or weight gain, lassitude and/or an increase or reduction in blood pressure. Any animals losing considerable body weight, i.e. more than 10 % body weight over a 3 day period, will be killed by a Schedule 1 method. *4. Administration of substances:* The administration of substances will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm. *5. Intracerebral injections:* Animals may experience post-operative weight loss, dehydration and/or lassitude. If animals lose more than 15% body weight or show signs of distress, they will be killed by a Schedule 1 method *6. Terminal general anaesthesia.* In the terminal phase of the procedure, animals will be insentient throughout. At the end of the experiments, animals will be terminated by Schedule 1 methods or transferred for continued use in another protocol under this or another project licence.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	The complex nature of the brain makes it difficult to study using non-living models. The rodent brain functions in many similar ways to that of the human

use non-animal alternatives	brain. Many aspects of AD can be accurately modelled using genetically altered rodents and can be used to test potential new treatments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals to be used in the project has been calculated by power analysis to provide the minimum number of mice sufficient to support robust statistical analysis by standard methods such as Analysis of Variance, Students t-test and linear regression.
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 and rats are the model organism of choice to study AD, because the structure and function of the rodent brain is similar to that of the human. Further, the mouse and rat genome can be easily used to make genetic alterations that replicate features of human AD. Rodents also breed easily, with a short generation time, facilitating multigenerational and ageing studies. Finally, protocols for rodent husbandry and health management are well established. The research procedures in the project will not exceed the Moderate severity level. To minimise suffering, all animals will be assessed daily for signs of distress or ill health. Vigilant monitoring will be done in animals following surgical procedures. Any animals exhibiting reduction in weight gain of 10 % body weight over a 3 day period, or showing signs of distress and/or pain will be killed by a Schedule 1 method. Handling will be minimised to routine husbandry and procedures required for the project.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Regulation of normal and leukemic blood cell development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Leukaemia
Stem cells
Transplantation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
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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>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>1. To better understand the normal process by which bone marrow stem cells produce mature blood cells during adult and embryonic development

2. To explore how growth “hormones” and other bone marrow factors influence the normal functioning of the bone marrow

3. To study and characterise the impact of damaged genes on the development of blood cancers

To achieve our goals we will study mechanisms of how normal stem cells survive, self-renew and generate normal blood in normal or other mouse strain models. To unravel the processes leading to blood cancers, we will either induce leukaemias in mice or inject the mice with human cells from patients. This will enable us to mimic blood cancers in mice and study in detail the disease pathways.</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>There are a number of key areas where this research will have an impact on science and might improve treatment of patients with blood diseases:

1. To help further refine and improve bone marrow transplantation approaches which are currently associated with very considerable morbidity and mortality. For example we hope to be able to specifically</td></tr>
</table>

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

	identify drug targets that can enhance the speed of recovery of different types of blood cells 2. To improve our understanding of blood cancers with particular focus on “leukaemia stem cells”; the cell type which is responsible for causing relapse in patients. 3. To improve techniques that might be used to generate blood cells from stem cells either in the laboratory or in patients with disorders of blood cells.
What species and approximate numbers of animals do you expect to use over what period of time?	39800 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The protocols in this application are all of moderate severity. The potential adverse events primarily relate to: • Immunesuppression and resulting infection • Irradiation • Surgery; laparotomy, accessing the thymus gland and kidney • Leukaemia development • Adaministration of substances We do not anticipate mortality or significant morbidity in any of the protocols at a frequency >5%. Welfare of animals at risk will be carefully and regularly checked. If some animals are in pain or exhibit other adverse effects, pain-killers or other treatments may be given under veterinary direction or humanely culled. Mouse strains showing any unexpected ill-health will be humanely culled. Most animals will be killed by a schedule 1 method at the end of the protocol, in all cases ≤15 months of age. Following identification of genetic status, genetically altered animals produced under the authority of this project and not used in other regulated procedures may be supplied to other projects with authority to use genetically altered animals of this type.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Blood formation and leukaemia development are precisely controlled processes that require the living environment, such as bone marrow. HSCs are known to interact with other bone marrow cells, and when exposed to culture (non-living conditions), they change their properties. Therefore, these processes have to be

	investigated using animals. The mouse is the most widely used system to study the formation of normal blood and blood cancers. Mouse models have demonstrated to be highly relevant and essential for development of an understanding and clinical application of the blood forming system in man, not the least application of bone marrow transplantation and understanding of leukemia since mouse and human stem cells share similar properties. Other advantages of the mouse model (apart from it being mammalian) include the availability of laboratory reagents to study blood functions. Furthermore, availability of various mouse strains allows the study how genes of interest function in the blood system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The laboratory has a number of systems in place to ensure minimal numbers of animals are used: • Limit cage numbers through weekly checks • Determining the use for animals in all cases prior to weaning • Use of appropriate number of animals in each experiment with careful experimental planning and statistical considerations to maximise the amount of information obtained from each animal e.g. serial blood sampling • Maximising yields of blood cells from each mouse for experimental use, for example, through optimal use of antibodies and nanofluidic molecular platforms developed in the laboratory allowing analysis of lower numbers of cells. • Cryopreservation to maintain smaller colonies; by collaborating with the embryo and sperm freezing service team in the institute, we maintain smaller but still healthy colonies without the risk of losing the strains to disease and any unforeseen circumstances.
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.	When undertaking work with animals to achieve our aims we have carefully chosen the least severe procedures to minimise the pain and adverse effects for the animals. Another refinement is to always ensure technical competent staff performing the procedures. To minimise adverse effects such as infections, animals will in all cases be housed in individually ventilated cages. Cages, food, water and bedding will be sterilised. Specific examples of refined procedures in the laboratory include: *Conditioning of haematopoietic cell transplant*

	recipients with split radiation dosage In order to detect the activity of the transplanted cells, the host animal's own haematopoietic system must first be depleted by irradiation, in the same way as is done with humans receiving bone marrow transplantation as a therapeutic modality. In order to minimise the morbidity and mortality associated with irradiation, a split of two half doses of irradiation, rather than a single full dose which, in association with temperature and noise monitored housing, provision of moist food and extra bedding, and rigorous monitoring has resulted in further reduced and very low levels of morbidity and mortality. *Housing of animals* We house all our animals in individually ventilated Cages (IVCs) which keep grouped animals separated from other animals and possible exposures, including exposure by air. *Training of PIL holders and staff* To ensure that these protocols are carried out to the highest standard by competent, extensively experienced individuals, a rigid, formal process of training of all PIL holders and staff is are continued to be in place. *Administration routes* Drugs and biologically active agents will be administrated by the least invasive route when multiple routes are available for example orally in water or feed rather than by intraperitoneal route in the case of tamoxifen. *Administration of substances* The types of substances used are described in the project plan. In some cases, this might involve use of an agent which has not previously been used in our laboratory where the optimal route and timing for the required experiment may not be clear. In such cases, a pilot study will be carried out to assess the feasibility and optimal protocol. For example, we have recently and successfully carried out such a pilot study for the use of cyclophosphamide (a cytotoxic drug) for haematopoietic challenge in vivo.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Genetics of embryonic cell differentiation in mice</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Stem cells, embryo development, placenta 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)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
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<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 goals are to gain new knowledge about the genetic programme that underpins the development of the single fertilized egg cell into a complex embryo comprised of many terminally differentiated cell types organized into specialized tissues and organs. The DNA of our genomes is estimated to code for 28,000 individual genes and of these about 25% are required to build a new, free living individual during pregnancy. We are seeking to understand how these genes are co-ordinately controlled and how they work with each other to direct primitive embryonic cells to become specific cell types such as muscle and blood and what signals cells respond to in their environment that cause them to form the correct cell type in the correct location within the three-dimensional architecture of the developing embryo.</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>Understanding the pathways that normally control the behaviour of cells in the embryos has many applications including understanding the basis of spontaneous fetal loss during pregnancy (in the human population between 8-20% of pregnancies result in miscarriage before 20 weeks of gestation), the causes of congenital defects as well providing insights into how these pathways go awry in the adult to result in diseases such as cancers. Our specific objectives seek to gain an indepth understanding of how one set of signalling “messenger” molecules called the TGFb growth factors control cell growth, movement and instruct cells what functions they need to fulfil in order to</td></tr>
</table>

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

	build an animal. One of the questions we are asking is how the placenta, the complicated specialized organ that supports the growth of the embryo in the uterus, is first formed then grows together with the fetus. Placental defects in the human population underlie complicated medical complications of pregnancy such as pre-eclampsia (which affects 1 in 200 pregnant women in the UK), and growth retardation of the fetus. Learning more about the fundamental basis of embryogenesis will inform the design of stem cell and other therapies to treat human disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Our experiments make use of the laboratory mouse. We generate specific genetic alterations in cultured embryonic stem cells and then transfer these alterations into the germ line of the mouse to study the effect their loss has on the development of tissues and organs in the developing embryo. Over the course of the next 5 years we anticipate using 40,000 mice and embryos. It should be emphasized that the vast majority of the mice we use are simply bred and killed for tissue and embryo harvest and not subject to any invasive procedures.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For the generation of genetically modified animals the procedures we use are for the most part mild (animals are interbred and pregnant females killed humanely to collect embryonic tissue). We use keyhole surgery to reimplant modified embryos into the reproductive tracts of females to obtain healthy offspring, a procedure classified as “moderate”. Following surgery under general anesthesia the animals are monitored very closely and any that show signs of ill-heath are euthanized. Appropriate post-operative analgesia is used for all surgical procedures. A limited number of mice (10 per year) will be injected with tumour cells. The expectation based on over 20 years of experience is that the cells will grow together as a coherent mass and will not metastasise to other part of the body. The animals will be killed before the tumour growth has any adverse affects on the well being of the animals. The genes we study are required in the embryo and adult animals carrying mutations in these genes are normal and fertile, and suffer no phenotypic symptoms. Pregnant females are killed for experiments. Males and females that have exceeded their useful reproductive lifespan and humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	There are no other alternatives available other that the use of animals to study the development of the fertilized mammalian egg into a free-living

use non-animal alternatives	organism. Since the process of embryonic development is highly complex and cannot be mimicked by in vitro systems, we have to use laboratory mice for our experiments. Where ever possible we test our hypotheses about genetic networks in mammalian cell culture systems prior to exploring their roles in the living animal. However in order to precisely unravel the complexities of embryonic development we need to work with the embryos themselves.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All of the experimental data we generate in the laboratory is subjected to review by peer scientists in the field before it is published and released into the public domain for use by other researchers. Scientific rigor requires that all of our findings are accurate and reproducible. To this end all of our experiments are carefully designed to generate data sets that are statistically valid including power calculations, while at the same time using the minimum number of animals. We breed the minimum numbers of animals at any one time and mouse strains that are not in use are archived as frozen sperm or embryos.
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.	Our experiments make use of the laboratory mouse for two reasons. First since mice are mammals, lessons learnt from their study may be applicable to humans. Second the laboratory mouse has proven to be a highly tractable genetic model organism, and techniques have been developed that allow the ready addition or deletion of genetic material. We generate specific genetic alterations in cultured embryonic stem cells and then transfer these alterations into the germ line of the mouse. By studying the phenotype of the resulting mutant embryos carrying the genetic alteration we can directly test the role of any given gene during embryonic development. We do not anticipate that any of our genetic alterations will adversely affect the post-natal animal. Every new strain we generate is very carefully monitored to ensure the genetic manipulation has no adverse side effects. Moreover all of our animals are housed under pathogen free, environmentally controlled conditions. Animals are routinely monitored for the presence of pathogens that could potentially lead to infections.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Genetic control of antigen presentation in mouse</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Autoimmunity, diabetes, T-cells, MHC class II</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[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>Our immune systems have evolved to protect us from a variety of infectious agents including bacteria, viruses and fungi. The cells that perform the function of killing infected cells and tissue of the body must be able to recognize so-termed “self” (i.e. our bodies own proteins) versus the unique “non-self” proteins present on the pathogens. However in certain situations the cells of our immune systems recognize normal tissue and attack and destroy normal healthy cells, a situation referred to as autoimmunity. Our objectives are to understand how the mechanisms that normally allow immune cells, such as T-cells, to ignore self tissues are derailed in autoimmunity.</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>Autoimmune diseases such as lupus, multiple sclerosis and diabetes affect a significant proportion of the human population. These diseases are difficult and expensive to treat. Obtaining new knowledge into the underlying genetic causes of immune system failure has the potential to advance our understanding of these diseases and hence guide future research into new treatments and therapies.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Our experiments utilize the laboratory mouse. We generate specific changes in the genes that regulate our immune systems in cultured stem cells which are then used (by our collaborators) to make new genetically altered strains of animals. Our subsequent studies of these animals allows us to understand the roles these genes play in the immune system. Over the course of the next 5 years we anticipate using a maximum of 52,500 mice.</td></tr>
</table>

[9] Delete Yes or No as appropriate.
[10] 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?	Our animals carry alterations in genes necessary for a fully functional immune system. Our facilities are extremely clean and we are careful to exclude all harmful rodent viruses and bacteria that have the potential to cause illness in the mice. In this environment having a compromised immune system does not compromise the health of the animals. For our experiments we sacrifice cohorts of age and sex-matched animals and collect tissues for analysis. At the end of their breeding life the animals are humanely sacrificed. A small proportion of our mice are from the NOD strain that provide us with a model of autoimmune diabetes. To monitor the onset of diabetes we check blood glucose levels at fortnightly intervals by collecting a single drop of blood by pricking their tail with a sterile needle. Any animals showing elevated glucose levels are humanely sacrificed before they develop any symptoms of the disease. It should be emphasized that the vast majority of the mice we use are simply bred and killed for harvesting different types of immune cells and not subject to any invasive procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to generate meaningful data on the intricate workings of the immune system we have to study intact animals. Because cells of the immune system circulate in the blood stream and migrate into organs and tissues to seek out pathogens, we cannot mimic this situation ex vivo. However whenever possible we perform experiments with tissue culture cell lines, such as immortalized B cells and T cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We employ best breeding practices. For all of our strains of GA mice we maintain a small number of breeding cages, and the minimum number of offspring to set-up new breeding pairs. In planning experiments we calculate the minimum number of animals required to produce statistically valid and reproducible data. Individual GA strains are then expanded to generate the appropriate number of age and sex matched animals for each experiment.
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 laboratory mouse provides us with a highly sophisticated model for studying the immune system. The ability to alter the genetic make-up of the mouse enables us to test our hypotheses about the way the genes that are active in cells of the immune system work together to mount an immune response. Our experiments also make use of a well described strain of mice (NOD) that are genetically highly prone to developing autoimmune diabetes. By altering the genetic make-up of these animals we have identified key genes of the immune system that contribute to development of

	the disease. By removing these genes we have reversed development of the autoimmune disease in NOD mice. For all of our experiments we carefully monitor the health of the animals. Any animals that show visible signs of ill-health or which develop high circulating glucose levels indicative of early onset of diabetes are humanely killed.

Radiopharmaceuticals for Cancer Therapy and Imaging

Cancer, Radiotherapy, Radiopharmaceuticals, Imaging

5 yrs

Basic research	Yes	~~No~~
Translational and applied research	Yes	~~No~~
Regulatory use and routine production	~~Yes~~	No
Protection of the natural environment in the interests of the health or welfare of humans or animals	~~Yes~~	No
Preservation of species	~~Yes~~	No
Higher education or training	~~Yes~~	No
Forensic enquiries	~~Yes~~	No
Maintenance of colonies of genetically altered animals[11]	~~Yes~~	No

Localised cancers can usually be treated by surgery and/or radiotherapy. Once a tumour has spread, chemotherapy is often the treatment of choice. Conventional chemotherapy drugs cause harm to normal as well as cancer cells. Cancer cells are often resistant to chemotherapy. Therefore, in the last two decades, research has turned to a more targeted approach, focused on developing treatments with improved efficacy and reduced toxicity. Molecularly-targeted radionuclide therapy is one such approach. By linking a radioactive atom to a tumour-seeking carrier, it is possible to selectively deliver radiotherapy to cancer cells. Conventional radiotherapy can only be applied to a localised tumour. However, radionuclide therapy is suitable for disseminated cancer.

Knowing the extent of a cancer (its 'stage') allows the optimum treatment to be selected. Imaging, using conventional methods such as CT scans, looks for abnormal structures in the body, but is sometimes insufficient because malignant tissue may only be seen imprecisely. A new approach is molecular imaging which uses tracers that specifically home into cancer cells – showing where they are in the body but also giving information about the nature of the cancer. Molecularly-targeted imaging tracers may be particularly useful in monitoring response to treatment, and so allow early cessation of treatment that isn't working well and/or introduction of a different treatment. This approach exemplifies the concept of 'personalised medicine' and saves patients from the toxicity of ineffective drugs. Our research concerns the synthesis and testing of new molecular imaging probes.

This project will design and test new radiopharmaceuticals (radioactive drugs) for the treatment and diagnosis of cancer. Eventually promising radiopharmaceuticals may be formulated for human use and tested in cancer patients participating in clinical trials. New molecular imaging probes will be developed. These may be used to locate and characterise cancers. They may give information about whether a cancer is responding to a particular treatment or not.

All the animals used in research under this licence will be mice. Approximately 2000 mice will be used over 5 years. This species has been chosen because of the availability of a variety of tumour cell types that can be successfully implanted into them for the growth of solid tumours that mimic human cancers.

In an effort to significantly reduce the number of animals used in these studies, we use imaging techniques such as nuclear medicine imaging (e.g. PET), MRI, and ultrasound. The advantage of these techniques is that animals can be scanned at different times to evaluate tumour size and characteristics. In this way, much data can be obtained from a

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

small number of animals.
In the context of what we propose mice will be tumour-bearing or not, and may receive anticancer radiopharmaceuticals or anticancer drugs or external radiation, and/or may have imaging performed. The expected level of severity is Moderate. The expected adverse effects that may occur are those associated with anaesthesia, surgery (used to establish some tumours), the presence of a tumour or side-effects of specific drugs or tracers. All animals will be humanely euthanized.
Where possible we grow cancer cells in the laboratory, including in 3D structures which mimic tumours. We test the effect of the new drugs on these cells. Only promising new agents are taken forward for testing in mice. Cell cultures do not faithfully reflect the complexity of tumours growing in intact animals and cannot completely replace murine models of cancer.
When first using a new tumour model, we perform pilot studies in a small number of mice to learn about the models e.g what rate it grows at. We use this information to design experiments that involve as few animals as possible but which are sure of giving us a reliable result. We have shown that repeated imaging of the same mouse over time is safe, and this means that we can obtain much information about how a tumour develops over time or responds to treatment over time -– all from a single animal.
All animals under this licence are mice. Wherever possible we use cancer models that are well-characterised with known effects. When we use a new model, we perform pilot studies in small groups of animals to confirm tolerability before proceeding to definitive investigations. We make use of use imaging to track tumour growth and to monitor the effect of anticancer agents on tumours. Animals are euthanized before tumours cause pain/distress. Animals are allowed to recover fully from one imaging session before proceeding to the next. Drugs are administered in the smallest volume as possible. For external radiation procedures, dose outside the intended field is minimised by shielding or we use a precisely-focused micro-irradiator.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Characterization and inhibition of norovirus infection</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Norovirus, gastroenteritis,</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)[12]</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[13]</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">Aim: The aim of this project is to better understand how noroviruses causes infection in the gut of the host using a mouse model system to determine which features of the virus and host are required for infection, as well as to develop potential methods of inhibiting virus replication in the host.

Background and scientific unknowns: Noroviruses are the major cause of viral gastroenteritis in the developed world and despite the significant economic impact of these viruses we know little with regards to how the viruses work. This is largely due to the fact that the viruses which infect humans do not grow well in the laboratory. However a very closely related mouse virus grows very efficiently in the laboratory and so we now plan to use this virus as a model system to identify features of the virus which allow infection in the host.</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">We expect to understand norovirus infections better and then using this knowledge to develop agents that protect against infection.

We anticipate that by changing the virus genetic sequence we can make a virus which can no longer infect and cause disease in the host but which can then be used as a vaccine to protect against subsequent infection. We also plan to develop novel therapies that can either inhibit or protect against norovirus infection</td></tr>
</table>

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

What species and approximate numbers of animals do you expect to use over what period of time?	We will exclusively use mice for our studies which vary in age from 4-8 weeks old. We have calculated that we will require up to a maximum of 6450 animals over the 5 year period of the project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals are unlikely to experience any adverse effects from infection with the virus as many studies have indicated that over 25% of laboratory mice harbour this virus with no signs of ill health. The animals may suffer mild gastrointestinal disease which should then resolve prior to establishing a long term infection. Immunocompromised mice infected with certain strains of MNV may show moderate clinical signs such as ruffled fur, hunched posture and weight loss and these will be killed if the clinical signs progress or do not resolve. At the end of the experiment all the animals will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is necessary to use animals in this study as this is the only method of determining the ability of a virus to infect and cause disease in it's natural host – in this case the mouse. We will however perform a wide range of analysis in the laboratory prior to deciding which viruses to use in the animal model. At present only mice and pigs have been used as model systems for the study of norovirus biology and of the two, the mouse model is more amenable to use in the laboratory.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal studies will only be performed after extensive characterisation of the genetically modified viruses in the laboratory. Therefore the majority of the viruses generated in the laboratory will not be analysed in animals. Where possible we will combine experimental groups e.g. using one set of animals infected as with wild-type virus for multiple experiments. The majority of our work requires the use of non-invasive sampling i.e. collection of faeces from live animals. This enables multiple sampling of individual animals over a period of time
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse model is the most appropriate as it is the natural host of murine norovirus. The ability to examine a pathogen in its natural host provides the best method to understand host-pathogen interactions. The only animal model for human norovirus is gnotibiotic pigs, which are costly and require specialist facilities. During the experiments animals will be monitored daily, they will be weighed on a regular basis – initially daily at the

	start of the experiment then weekly during the longer phase.

Project Title (max. 50 characters)	Cognition and behaviour in the normal and abnormal brain: understanding and treatment
Key Words (max. 5 words)	Cognition, behaviour, neurological disorders
Expected duration of the project (yrs)	5
Purpose of the project (as in Article 5)[14]	Basic research Yes ~~No~~ Translational and applied research Yes ~~No~~ Regulatory use and routine production ~~Yes~~ No Protection of the natural environment in the interests of the health or welfare of humans or animals ~~Yes~~ No Preservation of species ~~Yes~~ No Higher education or training ~~Yes~~ No Forensic enquiries ~~Yes~~ No Maintenance of colonies of genetically altered animals[15] Yes ~~No~~
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim of this project is to determine the functions of brain circuitry in the rat and mouse in the control of cognition and behaviour, with particular relevance to human neurological disorders and their treatment. Thus although some of our research addresses fundamental questions, it is all highly relevant to neurological and neuropsychiatric disorders—including dementia, Huntington's disease and schizophrenia—and age-related cognitive decline.

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

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By designing precise behavioural tests that are relevant to differences observed in neuropsychiatric disorders and age-related cognitive decline we can provide suitable 'models' of cognitive dysfunction in humans (e.g., object recognition memory in dementia, paired associate learning in schizophrenia and reversal learning in Huntington's Disease). This will lead to a greater understanding of the brain structures and processes involved in cognition, and the subsequent application of this understanding to neurological and neuropsychiatric disorders (e.g., via the development of pharmacological or other treatments that capitalise on the newfound mechanistic understanding).
What species and approximate numbers of animals do you expect to use over what period of time?	We use rodent behavioural models (both rats and mice). We use the minimum numbers of animals possible to achieve biologically and statistically meaningful data. We anticipate that we will use fewer than 6770 rats and 10620 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For the majority of our animals, we anticipate no more than transient discomfort and no lasting harm. In most cases, animals will undergo mild food or water restriction. Some animals will have drug treatments, some will undergo surgery, and some will have genetic modifications. The potential adverse effects of these procedures are relatively mild, and everything possible is done to minimise any adverse effects that do occur. We have specified clear endpoints, which will give us specific guidelines as to when we should humanely kill any animal who is seen to be suffering (which, again, should only happen rarely), thereby preventing any animal from suffering for more than a very short time. In the case of surgery, after the post-operative recovery period of about a week, the animals rarely will experience any adverse effects. For the majority of their lives, the animals will be participating in behavioural tasks that involve exploration of places and objects, and problem-solving for food reward: activities that are in all likelihood quite enjoyable for rats and mice.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This research is only possible with the use of animals. Human studies (e.g. brain imaging studies) are useful, but can only provide correlative data that do not address causation. Furthermore, it is not ethically possible to study the genetic and/or environmental factors that underlie neurological disorders in humans. Similarly, it would not be possible to develop new treatments for brain disorders without testing them in animal models

	first. In vitro models (e.g. brain slice preparations) or computer simulations are used in some instances but currently cannot replace animal use because the modelling of the brain and behaviour in these systems is not sufficiently advanced.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are fully committed to using the minimum number of animals required to obtain data that are statistically and biologically meaningful. We carefully design our experiments to maximise the behavioural data collected from each animal, and to minimise distress.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use rats and mice because they are the least sentient species that can model cognition and behaviour in neurological disorders. The brain circuitry implicated in many neurological disorders is highly conserved between rodents and humans, and the behavioural tasks that we have developed are widely recognised as modelling specific aspects of these disorders. We take the welfare of the animals very seriously. Most of our animals run in long-lasting behavioural experiments in which they perform tasks for food reward, and experience procedures, e.g. injections, that produce only transient discomfort and no lasting harm. Animals are monitored frequently and any adverse effects are discussed with the named veterinary surgeon. If these cannot be quickly ameliorated then animals are euthanized to prevent suffering.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Understanding the role of signalling networks in the immune system.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immunology, autoimmunity, signalling</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)[16]</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[17]</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>While significant advances have been made in our ability to understand functioning of the immune system, our knowledge of some aspects of immunity, especially at the levels of what occurs within an individual cell, is still very basic. Given the critical roles immune cells play in both fighting infection and, when not correctly regulated, in the development of many common diseases this is an important gap in our knowledge. To address this we will use genetically modified mice to understand some important unanswered questions about the function of specific proteins in the 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>Autoimmune disorders, which include rheumatoid arthritis, multiple sclerosis, lupus and vasculitis, are chronic and severely debilitating diseases. Together, autoimmune disorders affect a similar number of people as cardiovascular disease and cancer and represent a major healthcare issue. Despite advances, autoimmunity remains difficult to treat. Current therapies only arrest or slow disease progression and do not provide a cure to the underlying cause. As a result long-term or life-long treatment is required, which carries a major risk of the development of adverse side effects. In addition a significant proportion of patients do not respond to current drugs, and for some that do respond the drugs become less effective over time. There is therefore a pressing need to develop better drugs for these conditions. Autoimmunity is driven by a loss of control of cells in the immune system. In order to understand how autoimmune disease develops, we need to better understand how the immune system operates and how it goes</td></tr>
</table>

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

	wrong during the development of disease. This project aims to use genetically modified mice to understand how specific proteins in the immune system control its function. Through doing this we hope to identify new targets that can be used to develop novel drugs to treat autoimmunity. While this is a long term aim, two drug development programs have already been started based on work carried under our previous license.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will only use mice. Most of the mice will be used either for the breeding of gene targeted mouse lines or the provision of mice for the isolation of cells or tissue for further study. Up to 35000 mice will be used for this over 5 years of the project. A subset of these, in the region of 1000 to 1500, will be used in experimental protocols to examine their immune function.
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 majority of the gene-targeted lines to be used do not exhibit adverse welfare effects. A small number of the lines to be used may develop autoimmune disorders with symptoms similar to inflammatory bowel disease or lupus. The number of mice exhibiting these symptoms is likely to be less than 5% of the total number of animals used. Typically these adverse effects will occur in older animals and to minimise this mice will be used at a young age as possible. Where possible, in conjunction with the named vet, treatment programs will be used to further minimise and adverse welfare effects in these lines. Animals will be terminated by an approved method at the end of their use.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	For the majority of this work we will study cells isolated from the immune system of these mice, as this will allow us to complete much of our work without the need for experiments of the live animals. Mice will be used, as the ability to use genetically targeted mice to study the function of specific genes is essential for this work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Breeding programs will be kept to the minimum required to maintain the line and provide mice for experiments and cell isolation. Cryopreservation will be used to archive lines that are not required for on-going research. While whenever possible we will use studies on isolated cells or tissue, due to the complex nature of the immune system it will be necessary to test some of the predictions made from these studies in mice. For experimental models accepted statistical methods will be used to establish the minimum group sizes necessary for the work. In this way we will minimise the numbers of animals in which a

	direct experimental intervention is 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.	Mice will be used due to the ability to carry out gene targeting and the availability of research reagents. The majority of the mouse lines used for this project do not have apparent adverse effects on the animal's welfare. For the small number of lines were this does occur, protocols will be put in place in conjunction with the named vet in order to minimise any adverse effects. For in vivo experiments, end points with the lowest severity possible to answer the scientific questions will be selected.