Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 22

Project Titles and key words

- Cell signalling in immunity, infection and cancer

  Cell, signalling, Immunity, infection, cancer

- Novel treatments for metabolic diseases

  Metabolic disease, diabetes, obesity

- Brain Control of Peripheral Glucose and Energy Metabolism

  Diabetes, Hypoglycaemia, Brain

- Manipulation of Sodium and Chloride Ions to Treat Abnormal Epithelial Secretion

  Respiratory disease airway bio-marker

- Cardioprotection

  Cardioprotection; Ischaemia; heart

- Role of AMPK in cancer and in glycogen homeostasis

  cancer, glycogen, protein kinase

- Encoding of reward prediction

  attention, cortex, motivation, striatum, reward

- Mouse Rederivation and Cryopreservation

  Cryopreservation rederivation

- Reduction of cardiac ischaemia/reperfusion injury

  Heart; drug development; regulatory; ischaemia

- Identifying cytoskeletal regulators of metastasis.

  Cancer, invasion, cytoskeleton

- microRNAs in CNS vasculature and barrier function

  microRNA, blood-brain barrier, neuro- inflammation, aging

Cell signalling in immunity, infection and cancer

Cell, signalling, Immunity, infection, cancer

Summarise your project (1-2 sentences)

This project will help elucidate the role of the PI3Ks, a family of enzymes, in cells of the immune system.

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 immune system affects our health in many ways. First and foremost, the immune system provides an adaptable defence against pathogens. Vaccines help boost the immune defence against particular pathogens. Increasingly, efforts are also made to harness the immune system to fight cancers. In some individuals, however, the immune system attacks different tissues or organs and causes autoimmune and inflammatory diseases such as arthritis, lupus, asthma and diabetes. This project is focused on a family of enzymes called the PI3Ks. These are enzymes that generate messenger molecules inside cells. By blocking these enzymes, cells will not respond as they normally would to cues from the outside. We will explore how we can manipulate these enzymes to enhance immunity to infection and cancer while preventing harmful immune responses to tissue antigens and harmless environmental antigen (eg allergens).

Outline the general project plan.

The project is based on measuring different types of immune responses to pathogens, tumours and self antigens in mice with genetic or drug-induced alterations in the PI3Ks and other genes that complement or synergise with the PI3Ks.

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 mice breed under this protocol will be killed humanely and their tissues used for experiments in the lab. About 25% of the mice will be challenged with a pathogen, a tumour or a vaccine and the resultant immune response measured by various means (eg by drawing blood or analysing cell in different tissues obtained from the mouse after it has been killed at the end of an experiment.

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

It is of fundamental biological interest to understand how the PI3Ks work and how they have diversified and adapted through evolution to carry out cellular functions required by complex multicellular animals, such as humans and other mammals.

The most immediate practical benefit is that the research will guide the development and use of drugs that inhibit or modify PI3K signalling to treat cancers, inflammation and autoimmune diseases.

Secondly, many patients have mutations in different components of the PI3K signalling pathways. By modelling how these mutations affect health and disease at the cellular level, we may be able to treat such patients better.

Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

About 40,000 mice will be used over a five year period. The immune system in mice is similar enough to the immune system in humans that valuable parallels can be drawn. The availability of different genetically modified mice and reagents that recognise mouse cells means that this species can be used more efficiently than any other species to ask questions about the role of particular genes in the immune system. The breeding of the mice will be planned and monitored carefully to ensure that we only produce the mice needed for experiments. The majority of the mice will be used to provide immune organs harvested for lab-based assays. Other mice will be immunised, infected or will be challenged with tumour cells, and the results immune response monitored. In each case, the lowest number of mice that produce robust reproducible (statistically significant) results will be used.

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.

Non-animal studies will be used when possible. This may involve drawing blood from human donors or culturing immortalised cell lines. However, these approaches are limited in that they do not allow for accurate modelling of the interactions by diverse cell types within anatomical tissues during immune cell development, homeostasis and activation. In addition, cells adapt to culture conditions and use different genes and pathways than what is found in manipulated cell cultures.

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

Each study involves a progression of steps, from lab-based assays that characterise specific proteins, to the generation of appropriate mouse models, harvesting of tissues from these for lab-based studies. Only once these have shown the potential for novel and important discoveries are mice subjected to immunological challenge by vaccines, pathogens or tumours. Where a potential drug target has been identified, then mice autoimmune or inflammatory diseases will be generated with the aim to treat or alleviate the causes and symptoms.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Novel treatments for metabolic diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Metabolic disease, diabetes, obesity</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)[1]</td><td colspan="3"><table>
<tr><td>Basic research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></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><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td><s>Yes</s></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Identification of novel agents for the treatment of human metabolic disease including diabetes; identification of novel mechanisms involved in these diseases which can be targeted.

According to the World Health Organisation (WHO) there are 346 million people with diabetes worldwide in 2011, and in 2004, 3.4 million people died as a consequence of uncontrolled high blood sugar levels. This death rate is projected to double between 2005 and 2030. In addition, at least 2.8 million people worldwide die each year as a result of being overweight or obese. The WHO estimates that 35.8 million disability adjusted life years (DALYs) are lost as a consequence of individuals being overweight or obese, which is 2.3% of all global DALYs. Being overweight and obese leads to adverse metabolic effects on blood pressure, cholesterol, triglycerides and insulin resistance. Risks of coronary heart disease, ischemic stroke and type 2 diabetes increase steadily with increasing body mass index (BMI), a measure of weight relative to height. Raised body mass index also increases the risk of cancer of the breast, colon, prostate, endometrium, kidney and gall bladder.

Type 2 diabetes (T2D, formerly non-insulin dependent diabetes (NIDDM)), has a complex pathophysiology and the development of drugs to treat this disease is challenging. According to the WHO the consequences of poorly controlled type 2 diabetes include:</td></tr>
</table>

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

	• Increased risk of heart disease and stroke: 50% of people with diabetes die of cardiovascular disease (primarily heart disease and stroke). • Combined with reduced blood flow, neuropathy in the feet increases the chance of foot ulcers and eventual limb amputation. • Diabetic retinopathy is an important cause of blindness, and occurs as a result of long-term accumulated damage to the small blood vessels in the retina. After 15 years of diabetes, approximately 2% of people become blind, and about 10% develop severe visual impairment. • Diabetes is among the leading causes of kidney failure. 10-20% of people with diabetes die of kidney failure. • Diabetic neuropathy is damage to the nerves as a result of diabetes, and affects up to 50% of people with diabetes. Although many different problems can occur as a result of diabetic neuropathy, common symptoms are tingling, pain, numbness, or weakness in the feet and hands. • The overall risk of dying among people with diabetes is at least double the risk of their peers without diabetes. Despite these clear clinical needs, pharmacotherapy remains inadequate. First line treatment with insulin sensitising agents (i.e. metformin) can provide some benefits, but for a large number of patients the current repertoire is not sufficient.
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)?	New treatments for human metabolic disease including diabetes and obesity, and an increased understanding of the mechanisms responsible for these diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	4000 adult mice and 2000 adult rats 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 majority of animals used in this project would be expected to experience mild severity. The experiments we plan to perform will mostly involve measures of metabolic function, such as the monitoring of blood glucose following a bolus of sugar solution (OGTT – oral glucose tolerance test), or measurement of food intake and

	bodyweight in normal or obese animals undergoing treatment with a novel agent. Some animals will be used in experiments looking at the consequences of metabolic dysfunction such as kidney disease. These animals may have surgery or treatment which will bring on nephropathy (kidney disease) in conjunction with the metabolic disease, and we will assess new potential treatments which could reduce this injury.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is currently no in vitro or in silico system capable of simulating complex whole animal physiology and metabolism. Metabolic diseases have a complex pathophysiology with multiple components interacting to manifest the disease. Our therapeutic agents target specific biochemical responses or physiological mechanisms that in vitro systems cannot replicate. Individual mechanisms can be probed in vitro, and we conduct extensive studies to characterise these as far as possible before conducting in vivo experiments. In this case we expect to access human tissues and cell lines and use these to understand at a basic level what mediators and mechanisms are involved. Regulatory authorities such as the FDA and EMEA require compelling data packages to support the development of a new medicine in humans. In vitro potency data are seldom sufficient to provide confidence of efficacy in man, and demonstration of activity (and mechanism) in animal models is becoming increasingly important.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use relevant statistical tools (e.g. power analysis) to guide the design of our studies. Reference will be made to key texts (e.g. Festing, The Design of Animal Experiments, RSM Press 2002). Study designs will be consistent with accepted scientific methods, and will include relevant positive and negative controls as applicable. For example, we will minimise unwanted sources of variability by ensuring that wherever possible experimental and control animals are studied side-by-side on the same day by the same person. We have access to in house statisticians with whom we consult as necessary when planning in vivo studies.
3. Refinement Explain the choice of species and why the animal model(s)	Mice and rats are small and easily handled species with a highly characterised immune system and well defined biology.

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.	Mouse and rat models of metabolic disease have been established by other groups and reported in the literature. The inclusion of mice enables us to use mutant or GM animals for early hypothesis testing, target validation and humanization of target as necessary. Our models will be of the minimal severity consistent with the objectives. Pilot studies will be conducted for new protocols to ensure the methods used provide for the maximum animal welfare in relation to the experimental objective. We will consider ways to reduce further the welfare impact while retaining our key disease phenotype. Best practice, for example the use of analgesics after surgical implantation of continuous delivery devices, will be employed to minimise suffering.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Brain Control of Peripheral Glucose and Energy Metabolism</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Diabetes, Hypoglycaemia, Brain</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>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall objective of our work is to characterise how brain helps control blood glucose and other important aspects of peripheral metabolism such as blood lipids and body weight.

1) Some people with diabetes lose defences against a falling blood glucose putting them at risk of suffering from severe episodes. The mechanisms by which this occurs are unknown. We will examine how brain controls defensive responses to a low blood glucose and how this may become altered by diabetes
2) There is a global explosion of diabetes and the related “metabolic syndrome”/ obesity. We are also examining more broadly how brain controls blood glucose and other important aspects of blood metabolism such as levels of fats in blood stream. We will also look at whether any of the mechanisms we identify have effects also on control of body weight</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>All of the above may lead to the identification of potential novel targets for therapies addressing clinical problems to be then tested in human studies.
1) To try to reduce the burden of low blood glucose – particularly occurring without warning- in people with diabetes
2) To identify novel therapeutic targets for treating diabetes and/or managing obesity</td></tr>
<tr><td></td><td>Rats and mice.</td></tr>
</table>

[3] Delete Yes or No as appropriate.
[4] 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?	Maximum numbers listed in this licence are 21,500 mice and 4 500 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?	Most mice on the licence will be used for breeding/ maintenance of colonies. For those mice and rats undergoing more scientific procedures, these will mostly be of moderate severity. Typically this may involve injections of insulin to lower blood glucose or glucose to raise blood glucose, measures of feeding behaviour and activity. Some animals will undergo recovery surgery under general anaesthesia for implantation of tubes to allow subsequent infusion of test substances into brain and or blood sampling. One protocol is graded as severe which involves surgical implantation of tubes into blood vessels and subsequent “clamp studies”. During these studies where blood glucose is altered by infusion of insulin with frequent sampling of blood. They provide very detailed information about metabolism especially when combined with infusion of radioactive markers so that we can trace movement of glucose in the body into muscle and out of liver for example. The protocol has been graded as severe because the surgery is challenging in small animals and we typically see that 1 in 3 die before reaching study days. We have refined this over the last 5 years so that most of these deaths are while the animal is under anaesthesia. Animals will be killed at the end of protocols. We will often collect body tissues such as brain which allows us to examine patterns of brain activation in more depth
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	1) Control of energy balance and metabolism occurs at the level of the “whole body” and not simply at a cellular level. We can only partly replace animals with cell culture 2) There are technical limitations to examining brain metabolism in humans with techniques such as non-invasive brain imaging which are still too crude to visualise changes in small brain cell populations.
2. Reduction Explain how you will assure the use of minimum numbers of animals	1. We will use validated and standardised procedures to reduce experimental variability/ duplication of efforts. 2. Where possible, we will try to maximise the data obtained from each animal undergoing procedures to reduce overall numbers of animals

	needed
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	. Rats and mice are the lowest vertebrate groups on which well-characterised studies have been performed examining brain metabolism for extrapolation into humans. Lower life-forms, eg. fish or insects, regulate metabolism differently from mammals ‘ We will use both healthy animals initially but also then use validated rodent models of human disease- for example rodents with diabetes in order to allow us to extrapolate data back into the clinical setting of human disease. Most of the procedures are of moderate severity. We routinely use painkillers, anaesthesia and careful monitoring of animal welfare during procedures and we talk regularly with other similar research groups around the world in order to continue refining our methodology to improve animal welfare.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Manipulation of Sodium and Chloride Ions to Treat Abnormal Epithelial Secretion</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Respiratory disease airway bio-marker</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>3</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[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>This project aims to identify substances which can treat respiratory diseases characterised by abnormal airway secretions, such as cystic fibrosis and bronchiectasis.</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>Identification of substances that down-regulate the electrical potential difference across the airway lining will lead to subsequent development of these substances as drugs to reduce the cough, mucus accumulation, and lung degeneration suffered by patients with chronic respiratory diseases.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Rats and guinea pigs a maximum total of 800 of each and pigs a maximum total of 150 over 3 years, the duration of the project.</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>Should mild discomfort be observed, pain killers will be given. In the unlikely event of severe discomfort being observed the animal will be immediately killed by a humane method. Animals will be terminally anaesthetised for surgery and data collection so that no pain or distress is involved.</td></tr>
<tr><td></td><td></td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
<tr><td>1. Replacement
State why you need to use animals and why you cannot</td><td>Health regulatory authorities across the world set stringent regulations for evaluation of new pharmaceutical compounds. These include</td></tr>
</table>

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

use non-animal alternatives	requirements that all pharmaceutical compounds intended for use in humans are proved effective and safe in animal studies before being made available for use in patients. Currently there are no *in vitro* models available to replace the use of animals in these effectiveness studies. Animals are only used where no suitable alternative is available.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used will be reduced by screening substances to be investigated in *in vitro* cell cultures to identify those that are most active. Only these will then be tested in animals. Also preliminary experiments will be performed to increase the sensitivity of the measurement method (increasing the signal-to-noise ratio) which will reduce the number of animals used for each subsequent experiment. Statistical method will be used to calculate the number of animals in each group that will be required to show that a substance is sufficiently active to be taken through into development as a potential drug treatment.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats, guinea pigs and pigs have been chosen for this work because there is published evidence and experience that the bio-marker (electrical potential difference) can be measured in this species. The justification for use of rats, guinea pigs and pigs is based on available literature evidence of the suitability of the species, and previous data generated by Mucokinetica. And the pilot experiments choosing a strain in which the signal-to-noise ratio is increased will also determine the strain to be used. During and after all procedures, close monitoring of animals will be carried out to identify and minimise pain, suffering and distress – and appropriate use will be made of pain relievers.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Cardioprotection</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cardioprotection; Ischaemia; heart</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td></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>Patients suffering from many cardiac and non-cardiac diseases would benefit from increase in myocardial resistance to metabolic stress. Ischaemic heart disease (IHD) is the first and the most obvious target. IHD is a condition in which fatty deposits build up in the linings of the walls of the coronary arteries, causing a narrow artery and reduced blood flow to the heart muscle. This leads to the inability to provide adequate oxygen to the cardiac muscle and therefore an inability to meet demands upon the heart. IHD is the leading cause of death and disability among adults in the UK and developed world. By the age of 40, the lifetime risk of developing IHD is 50% for men and 33% for women. Currently, ~2.5 million people in the UK suffer from this disease and ~200,000 die annually. More than 65 million incapacity days per annum in the UK alone are thought to be attributed to IHD with an estimated economic burden of greater than £7 billion in 1999.

Heart damage in IHD patients can be slowed/halted with lifestyle changes, medication or surgery. Medications are prescribed according to the nature of the patient's disease and other factors, and act by improving blood flow to the heart or by decreasing heart metabolic demands. These include aspirin, statins, beta blockers, calcium channel blockers and angiotensin converting enzyme inhibitors or angiotenzine receptor blockers. At the moment, there is no therapy of IHD that would be based on cardioprotection.</td></tr>
</table>

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

	A therapy based on cardioprotection does not compete with these medications, but rather is complementary - an IHD patient on any regime would benefit from such therapy. Therefore, it is a consensus view that therapy of IHD and other heart diseases based on cardioprotection is warranted. We plan to determine: 1. Whether particular types of membrane-bound ion channels mediate cardioprotection; 2. Whether specific intracellular signalling molecules are involved in cardioprotection; 3. Whether modulating the functions of either of these classes of proteins, by drug treatment or by gene therapy, can result in much enhanced protection against the damage caused to the heart muscle by acute or chronic lack of oxygen. To do that we will manipulate levels/activity/function of signalling factors using transgenic technology and/or plasmids/viral constructs and or drugs at the whole organism/organ and/or cellular level. The outcome of this manipulation would be assessed by measuring range of parameters using many different biomedical *in vivo* and *in vitro* techniques. Based on obtained data we will develop a novel therapies based on cardioprotective signalling, which safety and efficacy will be further tested in *in vivo* and *in vitro* models of heart diseases.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The planned research will provide important information about cardioprotective signalling pathways. In turn, this will provide bases for novel, more efficient and safer, therapies against ischaemic heart disease and other cardiovascular diseases in humans and animals.
What species and approximate numbers of animals do you expect to use over what period of time?	For this 5 years-long programme, we estimate that we will breed up to 5000 mice, use up to 2000 for heart harvesting and up to 200 for telemetry. We also plan to use up to 200 rats and 300 guinea-pigs for heart harvesting.
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?	Genetically modified mice will be bred and maintained and animals will be killed humanely in order to harvest cells, tissues or intact hearts for laboratory investigations. Some animals will be used for the assessment of heart function in vivo, using echocardiography and wireless recording from implanted devices monitoring ECG or blood pressure. Some of the animals could be exposed to ~~mild changes in environmental oxygen tensions as well as to~~ exercise on a treadmill. Some mice may

	be treated with potentially protective drugs or genes. Some genetically modified mice might have decreased life-span and increased risk of cardiovascular diseases (atherosclerosis, cardiac hypertrophy etc.) but, under this licence, they will be killed humanely for detailed analysis before these conditions might impact on animal welfare. The implantation of the telemetry devices for ECG / blood pressure recording, like any surgical procedure, is associated with some risk, but this will be managed by a scrupulous attention to detail.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Hearts and heart tissue may be obtained ethically from humans only following rare heart transplantations, making this option impossible. We use heart-derived cell lines for initial experiments, but they are not entirely representative of adult cardiomyocytes. lines. Therefore, for this project, animal hearts/cells have to be used.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Using data from our previous studies, we have calculated sample sizes for planned experiments and minimal number will be used to ensure statistical significance. For most of the planned experimental interventions, group sizes are likely to be about 8-10 animals. Where tissue extracts are being used, an extract from a single heart will be used for more than one biochemical experiments.
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, rats and guinea-pigs are well established experimental models to study ischaemic heart disease at different levels (cellular, whole heart and *in vivo* levels). The obtained results can be easily compared to those in literature and they are highly relevant for humans. Health of the genetically altered mouse colonies will be monitored regularly and the advice of NVS will be sought in the event of any distress being noted. For injections every care will be taken that animals are handled to minimise discomfort and a general anaesthetic will be used whenever, on balance, this would benefit the animals. Surgical implantation of telemetry devices will be carried out aseptically and under general anaesthesia. Post-operative pain relief will be administered as a routine.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Role of AMPK in cancer and in glycogen homeostasis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>cancer, glycogen, protein kinase</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)[9]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
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<tr><td>Maintenance of colonies of genetically altered animals[10]</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>The first aim is to determine whether AMP-activated protein kinase (AMPK), an enzyme that phosphorylates other proteins and thereby changes their activities, can prevent or inhibit the formation of tumours in mouse models of cancer. We also wish to investigate the mechanism by which AMPK-activating drugs can provide protection against tumour development. AMPK has many roles in the body and our second aim is to test whether it acts as a sensor that regulates the synthesis of glycogen (the form in which glucose is stored in the liver for future use) .</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 first part of the project should answer several important questions regarding the role of AMPK as a tumour suppressor, and the mechanisms for anti-cancer actions of AMPK-activating drugs, which have been proposed through epidemiological evidence to provide protection against cancer in humans. The results should be very informative in aiding the design of human clinical trials of drug efficacy in prevention or treatment of human cancer. The second part of the project is relevant to the problem of insulin resistance in humans, and should provide insight into the mechanism(s) by which insulin resistance correlates with cellular glycogen content.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We shall use mice, as there are a number of tumour-susceptible lines we can use to test our scientific hypotheses. We shall also be able to use mice altered in the expression of the AMPK gene to determine precisely the role of this protein. We expect to use about 15000 animals over the course of the licence, in order to assemble the right</td></tr>
</table>

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

	combinations of genetic alterations and to maintain the lines. The great majority of these mice will be used without the need for any further experimental intervention; they will be killed humanely and tissues and cells will be analysed in detail in the laboratory. Wherever possible, we will breed and maintain lines in which tumour susceptibility is normal, but can be enhanced by the administration of an inducing compound. We also wish to test our hypotheses that some compounds might actually reduce tumour susceptibility (and therefore be potential preventive or therapeutic agents in people). We expect to use a total of about 5000 mice (drawn from the breeding colonies) for these interventions.
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?	Some mice will develop tumours, either naturally or after the administration of an inducing compound. We will work with animals of ages at which significant welfare issues will be unlikely. If any mouse does develop a problem, it will be euthanased. In some experiments we will deliver agents that we believe may protect the mice against the formation of tumours. In others, they will be treated, as humans are, with drugs that are expected to inhibit or reverse tumour growth, We therefore do not expect any additional problems to arise in these animals. In the dietary experiments, mice may become moderately obese when fed a high-calorie diet, but are not expected to go on to become diabetic.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use live animals for these experiments because the hypotheses on which the project is based have already been thoroughly tested using cell culture models. Cancer is also a complex process involving interactions between many different cell types, and cannot yet be modelled adequately in in vitro systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In many cases, the breeding of sufficient animals with the correct genotype is anticipated to be the step that limits the numbers of experimental animals produced at any one time. In this case, experiments will be stopped as soon as a statistically significant effect is shown. Otherwise, experiments will initially be conducted using small numbers of animals (up to ten per group). If a statistically significant result is not obtained with this number, we will then make a decision either to abandon the hypothesis or, if the results suggest that there might be a small effect, we will assess the size of the effect and perform power calculations to estimate the number of animals required to get a 95% chance of showing a statistically significant effect.

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 anticipate that the experiments proposed should only cause suffering or discomfort if/when tumours arise. When simply testing for susceptibility for tumour formation, we will inspect the animals regularly, and those with obvious tumours, or showing other signs of discomfort, will immediately be killed humanely for post mortem analysis. When studying the possible treatment of tumours with drugs, we will stop the experiments as soon as it is clear that any tumours are not responding. . We have chosen to study mice because genetically modified strains, and tumour models, required for the project are already well-established and available in this species, while knock-in mutant technology required for the glycogen homeostasis project is also routine.

Encoding of reward prediction
attention, cortex, motivation, striatum, reward

- Summarise your project (1-2 sentences)

This project aims to increase our understanding of brain mechanisms involved in motivated behaviours and attention. The areas of the brain involved in these functions play a crucial role in mental disorders therefore a better understanding of this system will provide a neurobiological basis for interpreting human clinical 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.

The overall aim of the project is to understand the brain mechanism underlying reward-related learning and attention. The project will assess how sensory stimuli gain significance during learning and how the brain produces stimulus-response learning, the process through which an animal learns to emit an instrumental response in order to gain access to a reward. It will investigate the function of neurotransmitters known to play a crucial role in this process. The focus of the studies will be the function of normal healthy animals. This information will broaden the general understanding on how positively reenforced learning and motivation is implemented at the neuronal level. The information will also lead to a better understanding of psychiatric conditions in which the above functions are compromised, in particular schizophrenia and drug addiction.

- Outline the general project plan.

A combination of neurophysiological techniques and behavioural training will be used for these experiments. Rats will be trained to respond to sensory cues and we will record the activity of the cortex and related brain areas to the presentation of these cues using microelectrodes implanted in the central nervous system. We will also study how networks of brain regions represent the stimuli in their coordinated activity. Behavioural tests may include Pavolovian conditioning or sensory discrimination. A subset of rats will be required to press a lever to obtain rewards and we will study how the recorded neurophysiological activity maps onto the behavioural performance of the animals.

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

In all experiments the welfare of the animals will be of the highest priority. It is, in fact, not possible to train animals in reward tasks if they are under stress or if their health is compromised. Therefore the very success of the studies hinges on animals that are comfortable and motivated while performing the task. Once the animals have acquired the task, the activity of individual neurons in target areas of the brain will be recorded with microelectrodes. Mild stimulation will be applied to some brain areas to test the function of brain circuits. Neither recording nor microstimulation will result in discomfort or disrupt the animal's behaviour. The microelectrodes will be implanted under general anaesthesia and the rats will get post-operative care in accordance with modern veterinary practice. Rats will be motivated to perform in the behavioural experiments by reducing the

availability of food or water; however we will ensure that the animals obtain their required liquid and nutrients so that health is not compromised.

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

Understanding how the brain generates reward-driven behaviors is crucial to interpreting how complex biological systems adapt and survive in a constantly changing environment where vital reinforcers (food, etc.) must be detected based on learned cues. These behaviors are likely to depend on the coordinated activity of complex neuronal networks. Currently we do not have a good understanding of how these networks implement motivated behaviour in their coordinated activity at the microscopic cellular level. The proposed study will pin down these mechanisms, dissecting not only pathways and brain regions but also assessing the contributions of specific cell types to the behaviour of the whole organism.

- 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.

170 rats are estimated to be required for this project. This number is based on previous experiments from my lab where group sizes of ~10 rats were used for individual runs. Because the unit of analysis in these experiments is neural activity in overtrained animals, power analyses based on behavioural performance are not appropriate and the number requested is based on experience gained from prior experiments.

Rats are a good animal model of motivational systems, at the same time being the least neurophysiologically sentient species appropriate for these types of study. A huge body of experimental work exists on the rat sensory system and motivational circuitry. This provides information for fine tuning experimental parameters increasing the likelihood of achievement and minimizing variance of the result and hence the number of animals used. There is a wealth of comparison data to aid interpretation and a sound theoretical framework in which the project findings will be incorporated.

- 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.

Detailed understanding of the function of individual neurons or how groups of neurons interact cannot be achieved using currently available fMRI techniques because of the limits in the temporal, spatial and neurochemical resolution of these imaging techniques. The question of how organisms perceive and react to the significance of reward-predicting stimuli precludes the use of tissue studies. To date there are no computer simulation models to adequately account for the complexity of central nervous system processes underlying motivated behaviour. These limitations necessitate the use of awake animals trained to associate environmental stimuli with the presentation of reward.

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

The work will involve the implantation of recording devices in the central nervous system of rats under general anaesthesia, followed by behavioural training and neurophysiological recordings. Implantation will be carried out using aseptic

techniques to minimize tissue trauma and rats will receive post-operative care in accordance with modern veterinary practice. During behavioural training rats will respond for reward and will not be exposed to any aversive stimuli.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Mouse Rederivation and Cryopreservation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cryopreservation rederivation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[11]</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[12]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Our work focuses on the molecular mechanisms underlying normal cellular processes with the aim of improving lifelong wellbeing and healthy ageing. We employ genetic models in the mouse which can reveal both underlying molecular mechanisms within cells and their impact in terms of dysfunction and disease. The mouse shares much of its physiology and developmental characteristics with humans, and as such genetic alterations in the mouse often closely mimic disease states in man. Several wild type mouse strains are bred for use on site. The state of the art animal unit is staffed by dedicated and experienced animal technicians who make use of various breeding strategies to closely match production with demand, minimising wastage. This project aims support science at the establishment by:

• Establish and maintain breeding colonies of wildtype or genetically altered strains free of specific pathogens and without genetic drift by freezing embryos and by transferring embryos to pathogen free surrogates. This provides consistency in the animals supplied for use in breeding and experiments. Decreasing variability, increases consistency of results and can achieve a reduction in numbers of animals used.
• Facilitate the import of genetically altered lines where Project Licence, authorisation is only required for short-term breeding and maintenance</td></tr>
</table>

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

	• Allow quality control of stocks of cryopreserved lines, reagents equipment and procedures used in the first aim, • Develop new methodology that will improve the efficacy or scientific potential of methods to achieve these aims, In particular addressing the principles of Replacement, Reduction and refinement in animal research.
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)?	• Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project. • Reduction in numbers of animals used, as supplying mice of high health status and consistency for breeding and experimentation, maximises the statistical significance of experimental results • Insurance against disease or damage to the animal unit by archiving embryos and sperm benefitting the whole site • Improves welfare by avoiding stress to live animals by transporting strains as frozen sperm or embryos • Reduces the number of genetically altered animals that need to be kept alive and breeding through cryopreservation • Reduces the number of breeders by utilising in-vitro techniques for rapid expansion of colonies from only a few individuals. • Potential for reduction and refinement site wide if novel techniques for cryopreservation and embryo transfer are demonstrated to be a refinement or more effective than current techniques Other projects or establishments which use genetically altered mice but lack the facilities or expertise to re-derive them would benefit from the services of skilled technicians.
What species and approximate numbers of animals do you expect to use over what period of time?	This project may use up to 4790 mice over 5 years. Approximately 15% or less of the animals used will be genetically altered and most of these will have no harmful effects from the genetic alteration.
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?	Infections arising from hormone injections are exceptionally rare. As the females are usually under 6 weeks old there is a slight risk of injuries caused by overlarge or aggressive stud males. After mating hormone treated females are culled by a schedule I method for collection of embryos. The expected level of severity is mild. Adverse effects from surgery or non-surgical embryo transfer for example wounds re-opening or

	infections are very rare <1% with animals recovering quickly. The expected level of severity for animals undergoing surgery is moderate. Non-surgical embryo transfer should cause no more than mild discomfort. After litters from embryo transfer are weaned the recipient females will be culled by a schedule I method, or may be sent to a health screening laboratory where they will be culled humanely. Vasectomised males will be culled by a schedule I method or sent to a health screening laboratory where they will be humanely killed. In rare cases genetic alteration may lead to abnormalities that affect the animals' welfare. Almost all genetic alterations will produce no worse than mild phenotypes. Genetically modified animals may be transferred to other projects on site or elsewhere
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Live animals are essential to study gene function in a complex physiological environment. Pilot studies involving culturing of embryos to hatched blastocyst stage will be used to show that the novel equipment, media, or protocols being tested are not harmful to pre-implantation stage embryos before proceeding to embryo transfer. The harvesting of the embryos for culture requires using animals but superovulation is a relatively harmless procedure and allows maximum embryo yield from the fewest possible mice
2. Reduction Explain how you will assure the use of minimum numbers of animals	A Statistician will be consulted on experimental design to ensure a high level of confidence in the results while using the fewest animals possible The number of embryo transfers performed will be reduced by implanting optimal numbers of embryos maximising the number of healthy offspring from each litter Superovulations will be reduced by using animals of optimum age and weight to maximise embryo yields
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the mammalian species of choice for our scientists: they study physiology, ageing and disease processes in mammals and this is the model where these methods are most advanced and for which most existing genetically altered strains are available Superovulation performance is monitored and reviewed, technicians receive regular refresher training to minimise distress to the animals and ensure consistent results Surgery i.e.embryo transfers and vasectomies are

	performed by a small team of skilled technicians with excellent success rates. Appropriate analgesia is always given to animals undergoing surgical procedures. When appropriate to the stage of embryo non-surgical embryo transfers are used as a refinement over surgery. Numbers of embryos implanted are optimised for maximum numbers of healthy pups from fewest procedures. Vasectomies are performed using the least invasive technique possible. In a small number of strains genetic modification may adversely affect animal welfare. However close health monitoring, provision of appropriate treatment under the guidance of the NVS and adapting husbandry routines to the needs of the animal will be used to ameliorate these effects.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Reduction of cardiac ischaemia/reperfusion injury</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Heart; drug development; regulatory; ischaemia</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>3 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</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[14]</td><td>~~Yes~~</td><td>~~No~~</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The purpose of the present project is to determine if and how a novel drug can prevent the development of tissue death in the heart muscle after a heart attack.</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 present project will produce data to decide whether this drug will progress into early clinical trials in humans to be further developed into clinical use.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Pigs up to 80 over 3 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>Non-recovery</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
<tr><td>1. Replacement
State why you need to use animals and why you cannot use non-animal alternatives</td><td>All efforts to establish a reproducible and reliable cell model of tissue death have failed so far. Only very basic information can be obtained from cell experiments or experiments using isolated organs. Furthermore, effects of given drugs on cardiac</td></tr>
</table>

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

	rhythm, blood pressure or cardiac function cannot be assessed using cell models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Published data will be reviewed and the surgeon will work with other experienced teams from around the world prior to starting this project. This will reduce variability in the surgical techniques and reduce animal numbers used for a valid statistical interpretation of the data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The drug licensing authorities require confirmation of previous results in rodents in a larger species prior to the use first in humans. Pigs are the most suitable and best studied species for the translation of cardiac drugs towards human use. There is no marker available which would allow testing of this novel drug in humans before a suitable target dose is established. All procedures are to be performed are non-recovery and performed under general anaesthesia.

Project Title
Identifying cytoskeletal regulators of metastasis.
Key Words
Cancer, invasion , cytoskeleton
Expected duration of the project (yrs)
5
Purpose of the project (as in Article 5)1
Basic research
Yes
Translational and applied research
Yes
Regulatory use and routine production
No
Protection of the natural environment in the interests of the health or welfare of humans or animals
No
Preservation of species
No
Higher education or training
No
Forensic enquiries
No
Maintenance of colonies of genetically altered animals2
No
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)
There is a driving need to find novel therapeutic targets to inhibit metastatic spread. Currently , localised tumours can be treated with radical surgery, chemotherapy or radiotherapy but once the cancer has spread it is almost impossible to eradicate. Whilst there have been a number of important advances in the development of anti-proliferative agents the study of metastatic spread has lagged behind . This is largely due to the complex inter-relationship between the migrating cancer cell and the local microenvironment. Advances in matrix production and 3D organotypic modelling have helped us to understand some of this complexity but cannot yet fully compensate for animal studies. This project aims to identify the key proteins that regulate cancer cell dissemination using zebrafish as our model system. We will use a zebrafish model of melanoma to image in real time the movement of these cells away from the primary tumour site. We can then use specific inhibitors and/or manipulate the level of key proteins in these cells and monitor how this effects their ability to move away from the primary tumour. In our second model we will inject a ball of human cancer cells into the zebrafish embryo and test the ability of these cells to disseminate thought-out the embryonic body. Again we can modulate these cells as above to identify those proteins that are essential to the dissemination process.
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 is important because it will help to validate key cytoskeletal regulatory proteins as potential anti-metastatic targets and also develop a widely applicable in vivo system for validation of other anti-invasive targets. This could be applied to other potential drug candidates. Ultimately, this will make a significant contribution towards the development of anti-cancer drugs.
What species and approximate numbers of animals do you expect to use over what period of time?
Zebrafish , 5 years , 7,600 animals
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 expected level of severity for breeding is mild. Some of the tumorogenic fish will develop tumours that could cause discomfort and distress. If there is a visible tumour the fish will be immediately humanely culled using a schedule 1 method. The expected level of severity for protocol 1 is mild – in rare instances some embryos might experience distress when embedded in agarose. If the embryo becomes distressed (change in cardiovascular function, twitching) it will be humanely culled immediately using a schedule 1 method. All other protocols are non-recovery.

Application of the 3Rs

1. Replacement

State why you need to use animals and why you cannot use non-animal alternatives

Advances in matrix production and 3D organotypic modelling have helped us to understand some of the complexity of cell migration in vivo but cannot yet recapitulate tissue architecture, provide local vasculature for long distance dissemination and thus fully challenge the migration of cancer cells.

2. Reduction

Explain how you will assure the use of minimum numbers of animals

We have calculated the number of animals we need to obtain statistically significant data in all our studies in collaboration with a Statistician.

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.

Zebrafish is considered, by many, less sentient than other vertebrate model organisms currently used e.g. rodents, ungulates, and primates. When use of vertebrate models is dictated by the scientific agenda and permitted from the cost/benefit analysis, zebrafish should be employed wherever practicably possible. All procedures listed here follow a non-recovery of anaesthetised animals scheme. Within the breeding programme if a tumour develops the animal will be killed humanely, without delay.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>microRNAs in CNS vasculature and barrier function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>microRNA, blood-brain barrier, neuroinflammation, aging</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 year</td></tr>
<tr><td>Purpose of the project (as in Article 5)[15]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></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[16]</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>The blood vessels of the brain and spinal cord exert a tight control over the blood-borne molecules that can access the neural tissue so that this stable environment facilitates transmission of electrical signals between nerve cells. Indeed, the cells that form the blood vessels are called endothelial cells and form tight seals between them, an anatomical structure known as the blood-brain barrier (BBB). In many neurological diseases such as multiple sclerosis and in normal ageing, these seals between endothelial cells malfunction leading to leaks between the blood and neural tissue that contribute to tissue damage. The molecular mechanisms by which this leakage occurs in disease remain elusive. In this project, we aim to determine the role of one type of endothelial molecules termed microRNAs in BBB dysfunction in CNS pathologies and in normal ageing. The project involves first identification of microRNAs deregulated at the BBB in ageing and neuroinflammatory models and, second, modulation of microRNA levels to determine their effects on BBB dysfunction.</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 information obtained should have a high value and it is hoped that this will allow direct-translation of the laboratory findings to the clinic and provide new knowledge regarding BBB dysfunction and repair, which will in the longer term, contribute towards the development of additional treatments to improve the lives of people with CNS pathologies such as multiple sclerosis and allow ageing</td></tr>
</table>

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

	population to enjoy quality of life.
What species and approximate numbers of animals do you expect to use over what period of time?	3000 mice during 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 inflammation model, immunoregulating agents (e.g. lipopolisaccharide, cytokines such as TNFα) will be administered at defined doses. It is anticipated that animals will show transient mild to moderate flu-like adverse effects such as shivering and mild to modest sedation. We will adjust the doses to obtain reproducible systemic inflammation and avoid substantial adverse responses. Any animal showing evidence of adverse events of unexpected toxicity (abnormal behaviours (e.g. seizures), non-transient (more than 2 hours) mild abnormal behaviours (e.g. reduced movement) or more than 15% loss in weight body over the 3 day procedure will be killed by schedule 1 methods. For the aging model, likely adverse effects will be age-related distress such as weight loss, hair loss, changes in habits of eating and drinking, general and social activities. Animals will be regularly monitored, and animals will be killed by schedule 1 methods if deemed necessary. To assess the function of the blood-brain barrier (BBB), administration of tracers into the bloodstream is usually done hours before termination of the experiment and is unlikely to cause lasting harm and is minimised by good handling and laboratory technique. Intravenous injection may cause collapse of the vein (0.1%), this is limited by using small bore needles. Any discomfort from multiple injections will be reduced by not injecting repeatedly into the same site/vein. Radioactive labelled tracer (e.g. ^{125}I-albumin) compounds will be limited to the maximum dose of 10^7 cpm, and handling of radioactive substances will strictly follow the EPR2010. Bleeding after injection will be controlled by local pressure. Defined end points will be designed accordingly, animals will be subjected to terminal general anaesthesia to be perfused or killed by schedule 1, and tissues will be taken for RNA, protein extraction, quantitation of tracers and/or fixed for morphological analysis.
Application of the 3Rs	
1. Replacement State why you need to use	The complex clinical picture of neuroinflammation and of normal ageing cannot be currently

animals and why you cannot use non-animal alternatives	completely modelled in cell-culture or computer-based models, and the use of live animals is needed. Mice are well-known and broadly accepted species for establishing animal models of systemic inflammation and of ageing.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Written protocols will be provided for each experiment including methods of analysis, to minimize experimental variation, by which we can limit the number of animals in the control group to a maximum of 10. We will use systemic inflammation for screening for therapeutic treatment which is highly reproducible, thus reduce the animal numbers in substantial procedures. For the study of ageing, we will mainly focus on 4 age-groups: 3 months, 12 months, 18 months and 24 months, limiting the number of animals in each group to a maximum of 10, occasionally younger or older animals will be used. Sample sizes have been set using power analysis, generally using a significance level of 5%, a power of 80%, and a least practicable difference between groups of 25%. Otherwise, we will use the least number of animals to provide an adequate description, generally on the basis of previous experience.
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.	Multiple sclerosis exhibits many pathological processes including relapsing and secondary progressive disease. Researchers aim to model some of the features of multiple sclerosis with models in rodents and primates who develop autoimmune mediated disease of the CNS (termed experimental autoimmune encephalomyelitis, EAE), but few exhibit reproducible relapsing disease and progression, and in addition the model is of substantial severity. In comparison to many researchers that use EAE to study autoimmunity, we study BBB dysfunction which is obvious during the early stage of the disease. Therefore, our chief mouse model will involve a systemic inflammatory challenge for a short period of time that has proved reliable and far superior to most EAE models in the context of blood-brain barrier dysfunction due to its reproducibility and the absence of major CNS inflammation thereby avoiding the establishment of long-term clinical disability. To investigate dysfunction of the BBB, we may examine mouse strains other than C57BL/6 although this is the strain of choice as it is the source for specific microRNA deletions which will considerably reduce the number of animals used in order to determine

	the role played by microRNAs in BBB dysfunction. We aim to continue to refine this model to detect effects of knock-down/over-expression microRNA(s) and enhance the utility of the model. Through the use of a reproducible system and defined endpoints for each objective in the absence of clinical disability, we can limit the time in procedure and as a result the suffering that the animal would accumulate as a result of autoimmune attack in other animal models of neuroinflammation.