Home Office

# Animals (Scientific Procedures) Act 1986

## Non-technical summaries granted during 2013

## Volume 29

## Project Titles and key words

- Regulation of breathing by hypoxia and hypercapnia

  AMPK, LKB1, hypoxia, breathing, pulmonary

- Functional magnetic resonance imaging in mice

  Functional magnetic resonance imaging, fear memory, rodents,

- Cross-talk between signalling intermediates

  Autoimmunity, cell signalling, tumours, lymphocyte biology

- Function and regulation of the immune system

  Respiratory viruses, bronchiolitis, asthma, immunology

- Multiple sclerosis, remyelination and small vessel disease

  Multiple sclerosis, remyelination, small vessel disease, oligodendrocyte

- Modulating and resolving inflammation

  Inflammation, Resolution, Cyclin-dependent kinase 9, Leucocytes

- Epigenetic regulation of gene expression

  development, aging, cancer

- Synaptic & network dysfunction in neurodegeneration

  Neurodegeneration, Alzheimer's disease, Aging, therapeutics, cognition

- Neuromuscular and neurodegenerative disorders: pathogenesis and therapy

  Motor neuron disease; peripheral nerves; muscle disorders

- The regenerative capacity of the mouse heart

  Heart, regeneration, stems cells, molecular biology

- Salmonella Virulence

  Bacterial, pathogen, virulence, immunity

| Regulation of breathing by hypoxia and hypercapnia |
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| AMPK, LKB1, hypoxia, breathing, pulmonary |

- Summarise your project (1-2 sentences)

This project aims to determine the role of two enzymes, LKB1 and AMP-activated protein kinase, in regulating blood supply to the lungs and breathing patterns, respectively, during hypoxia. These studies will advance our understanding of the mechanisms that underpin hypoxic pulmonary hypertension, sleep apnoea and sudden infant death syndrome.

- 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 aim to investigate two key processes by which our bodies adjust to changes in oxygen availability, in order to insure that we receive the oxygen we need.

At all times blood supply to our lungs is regulated to insure that the blood flows to areas of the lung that can best provide the oxygen we need. This process is called hypoxic pulmonary vasoconstriction, which drives the closure of blood vessels in areas of the lung where oxygen supply is low in order to divert blood flow to areas of the lung that are rich in oxygen. However, in disease, for example in cystic fibrosis or obstructive airways disease, hypoxia in the lung can be widespread and may thus trigger global hypoxic pulmonary vasoconstriction and pulmonary hypertension. The World Health Organisation's latest estimates suggest that survival time from pulmonary hypertension is between 3 and 6 years

The rate and depth at which we breathe is also highly regulated by oxygen supply. This is controlled by the carotid and aortic bodies, which monitor blood oxygen and signal the brain to increase the depth and rate of breathing when oxygen supply falls. Malfunction of this process has been proposed to trigger hypoventilation and central apnoeas in idiopathic sleep apnoea, altitude sickness, heart failure, preterm birth and polycystic ovary syndrome. Current therapies for such breathing disorder are poor

We will also investigate whether or not similar mechanisms regulate breathing and oxygen supply after birth and determine their role in sudden infant death syndrome (i.e. cot death).

Our studies will define the role of genes that code of two key enzymes that our previous investigations suggest may be involved in regulating breathing and blood supply to the lungs. Further work will improve our understanding of the physiology and pathology of these processes. Therefore, there is the potential to identify new drug targets which could lead to new therapeutic strategies. This is important as current therapies are poor.

- Outline the general project plan.

We will delete 3 genes, each alone and in combination, and study the effect of gene deletions on breathing patterns and blood flow to the lungs. The general project plan is to delete our target genes:

1. In the muscle cells that line blood vessels and thus determine whether the enzymes for which these genes code are required for to induce blood vessel closure during hypoxia, and thus hypoxic pulmonary vasoconstriction and the development of hypoxic pulmonary hypertension.

2. In cells that regulate the rate and depth of breathing in response to changes in oxygen supply to the body. Thereby we will determine whether or not loss of the enzymes for which the genes code trigger, for example, sleep apnoea.
3. In the cells that produce adrenaline after birth in order to trigger air breathing by the new born. Thereby we may identify the mechanisms responsible for sudden infant death syndrome.

- 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.

We will measure breathing patterns and associated brain function, and also assess blood pressure and blood flow in a low oxygen environment. We have observed no adverse side effects in previous studies. We will continue to closely monitor animals for any signs of harm, and we aim to reduce the disease severity. Therefore the majority of work will be breeding and maintenance of transgenic animals, in order to allow for post-mortem tissue collection and / or studies on animals under anaesthesia form which animals will not recover. There will be a limited number of surgical procedures from which animals will recover, but suffering will be minimised anesthesia during surgery and by post-operative analgesics. Surgery will be used to implant monitoring devices to allow for the measurement of the levels of gases of interest (e.g. oxygen) in awake animals that are behaving normally or to allow injection, in a pain free manner, into specific areas of the brain in order to determine how breathing is regulated by changes in measured gases.

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

Our studies will provide new therapeutic strategies for the treatment of sleep apnoea, sudden infant death syndrome and pulmonary hypertension. This is important because in each case current therapies are poor. For example, life expectancy for patients with pulmonary hypertension is between 3 and 6 years. Although less severe, sleep apnoea is a debilitating and progressive disease, with loss of wakefulness during the day and cognitive dysfunction, and at present the only treatment is by bedside CPAP (continuous positive airway pressure) machines.

- 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.

Approximately 6500 transgenic mice will be developed. Approximately 2000 of these will be used in experiments during the course of this project, about 500 mice per year. We are careful to use as few as possible to answer our questions, and aim to get the maximum amount of information from each animal to help us in our research. We do this by asking simple questions with cells/sections of animal tissue initially, and only progressing to live animal work for the most vital research questions that may provide for the development of new therapeutic strategies. Mice are the smallest mammals that are used to model human disease, and many drugs/therapies now in use in humans were first tested in mice and / or other rodents, giving more confidence that if they work in these animals, they may work in humans.

- 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.

Where possible we will study processes (e.g. protein-protein interaction) using cell culture techniques. However, we have to use transgenic mice because there is no non-animal

approach that will allow us to study the effect of gene deletion on breathing patterns, sleep apnoea and blood flow to the lungs and the rest of the body. The physiological systems that control these processes are complex and are arranged in three dimensional structures with many interacting cells, and we cannot model this yet in culture dishes. Only animals can help us study functions with this complexity.

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

The protocols and how they are carried out have been designed to limit suffering. Most studies are of mild to moderate severity. Those that require surgery will not include re-use of animals. We recognise that research experiments do not produce good, reliable and repeatable results if the animals involved are ill or suffering. Therefore, in order to answer important research questions, as well as for personal reasons, we are highly motivated to provide excellent care for our animals. Our experiments use very specialised equipment to limit injury, and provide for fast operations and excellent post-operative care.

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| Functional magnetic resonance imaging in mice |
| Functional magnetic resonance imaging, fear memory, rodents, |

- Summarise your project (1-2 sentences)

Functional Magnetic Resonance imaging (fMRI) is a powerful non-invasive technique that images the areas within the brain that are activated in response to a specific task, a technique that has proved invaluable in identifying abnormalities in processing in patients with psychiatric disease and cognitive decline. In this licence we wish to develop fMRI techniques that allow parallel assessments between rodents and humans, which will allow verification of animal models and assessment of potential therapeutic targets but will also advance the basic science knowledge underpinning psychiatric and memory disorders.

- 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.

The objective of this project is to develop and optimise fMRI protocols in conscious mice to determine the brain networks activated in response to emotional and other cognitive tasks. Developing such protocols will be of benefit to many clinicians and scientists interested in psychiatric and cognitive disorders. This is a non-invasive imaging technique that allows simultaneous assessment of regions of the brain activated in response to specific tasks. Furthermore, fMRI shows when activation occurs and how the activation changes over time (eg diminishes or increases) whilst the animal takes part in a cognitive task. In future studies, the technique can be used to validate animal models but also since the technique parallels that used in humans, therapeutic potential of novels drugs can be validated in animal models using a translatable test. Awake fMRI has been successful in rats in response to a learned task and the brain activation patterns resemble those seen in humans. We now wish to extend the fMRI protocols to mice, to take advantage of the plethora of genetic animal models of psychiatric disease and dementia.

- Outline the general project plan.

The essential first part of the research plan is to optimise and verify the habituation of mice to the scanner environment. Habituation will be considered successful when the mice exhibit minimal signs of stress from hormonal, heart rate and behavioural assessments. Only when the protocols are optimised will the mice be taken into the scanner. Novel cognitive tasks that involve activation of cognitive pathways associated with fear processing will be designed to identify the brain regions involved in the altered cognitive processing in animal models.

- 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 undoubtedly experience stress during habituation to the scanner environment (restraint, anaesthesia, loud noise). However, the main focus of this project will be to ensure that habituation is optimised and that consequently, stress is reduced to an absolute minimum.

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

The benefits of fMRI in mice is two-fold: First the assessment of global brain activation in response to performing a cognitive task in various animal models will further the knowledge of networks involved in specific cognitive tasks and how these are altered in animal models of psychiatric disease. Secondly, it will pave the way for future studies to assess novel therapies in a directly translatable paradigm.

- 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.

As with our previous rat fMRI project, for the habituation optimisation we will use groups of 4-5 mice. It is not possible to predict how many different habituation protocols will be tested, but we do not expect more than 4 different protocols (eg of varying restraint/noise combinations/exposure lengths). We predict 5 anesthetised mice will be used to establish the fMRI scanning parameters. We have already conducted successful fMRI studies in rats carrying out a learned task. Based on this previous work, we predict groups of 10 rodents will be the minimum needed to verify the success of imaging responses to the task. We will then perform fMRI in transgenic mice that have an over expression of the mineralocorticoid receptor in the forebrain and have been shown to have altered fear learning and memory relative to control littermates (again, 10 mice per genotype will be used). This will demonstrate the technique is sensitive enough to detect differences in brain activation during cognitive processing in transgenic models.

- 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.

It is only possible to study cognitive processing in animals or humans. And while it may be argued that fMRI in humans with anxiety disorders has greater translational value than fMRI in mice, anxiety prone humans may not be able to cope with the scanning environment (loud noises, confined space). Animal fMRI studies will aid future drug developing studies, since early stage candidate drugs cannot be administered to humans. Animal studies also enable control of many variables (such as age, genetic background, previous history of drug use) that may confound or increase unwanted variability in the data. Finally, genetically modified mice enable the study of causal risk factors for affective disorders.
Development of non-invasive fMRI techniques in rodents may contribute to a reduction in the numbers of animals used in research. For example, previous studies of neuronal activation in response to a task were carried out using electrophysiological techniques that give a snapshot of one neuronal pathway in each animal and so multiple animals would have to be used to identify the global networks. This information can now be acquired in one non-invasive scan using fMRI (though we accept that fMRI does not replace electrophysiological techniques).

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

The protocols are designed to optimise the training of the animal to the scanning environment so that they reduce stress to an absolute minimum, have the least impact on the wellbeing of the animal and hence increase the quality of the data obtained.

| Cross-talk between signalling intermediates |
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| Autoimmunity, cell signalling, tumours, lymphocyte biology |

- Summarise your project (1-2 sentences)

  The overall aim of this project is to identify molecules and cells that influence the development, function and dynamics of the immune system. We aim to gain an understanding of how an efficient and appropriate immune response to infectious organisms is mounted, how immunological memory is established, and how a state of tolerance to self tissue is maintained.

- 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.

Knowledge of the molecules and cell interactions that drive the immune system is vital for informing development and improved efficacy of vaccines. Improvements in vaccination strategy could also benefit human disease in the control of malignancies. Understanding why, in some instances, tumours are not recognised and developing strategies to prime appropriate immune responses against them would offer significant therapeutic benefit. On the other hand an understanding of why the immune system may stop being tolerant of self components resulting in the development of autoimmune diseases would be beneficial for the development of therapeutics to help with these debilitating diseases.

- Outline the general project plan.

For many years we have been interested in how cell signalling molecules influence the responses of white blood cells. Recently genetic screening studies have identified that mutations in a number of the molecules that we study are associated with several autoimmune diseases and disorders of the immune system. By making genetically altered mouse models we can mimic these mutations found in humans and ask how the immune response is affected. Our readouts are to look at the influence of these proteins on the ability of white blood cells to mount responses to infectious organisms or tumours and to initiate autoimmunity. Getting immune responses started frequently can only be done in the context of the intact animal, however subsequent detailed analysis of white blood cell responses will be further studied by culture outside the animal.

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

Most of the procedures used are mild and do not cause stress or pain to the animals. Many of our studies involve using tissues from genetically altered animals and mice are bred and maintained without further procedures and humanely killed to provide organs which we can further study in culture in the laboratory. Sometimes substances that modify the immune system are given by injection but these do not cause adverse effects. In some instances mice will be infected with organisms, like bacteria, so that we can follow immune responses, some infections may cause the animals to lose a little weight transiently but we expect few other adverse effects. In some instances we will be provoking autoimmune disease which are more substantial procedures and in the case of arthritis expect to see paw swelling, while in a model of multiple sclerosis (EAE), that we use infrequently, we expect to see partial paralysis of the tail and hind limbs. Occasionally we follow the immune response to tumours and tumours are induced by injection of tumour cells under the skin. Tumour size is monitored carefully and no adverse effects are generally seen before the experiment is terminated. Blood samples are taken from superficial vessels of conscious animals in amounts that do not harm the health and well-

being of the animal.

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

We aim to identify biochemical pathways and important molecules that can be targeted to modify immune cell behaviour. We want to be able to target pathways that reduce responses in the case of autoimmune disease or alternatively to boost responses, for example to try and fight off tumour cells or infections.

- 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.

Our experimental model uses the mouse as this is the best studied and has an immune system that is similar to that in man, numerous reagents for tracking immune responses are available, and there are advanced techniques for and availability of genetic modifications. An important part of our program requires the production and breeding of genetically modified mice that show little or no ill effect from the altered genes they carry, and form the bulk of the animals we report (max estimated usage 25,000 mice). Analyses of the effects of the genetic modifications are carried out mainly on tissue samples from the mice after they have been humanely killed. By using techniques that have been standardized over the last 20 years and group sizes that are statistically validated, the overall numbers of mice used are kept to a minimum. The numbers will be kept as low as possible by good experimental practice that reduces the need for multiple repeats of experiments, by vigilance of the breeding program, and by information gathered continually from our scientific colleagues.

- 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.

Some of the functions of immune and haematopoietic cells can, to some extent, be reproduced in cell studies; however information from such studies is limited for a number of reasons. Firstly, the cellular interactions involved in the immune system are complex and we do not understand how all the contributors interact so we cannot reproduce this complexity in culture. Secondly, we cannot study how specific genetic mutations influence the workings of a complex immune system as we cannot readily introduce mutations into genes specifically in cell lines. Wherever possible we carry out experiments in culture conditions and we aim to reproduce our findings in human cells as they are most relevant for the processes we are trying to understand.

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

The protocols have been adapted to ensure best practice and the least suffering to the animals as we are trying to recapitulate the workings of the immune response. Undue stress and/or pain would not only harm the animals but would influence how the immune system behaves and potentially provide us with a less representative picture of a normal immune system.

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| Function and regulation of the immune system |
| Respiratory viruses, bronchiolitis, asthma, immunology |

- Summarise your project (1-2 sentences)

Using mouse models of respiratory viral infections and allergic asthma, we will study the immune and inflammatory responses involved in virus induced bronchiolitis and asthma attacks. We will use this knowledge to modify these responses, in order to prevent, reduce, treat and shorten these conditions.

- 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.

Respiratory virus infections can cause a severe lung disease in infants called bronchiolitis, which can predispose to asthma, and they can trigger severe asthma attacks. There is no prevention or effective disease-modifying treatment available for bronchiolitis and most virus-induced asthma attacks. Excessive inflammation is thought to underlie both of these conditions. However, the immune mechanisms that lead to this inflammation and that underlie the increased asthma risk after viral bronchiolitis are not sufficiently understood to develop immune modifying treatments to prevent, reduce and/ or shorten excessive inflammation and consequent disease after respiratory viral infections.

- Outline the general project plan.

Mouse models of respiratory syncytial virus (RSV) bronchiolitis and of allergic asthma will be used individually and in combination to investigate immune mechanisms that lead to or prevent excessive inflammation and disease following viral infection in “normal” and “allergic” individuals. We will also investigate effects of immune modifying interventions, e.g. by depleting “harmful” immune cells with antibodies. To assess disease, lung function and body weight will be monitored. In some cases immune cells will be studied in live mice by labelling them with light emitting markers that can be used for live-imaging using an ultra-sensitive camera. In most instances immune cells, soluble immune substances and inflammation will be studied after mice have been killed assessing organ structure and inflammation by histology, counting of individual immune cell populations, and detecting immune substances and activation of their genes.

- 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.

Mice will be infected with RSV by applying droplets to the nose or the trachea under light anaesthesia. RSV infection usually does not result in disease, but in some cases can cause transient weight loss from which mice recover after 2-3 days.
In the asthma model, mice will be sensitised to an allergen usually by intraperitoneal injection followed by allergen challenges to the airways by aerosol inhalation or application of droplets to the nose or the trachea under light anaesthesia. Allergen sensitisation and challenges usually do not cause disease.

Changes in lung function (without clinical disease) can be detected after RSV infection and in the asthma model. Lung function will assessed in a chamber, in which the mouse can freely move, using minimal changes in chamber air pressure caused by breathing. Agents provoking short lasting deterioration of lung function will be aerosolised into the

chamber, resulting in short lasting discomfort, as experienced by patients during similar lung function measurements. In some mice lung function will also be measured invasively under deep surgical anaesthesia without recovery.

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

This project will advance science by increasing our understanding of how immune cells and immune substances work to cause lung inflammation in viral infection and asthma. This will provide targets and ideas for future development of immune based therapy and prevention of viral bronchiolitis and virus-induced asthma attacks.

- 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 up to 20,000 mice over the 5 year project. Although the disease processes are not completely identical to those in man, mice offer the best available model system in which a variety of relevant immunological, genetic and molecular tools are available to study viral and allergic inflammatory lung disease.
All experiments are designed to use the minimum number of animals to give statistically significant results or to obtain sufficient numbers of immune cells for ex-vivo analysis. To that end all groups and controls of an experiment will be run in parallel, all organs of interest will be used simultaneously from each individual and animals will be identified individually. Where appropriate we will use statistical tests to calculate the number of animals we will require based on how variable we expect the results of our studies to be and how big a difference we are looking for between groups. Where such calculations are not possible, e.g. in experiments to generate primed immune cells or organs for histology, we will use our previous experience with similar experiment and published data to determine minimal numbers of mice required.

- 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.

The mouse models outlined above are an integral part of my research programme, which uses laboratory based experimentation and clinical studies in humans wherever possible. We will assess lung secretions from infants ventilated due to viral bronchiolitis for immune cells. In parallel, we will study effects of respiratory viral infections on human immune cells generated *in-vitro.*
However, there is currently no laboratory based system available that allows us to study the complexity of immune interactions, within and between different organs, and the lung function changes in inflammatory lung disease induced by respiratory viruses and allergen sensitisation. We therefore have to use animal models of disease.

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| Multiple sclerosis, remyelination and small vessel disease |
| Multiple sclerosis, remyelination, small vessel disease, oligodendrocyte |

- Summarise your project (1-2 sentences)

This project seeks to find ways of improving repair of damaged brain in diseases such as multiple sclerosis.

- 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 aim to understand why the brain repairs poorly in diseases such as multiple sclerosis and other similar diseases, so we can design therapies to try and improve repair. This is important as there are absolutely no therapies which are effective for stopping the dying back of nerves in the brain in neurodegenerative diseases. So, patients with progressive multiple sclerosis or similar diseases become more disabled, and we cannot even yet slow this process down, let alone stop or reverse it. Nerves are covered with a substance called myelin, which has a similar role to the insulation on wires, protecting the underlying nerve. This is damaged in diseases such as multiple sclerosis, and there is evidence that if this is repaired, nerves are protected from dying back, reducing disability. This project aims to understand this repair of myelin and improve it.

  Another disease with brain changes similar to multiple sclerosis, but occurring mostly in older people is called “small vessel diseasewhich can cause memory problems. It is not understood why this happens, and what causes the disease. Without this understanding, it is difficult to design therapies to treat it. This project aims to increase understanding of this disease in order to find ways to improve it.

  Therefore, our project is to try and understand the processes of brain damage and repair in both multiple sclerosis and small vessel disease, and to design therapies to combat them.

- Outline the general project plan.

We aim to:
1) understand how repairing cells recognise damaged parts of the brain, how they travel to these areas and how they then replace the protective covering of nerves (myelin).

2) manipulate some of the molecules that we already know are involved in the repair pathway to try and improve how repairing cells reach areas of damaged brain, with the idea of developing drugs and therapies to do this better.

3) discover new signals involved in the pathway, especially in the recruitment of repairing cells to areas of damage.

4) understand how interactions between nerves, repairing cells, immune cells and blood vessels cause a type of neurodegenerative disease called small vessel disease.

- 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.

We will use animal tissue to obtain cells and sections of brain to grow in dishes to study how repairing cells of the brain function, and how we can improve how efficiently they can carry out repair. To understand neurodegenerative diseases better, we will also use live models of these diseases, to try and understand what causes the damage and how it is repaired, and to test possible therapies to improve the diseases. This involves using genetically modified rats and mice, and causing small areas of damage to parts of the brain by neurosurgical operations to mimic the problems found in these diseases in humans. These problems include weakness in a limb or blindness in one eye, which we then study and treat to try and improve repair. Some animals have more than one operation – first to cause the small area of damage and then to add a therapy to try and improve it. Some genetically modified rodents develop signs of brain disease spontaneously, causing problems with mobility, and again we then try and improve the course of their disease by encouraging brain repair, by adding therapies given by neurosurgical operations, injections or in the food. These animals are closely monitored for signs of harm, with the use of strict humane endpoints, and we aim to improve repair, and reduce the disease severity.

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

There are currently no therapies that are effective for neurodegenerative diseases such as the progressive stages of multiple sclerosis. By increasing understanding of the way that nerves die back in these diseases, and finding ways to repair the damage, we will start to fill a completely unmet need. Therapies to aid repair in the brain aim to reduce disability from these diseases. Work such as this from our lab in the last 5 years has produced two new targets for drugs to help repair, and these are now in the process of being developed in collaboration with pharmaceutical companies to make drugs suitable for testing in humans.

- 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.

To do this work, we will use about 500 zebrafish, mice and rats per year. We are careful to use as few as possible to answer our questions, and aim to get the maximum amount of information from each animal to help us in our research. We do this by asking simple questions with cells/sections of animal tissue initially, and only progressing to live animal work for the most promising possible therapies for disease and most vital research questions. Zebrafish are very useful for studying repair as they regenerate damaged areas very effectively, and we can try and understand why it is more limited in rodents and humans, and use this information to help repair work more efficiently. Rodents are the smallest mammals that are used to model human disease, and many drugs/therapies now in use in humans were first tested in rodents, giving more confidence that if they work in these animals, they may work in humans.

- 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 try and use human tissue where possible to study brain repair – using donated post

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| mortem tissue and also generating brain cells from stem cells in a dish. This technology is new but will be very powerful to test research questions and possible therapies. However, the brain is a three dimensional structure with many interacting cells, and we cannot model this yet in a dish, and so have to turn to animals to help with this complexity. |
| • Explain why the protocols and the way they are carried out should involve the least suffering.<br><br>Research experiments do not produce good, reliable and repeatable results if the animals involved are ill or suffering. Therefore, in order to answer important research questions, as well as for personal reasons, we are highly motivated to provide excellent care for our animals. Our experiments use much specialised equipment to ensure small areas of damage in the brain, fast operations and excellent post-operative care. |

| Modulating and resolving inflammation |
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| Inflammation, Resolution, Cyclin-dependent kinase 9, Leucocytes |

- Summarise your project (1-2 sentences)

We have identified an important pathway that fundamentally regulates inflammatory processes by controlling new protein synthesis. In this project, we will investigate, using several approaches including the use of new potential anti-cancer therapeutics, that modify this key pathway, to confirm and expand our knowledge of its role in inflammation with the idea to develop new, much needed, anti-inflammatory agents for the treatment of acute and chronic inflammatory diseases.

- 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.

Disordered inflammation is responsible for most of the diseases afflicting western society, including hardening of the arteries (causing heart attacks, stroke and peripheral vascular disease), lung diseases (including chronic bronchitis and asthma), crippling rheumatoid and osteoarthritis, and the commonest forms of liver and kidney disease that necessitate transplantation. Despite the huge burden of illness, loss of work and ultimately death, there is yet no effective drug therapy for most of these conditions. Previous approaches to drug development have been targeted at interfering at single points within a hugely complex process that involves thousands of mediators, many of which duplicate and triplicate each other's effects. With continuous research over the last twenty years, we have been taking an entirely different approach. While some forms of inflammation (pneumonia and acute gout, for example) cause dramatic inflammatory diseases, they have the capacity to resolve completely with no residual damage to surrounding tissues. Starting from this observation we have been studying how these conditions resolve in order to generate new therapies for the vast majority of inflammatory diseases that fail to resolve 'in the fashion nature intended' and therefore result in chronic inflammation and ill-health. Our major early discovery was that certain damaging white blood cells need to undergo a form of 'silent suicide' and be quietly removed by local scavenger cells in order for inflammation to resolve effectively. We spent several years working out the detailed mechanisms of this process in order to attempt to harness them for therapeutic gain. In the 'test-tube' we found many ways of 'driving' these suicide and clearing-up processes, but whenever we tried to reproduce the results in mouse models of human disease the beneficial effects were counteracted by the presence of powerful cell-survival factors in live tissues. The 'breakthrough' came when we showed that agents that not only drove the cell suicide process in the presence of survival factors in the test tube, but greatly accelerated the rate of resolution in mouse lung models of human inflammatory/scarring diseases. In the past four years, we have homed in on the specific molecules involved in this exciting discovery in order to build a strong scientific foundation for better-targeted, less toxic new drugs. We have now clearly identified a molecular pathway for close attention in our new research programme, and shown that new therapeutic agents under development for cancer treatment not only drive cell suicide but also cause the 'scavenger' cells to release anti-inflammatory agents, thus adding to the drug's potential therapeutic benefit.

- Outline the general project plan.

We will investigate this pathway mainly using isolated human inflammatory cells and

zebrafish larvae but will also determine its role in acute mouse (lung, skin and peritoneal) and chronic mouse (lung and cardiovascular) and chronic zebrafish (high fat diet induced) inflammatory models.

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

Most of the procedures are already well characterised and will cause mild to moderate inflammation. We predict that mild form of inflammation induced in the models will be equivalent to the symptoms induced by the common cold or mild skin irritation.

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

There is a much-needed unmet clinical requirement for new therapeutics for the treatment and reduction of symptoms associated with inflammatory diseases both in human and animals. We predict that our research will confirm and expand our understanding of an under-investigated pathway that, we have identified, controls key inflammatory processes and identify new drugs for the treatment of inflammatory 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.

Guided by our human cell work we envision the use of approximately 3-500 mice per year and a total of 1000 zebrafish over the course of the project. Mice have been chosen because we can use genetically modified animals in a variety of disease contexts and the inflammatory models are well characterised. Many of our experiments will use zebrafish larvae (up to 5 day) and are therefore not subject to Home Office regulations; these experiments will guide and thereby reduce the number of adult fish that we plan to use. Animal numbers will be minimised by ensuring correct statistical planning of experiments, gaining as many “readouts” from a single animal as possible and where possible replacing animal experiments with “test-tube” experiments using human cells.

- 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.

The majority of our work is performed using isolated human inflammatory cells in a test tube or on pre-five day old zebrafish larvae. These studies have and will continue to help us comply with the 3Rs. However, the complex responses seen in inflammatory conditions cannot be completely mimicked *in vitro* and hence we have chosen to perform some of our experiments using mainly mild models of mouse and zebrafish inflammation.

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

The majority of experimental protocols outlined are already well-established within our Institute and therefore the amounts of inducing agents needed and clinical and biological responses already known. We are therefore able to minimise suffering through the use of well-defined anaesthetic and treatment protocols and regular observation of animals.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Epigenetic regulation of gene expression</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">development, aging, cancer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[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 colspan="3">All mammalian cells contain the same genetic information encoding for ~25,000 genes. However, only specific subsets of these genes are active in specialized tissues. How gene activity is controlled and determined during development and how it goes wrong during aging and cancer is still poorly understood.<br>This project aims to investigate the function of two specific proteins (LSH and G9a) in the control of gene activity during development, tissue maintenance and cancer progression.</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">Inappropriate regulation of gene expression leads to variety of human conditions including congenital disease, premature aging and cancer. Better understanding of how specific proteins function to control gene activity and what the consequences of their deficiency are may benefit the design of novel therapies and drugs for more efficient treatment of cancer and other human disorders.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">Mice, 1510 a year</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td colspan="3">The protocols associated with this project do not exceed moderate level of severity and most animals used in the project are expected to have a normal healthy life. However, we will use animals carrying genotypes that predispose them to cancer and premature aging. In these cases, the animals will be monitored by daily observation of their</td></tr>
</table>

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

| | |
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| | clinical condition and early humane endpoints will be implemented to minimise suffering. A small minority of animals will undergo surgery using optimised anaesthetic protocols and ensuring provision of analgesia, as advised by the NVS, to ensure prompt recovery and minimise post-operative pain. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | Embryonic development and cancer are processes which require interactions between different cells in an embryo or in a tissue. Therefore development and cancer formation that cannot be studied without the use of animals. However, whenever possible we will isolate cells from genetically modified animals and tissues for further more detailed experiments that do not require animals. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | We will use robust statistical methods and the published literature to estimate the number of animals required for each experiment so that the data obtained is statistically significant. When working in a setting where the outcome cannot be predicted, we will undertake a small scale pilot experiments first to estimate the number of animals required for further experimentation. In all cases, we will ensure that we use appropriate experimental set up to generate statistically significant data in order to prevent using more animals than absolutely necessary. |
| **3. Refinement**<br>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 will use mice for our experiments for the following reasons:<br>(i) Mouse development is well understood and extensively studied. Therefore reliable protocols and guidelines are available. Genetically modified animals developing adverse phenotypes will be humanely killed before the symptoms exceed specified level of severity (moderate).<br><br>(ii) Mouse strains carrying genetic mutations predisposing to cancer have been established and are available from designated suppliers. We will closely monitor the animals that are likely to develop tumours and will adhere to the UKCCCR "Guidelines for the Welfare and Use of Animals in Cancer Research" (Workman et al., 2010). |

| Synaptic & network dysfunction in neurodegeneration |
| --- |
| Neurodegeneration, Alzheimer's disease, Aging, therapeutics, cognition |

- Summarise your project (1-2 sentences)

The project will examine how neurodegenerative disease, related neurological diseases and aging affect the functioning of the brain. We will investigate pathological changes at the level of single cells and at the level of complex networks of interconnect brain cells.

[Word count 40]

- 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.

Alzheimer's disease (AD) is a devastating neurodegenerative disorder that affects millions of people world-wide, for which there are currently no effective treatments. For over a century, two pathological lesions - neurofibrillary tangles and senile plaques – have been used to define AD pathologically but the relationship of these pathologies to dementia remains elusive. Here we will investigate how the proteins associated with plaques and tangles affect the functioning of specific brain cells, and disrupt communication in networks of brain cells. Such disruptions in the networks could underlie the cognitive deficits seen in the earliest stages of AD. Understanding these pathological changes will provide a new avenue for developing therapeutics.

[Word count 108]

- Outline the general project plan.

The project will make use of mice that have been genetically engineered to exhibit symptoms of AD. Colonies of these mutant mice will be bred onsite for use in experiments. The activity in the brains of the mice will be studied in various ways. We will sacrifice the mice and cut slices of brain tissue for recording of electrical activity (which can be done under special conditions for several hours after sacrifice) and for studying structural changes. We will also implant electrodes in the brains of living animals to allow us to record the how the pathology affects the activity in the networks of the intact brain. We will study the effects of drugs treatments on the functioning of networks of neurons. Finally changing the proteins made within the brains of the animals using genetic methods, will allow us to identify particular proteins or particular cells that are linked with the pathology.
[Word count 152]

- 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 majority of mice will be sacrificed by approved humane methods and no adverse effects are expected.

Brain surgery (to implant electrodes and/or deliver agents that change proteins) will be carried out under general anaesthesia and using approved aseptic techniques. In a small minority of animals, wounds may become infected. Veterinary advice will be sought immediately.

Once the electrodes are implanted and animals have recovered from surgery, the electrical activity will be recorded in freely moving animals by wireless transmitters. Animals will be housed in cages that reduce the likelihood of headstages becoming trapped, however, mechanical headstage damage may occur in a minority of animals.

Drug treatments will be administered to living animals by approved means (eg. through food/water, orally, by injection etc). Drugs will be administered at doses comparable to those used in humans to reduce side effects. However, animals will be monitored for signs of ill health and adverse responses during drug treatment.

[Word count 155]

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

  The findings of this work will advance our understanding of the causes of AD. Understanding how particular cells in the brain are affected provides a means of developing selective therapeutics. Moreover, an understanding of the changes in how brain cells communicate and the way brain networks function, could allow us to understand the changes to cognitive function seen in AD.

[Word count 60]

- 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 estimate that approximately 11000 mice will be used in the project over 5 years. This total includes experimental animals (~7000) as well as animals for the breeding colony. The experiments will compare transgenic mice that have exhibit AD-like pathology, to normal mice. In addition, other strains of transgenic mice will also be used to study how the particular cell types are affected by the pathology

These transgenic mouse lines recapitulate Alzheimer's disease age-related brain pathology that cannot be done without a living brain. Plaques and tangles similar to those in the human brain cannot be produced by cells in culture. Neither can cultured cells form the complex networks that are involved in memory and cognition affected in the disease

Animal numbers will be based on the minimum number of animals needed for statistical significance of findings. Where necessary, we will consult with statisticians to determine this number.

[Word count 147]

- 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.

  The project will investigate the functioning of brain cells within the intact brain, and how the brain changes with age. Non-animal alternatives cannot provide information on how brain cells in the intact networks of the brain function and communicate.

  The data generated by this project will form the basis for computer models that will be used to investigate how disease affects the brain.

[Word count 63]

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

  Protocols will be carried out in the most humane way possible. All surgery will be performed under general anaesthesia and pain relief will be administered during recovery to minimise distress. Protocols to monitor brain activity in living animals make use of wireless transmitters to reduce distress that may occur from cables limiting movement.
  Animals wills be housed in groups and cages will be enriched with tubes and objects for exploration. These methods have been shown to reduce stress in mice.

  [Word count 80]

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Neuromuscular and neurodegenerative disorders: pathogenesis and therapy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Motor neuron disease; peripheral nerves; muscle disorders</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>Yes</td><td></td></tr>
</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 research is focused on the pathophysiology of neuromuscular disorders, in particular Motor Neuron Diseases (MND), as well as disorders of nerves, such as peripheral neuropathy, and muscle disorders such as Inclusion Body Myositis. All of these debilitating disorders have a significant impact on quality of life of the affected individuals, many of them are fatal, and currently almost all of these diseases remain largely untreatable.<br><br>Despite many years of research our understanding of the underlying mechanisms of these diseases, our understanding of the underlying pathological mechanisms remains poor and there is still no cure or effective treatment for these diseases. The development of an effective therapy therefore remains an imperative in the field.<br><br>This project aims to i) advance our understanding of the pathological mechanisms that play a role in these disorders in order to ii) identify, develop and test novel therapeutic strategies that will be effective in modifying disease progression in these 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>We hope to improve our understanding of the mechanisms that play a role in these diseases which should help identify new targets for the development of novel drugs and therapeutic strategies.</td></tr>
</table>

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

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| What species and approximate numbers of animals do you expect to use over what period of time? | We will mainly use mice, but sometimes rats, often genetically modified to model aspects of disease. We expect to use a maximum of 29000 mice (including all genetically modified mice bred) and 1,900 rats. |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | Based on our experience, we expect to have very few adverse events, which are likely to be limited to mild discomfort after surgery, but more commonly deficits in locomotion associated with a neuromuscular disease phenotype, typically restricted to hindlimbs. The likely level of severity is moderate. At the end of these experiments, all animals will be humanely culled. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | Neurodegenerative and neuromuscular diseases involve complex interactions between different cells in the CNS as well as interactions in the periphery, in nerves and muscles. Furthermore, many of these disorders manifest late in life, on a background of aging. It is therefore difficult to model both the complexity of cellular interactions and aging in cells in culture. Where possible we do indeed use such cultures, to both model disease and to test new therapeutic agents. We will therefore continue to use cultured primary cells where possible rather than in vivo animal experiments |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | All of the in vivo experiments are based on our experience of similar work undertaken in our laboratory over the past 20 years, and have been calculated in consultation with statisticians. We have also taken several measures to reduce animal use wherever possible, and for example we routinely share tissues from individual animals so that different tissues from an individual animal can be used to support experiments by several researchers. This approach ensures that we keep our animal use to a minimum. |
| **3. Refinement**<br>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. | Most of the work will be undertaken in mice. The mouse is unique in that it is relatively easy to create mutations in any gene of interest and therefore dissect the mechanisms involved in specific diseases, and to generate models of disease in which to test new therapeutic approaches. Genetic. Furthermore, we have a good understanding of the mouse nervous system and behaviour, which enables us to build up the whole picture of disease pathology and disease-relevant changes. Our group also has a large body of background data on the normal functions and behaviour of the neuromuscular system, which has been largely gathered from mice, and which therefore significantly reduces the total numbers of animals that need to be used in this study. Finally, the |

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| | ultimate aim of our research program is to relate our findings back to human disease, and therefore we must work with a mammalian system. Thus, for all these reasons, rodents, and in particular the mouse, is the most appropriate species to use. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The regenerative capacity of the mouse heart</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Heart, regeneration, stem cells, molecular biology</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To investigate gene regulation (epigenetic mechanisms) in the heart to determine candidate genes for functional studies.<br>To modify the epigenetic machinery, in vitro and in vivo, in relation to its role in heart regeneration after heart attack.<br>To identify genes for drug targeting in association with heart attack and failure.</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 techniques used will increase understanding of the regulatory processes underlying cardiac development in health and disease. Subsequently, we will elucidate the molecular and functional basis of mouse cardiac cell differentiation and regeneration, which will form the foundation for understanding the pathology of human heart disease, and methods for treating heart attack and failure.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We plan to use approximately 6500 mice and 50 rabbits over the course of 5 years.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>Genetic engineering:<br>-Female mice will be used for egg production by hormone injection. After collection of eggs/embryos, the mice are culled (mild severity).<br>-Female mice will have embryos implanted to develop to birth or adulthood. Following birth or weaning, mothers will be culled (moderate severity). Pain will be controlled with appropriate medication. In the case of uncontrolled pain, or surgical complications, or general health deterioration, the animal will be culled.</td></tr>
</table>

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

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| | -Stud males may undergo vasectomy for the purpose of pairing with females to induce a false pregnant state. The vasectomy will require surgical anaesthesia. Adverse effects are similar to those stated above for such a procedure. Animals will be culled at 18 months (moderate severity).<br><br>Breeding and maintenance of genetically modified mice (mild severity):<br>-The health and viability of the animals is likely to depend on the type of genetic modification. Mice will be monitored for abnormal disease characteristics and appropriate control measures implemented. Tissue samples in order to establish the genetically modified status will be taken in the appropriate method of least severity. Animals, prior to or showing signs at the limits of health, will be monitored or culled.<br><br>Cardiovascular and metabolic studies. The following procedures will be carried out on mice in this study. Listed are their expected adverse effects (severe):<br>-Surgically opening of the chest - Collapsed lung, internal bleeding, ruptured suture, infection;<br>-Insertion of telemetric device, under the skin or into the body of the animal or minipump drug delivery- Internal bleeding, ruptured suture, infection, blocking of a vein/artery, nerve damage;<br>-Non-invasive imaging – reduction in body temperature;<br>-Ultrasound guided injection to the heart - Internal bleeding;<br>-Dietary modification – Obesity, failure to eat;<br>-Metabolic measurements - Fighting on regrouping;<br>-Heart attack/induction of enlargement of the heart - Heart failure.<br>-Modification of gene regulatory mechanisms may be associated with decline in fertility.<br>Animals will be culled following each study, or where several procedures will be carried out on one animal, the animal will be culled at the appropriate end point.<br><br>Preparation of anti-sera to investigate gene product (protein):<br>-This will involve bleeding and a course of immunisation. The immunising solution may cause inflammation of an abscess at the injection site. This procedure is classified as mild.<br><br>Culling of neonatal mice:<br>-Removing the heads of the mice with a sharp scalpel is the most humane was of culling the |

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| | neonates. This is classified as mild in severity. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | The power of rodent genetics gained through controlled breeding, availability of inbred strains, and their relative homogeneity compared to human disease characteristics, lends rodent studies a unique route to defining mechanisms of gene action unattainable through human disease gene study. Animals also offer better systems for studying mechanisms of action of genes, which can only be fully appreciated by investigating multiple physiological systems affecting whole body characteristics and cannot be replicated with cells alone. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | In order to minimise the number of mice to be used throughout the project, mice will be used only after preliminary studies have confirmed a likely role in the characteristics of interest.<br>Some procedures will be combined where possible, and the most refined techniques available will be used to reduce animal numbers through improved accuracy of measurements. |
| **3. Refinement**<br>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 is favoured for these cardiovascular studies due its longstanding use as a genetic and epigenetic-drug model.<br><br>The use of genetically modified mice for physiological investigations is a powerful tool for providing novel insights into mammalian physiology and for identifying genes relevant to human disease. Hence, the use of genetically modified mouse models is currently the most appropriate way to undertake the physiological analyses in the proposed work.<br><br>For *in vivo* studies, experiments will be designed to reduce the number of procedures used sequentially and to maximise recovery time between procedures. Where surgery is involved, aseptic techniques and antibiotics will be used to reduce the risk of infection. Analgesics will also be given to reduce pain and discomfort.<br><br>When inducing disease features such as heart attack the surgical procedure will be refined to reduce adversely affecting animals as a consequence of the surgery. Animals will be observed for wellbeing, but minimally disturbed during the follow-up period (3-5 weeks), following all surgical procedures.<br><br>Blood pressure measurements may be taken continuously *in vivo* using implantable telemetry transmitters, which will allow the animals' free |

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| | movement in their cages. For invasive blood pressure and ECG recording animals will be anaesthetised.<br><br>Mice undergoing experimental procedures will be monitored for signs of distress or ill health and action taken as appropriate to alleviate any distress or to improve health. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Salmonella Virulence</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Bacterial, pathogen, virulence, immunity</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes√</td><td>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[8]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>In this project we will study mechanisms by which Salmonella causes disease and how our immune system works to resist this. Infection of humans with Salmonella can lead to gastroenteritis and typhoid fever, depending on the strain type. It is estimated that over 90 million cases of Salmonella gastroenteritis and 13 million cases of typhoid fever (with approximately 130,000 deaths) occur globally each year. There is no vaccine against non-typhoidal Salmonella and current typhoid vaccines provide only partial protective efficacy.<br>Infection of humans with Salmonella Typhimurium usually results in self-limiting gastroenteritis but this strain causes a systemic disease in mice that is similar to human typhoid fever. This very useful model system has been exploited intensively over the years and has provided a great deal of information of the pathophysiology of the infection process, the basis of host defence and immunity, and bacterial virulence factors involved. Much of this information is known to be relevant to human disease and has been exploited in the design of a novel vaccine against typhoid fever, which has been shown to be safe and immunogenic in clinical trials in humans.<br>A large part of Salmonella pathogenesis is associated with its ability to grow inside host cells, and we make extensive use of cell lines of human and mouse origin to study the biochemistry and cell biology of infection. However, these systems can never fully represent the complex environment and host response to infection that occurs in vivo. For example, an essential aspect of the host immune</td></tr>
</table>

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

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| | response involves communication between different cell types and their activation. These interactions and responses are highly coordinated in time and space, involve cellular migration and occur both within and between different tissues. Therefore, some work on living mice is unavoidable to properly assess potential virulence defects, bacterial population dynamics and spread throughout organs as well as immune and other cellular responses. We will study the effects of deleting genes of *Salmonella* with respect to its ability to multiply and spread in mouse tissues. We will study the immune responses of mice to these strains, taking advantage of mouse strains that are already available and which have known immune defects. We will also exploit state of the art imaging techniques to be able to follow the infectious process over time in a non-invasive manner. Animal suffering will be minimised by regular checking of animals for relevant symptoms that constitute the end point of the experiment, and the use of mixed infections to eliminate mouse-to mouse variability and hence the number of animals required to achieve statistical significance. |
| 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)? | Through this research we are likely to discover new processes of pathogen and host cell biology, which could have implications for other important pathogens that propagate within our cells. Our work is also likely to provide valuable information for designing vaccines, which are still needed to provide effective long-term protection against *Salmonella* and other bacterial pathogens. |
| What species and approximate numbers of animals do you expect to use over what period of time? | Approximately 6000 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? | Depending on the dose, the inoculation route and the strain of mouse, between 2 and 180 days after inoculation the majority of animals inoculated with *Salmonella* will develop mild to moderate symptoms of systemic infection consisting of reduced activity, hunched posture and ruffled fur. We will assess animals for these symptoms and those that display all three symptoms will constitute the end point of the experiment and will be killed humanely. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | Our aim is to understand the pathogenesis of *Salmonella* (a bacterial pathogen which causes a wide variety of diseases in humans and other animals) and to learn more about host processes that influence the outcome of infection. An essential |

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| | aspect of this work involves testing the pathogenic potential of different bacterial strains in the well-established murine model of infection. This helps us to establish the importance of genes that play critical roles in the pathogenic process and provides information about their interactions and possible use in vaccine design. Much of our work involves experiments in which *Salmonella* grows in immortalized host cell lines. However, these systems can never fully represent the complex environment and host response to infection that occurs *in vivo.* For example, an essential aspect of the host immune response involves communication between different cell types and their activation. These interactions and responses are highly coordinated in time and space, involve cellular migration and occur both within and between different tissues. Therefore, some work on living mice is unavoidable to properly assess potential virulence defects, and immune and other cellular responses. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | All experiments will be designed so that the minimum number of animals necessary will be used. Breeding strategies are performed by qualified personnel and aimed to avoid unnecessary animal generation (animal surplus). A competitive index assay has been designed to evaluate the virulence of mutants in which a 1 to 1 mixture of wild type to mutant bacteria is inoculated into each animal. Because of the lack of inter-animal variation, significant differences in the virulence of strains can be obtained using fewer animals. Bioimaging will reduce the numbers of animals used due to serial imaging. |
| **3. Refinement**<br>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 most appropriate species in which to model systemic infection by Salmonella. The mouse model is especially appropriate as it enables (1) measurement of bacterial virulence by enumeration of colony forming units of bacteria at different time points and from different organs following inoculation by the oral, intraperitoneal or intravenous routes, (2) population dynamics of the pathogen to be analysed by new methodologies involving mathematical modelling and (3) whole body imaging of the process of disease progression using live animals. This form of imaging will reduce the numbers of animals used due to serial imaging, and refine the procedure by allowing infection to be monitored more closely. Animal suffering will be minimised by regular checking of animals for relevant symptoms that constitute the end point of the experiment, and the use of mixed infections to |

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| | eliminate mouse-to mouse variability and hence the number of animals required to achieve statistical significance. |