Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 35

Project Titles and key words

- Intestinal delivery of agents

  Oral delivery, diabetes therapy

- Novel Treatments for Neurodegenerative Diseases

  Neurodegeneration, Alzheimer's disease, Parkinson's disease, Models, Treatments

- Validation of an in vitro subcutaneous injection model

  Subcutaneous injection, in vitro replacement

- Reproductive & developmental toxicology

  Reproductive, developmental, toxicity, hazards

- Inflammation-induced pain mechanisms

  Neuropathic pain, inflammation, electrophysiology, nociceptors, repetitive strain injury

- Safety and efficacy in non-human primates

  Safety, Efficacy, Pharmacology, Primate

- Multimodality cancer therapy and imaging

  Cancer, imaging

- The Morphological Effects of Dietary Consistency

  MicroCT; PET; Morphology; Dietary Consistency

- Rodent regulatory genotoxicity

  Genotoxicity, Cytoxicity, Rodents

- Molecular mechanisms of pain

  Nociception; pain; sensory neuron; ion channel; G protein coupled receptor

- Interneuron development in the mammalian forebrain

  Brain development, autism, schizophrenia, nerve cells, network function

<table>
<tr><td>Project Title (max. 50 characters)</td><td>INTESTINAL DELIVERY OF TEST AGENTS</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Oral delivery, diabetes therapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>We are examining the potential for rationally designed molecules to alter the permeability properties of the small intestinal for the improved uptake of biopharmaceuticals that currently must be administered by injection.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The potential benefit of these studies would be the identification of a method to manage diabetics using oral dosage forms for drugs that are currently must be given by subcutaneous injection. The information obtained from these studies may also provide an improved understanding of how the small intestine functions in health and disease.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Rats will be used. We plan to use approximately 200 animals to determine the potential of several novel agents for their ability to modulate the uptake of biopharmaceuticals across the mucosa of the small intestine. If toxicity of a modulating agents is observed, we will use up to 100 additional rats to find non-toxic doses of these modulating agents for subsequent testing.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>All of the studies to be performed will maintain animals under anaesthesia thru out the study. While it is possible that some of the treatments might result in hypoglycaemic events of some kind of unexpected toxicity, the animals will not experience any of these issues. At the termination of each study, the animal will be euthanized without regaining consciousness.</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>Only animals provide the complexity of the small intestinal barrier organized with portal and systemic circulations.</td></tr>
</table>

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

use non-animal alternatives	
2. Reduction Explain how you will assure the use of minimum numbers of animals	All of the modulating agents to be tested *in vivo* have first gone through extensive *in vitro* studies that screens out those that are not effective or are cytotoxic.
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 are the smallest animal with anatomy and physiology to man that can be used to place catheters in the portal and jugular veins that can be used to monitor blood levels of a biopharmaceutical before and after the liver once it has been absorbed across the intestinal mucosa. All studies will be performed with animals under anaesthesia. At the termination of each study, the animal will be euthanized without regaining consciousness.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Novel Treatments for Neurodegenerative Diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neurodegeneration, Alzheimer’s Disease, Parkinson’s Disease, Models, Treatments</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>
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<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>Alzheimer’s disease and Parkinson’s disease are chronic neurodegenerative conditions. There are no medications that slow or prevent these conditions and the medications that do exist only serve to provide some symptomatic relief in the early stages of the disease. As these diseases progress they create huge medical, social and economic issues. We will seek to develop animal models that allow us to understand the biology of these diseases and develop and test molecules that slow the progression of these diseases.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The work will lead to improved understanding of the biological pathways leading to neurodegeneration and create improved models that better reflect the human disease. We will test new drugs that if successful could advance into clinical trials and may lead to new drug for treating neurodegenerative diseases. This would allow people to be more independent, live longer and healthier lives and this should also reduce care burden and nursing home costs.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>It is estimated that about 1000 rats and 2000 mice will be used per year over the course 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</td><td>Many of the models used in the project will involve surgery and when animals are subjected to surgical techniques, these are always carried out under general anaesthesia with post-operative analgesia</td></tr>
</table>

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

level of severity? What will happen to the animals at the end?	and observation. Very occasionally (<0.05% incidence), administration of novel compounds can result in unexpected adverse effects that might require animals to be immediately and humanely killed, for instance seizures or respiratory distress. Should any adverse effect occur the animal will be immediately and humanely killed. At the end of the studies the animals will be humanely killed and tissues used for biochemical and histological assessment to understand the model and the effects of treatment on key molecular pathways.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible we will use *in vitro* and *in silico* methods to test compounds first and only a limited new of compounds will be tested in animals. However, the brain is complex, with several neuroanatomical loci interacting with each other in a manner that cannot be adequately modelled *in silico* or *in vitro* and responses to new compounds cannot be reliably predicted from such tests. In addition, many of the drugs used need to be tested under conditions where absorption, up-take into the brain and metabolism are present in order to see if they can indeed work under physiological conditions. Moreover, many of the models utilised in the current licence are complex in nature and involve interactions of various molecular and neuronal pathways and anatomical loci and are not possible to model *in vitro.* In addition, many of the drugs used need to be tested under conditions where absorption, up-take into the brain and metabolism are present in order to see if they can indeed work under physiological conditions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals used will be kept to a minimum by the use of good experimental design and statistical principles. Many of the behavioural tests used will utilise computerised testing procedures and allow the collection of data on multiple variables within each experiment so that any potential confounds in the interpretation of the outcome can be more easily avoided. Where possible we will use unilateral lesion or infusions so that the other hemisphere can be used as a within animal control. In the majority of experiments we will measure multiple ex vivo experimental (biochemical and histological) end-points and therefore generate multiple experimental data from the same animal. Where possible we will use a combination of behavioural testing followed by subsequent histological and/or neurochemical measures. This will allow us to use fewer animals,

	compare the effects of the manipulation and drug on both the pathology and on the function in the animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use rats and mice and focus on models that are informed by genetics, anatomical location and/or molecular mechanisms linked to the human disease states. For key proteins (amyloid, tau, synuclein) that are believed to contribute to pathology we will use transgenic mice/rats that express these proteins in key brain regions and produce relevant pathology. To refine models further we will in some cases surgically deliver a neurotoxin, gene product or pathological extract into discrete brain nuclei and "networks" that we know are implicated in a disorder (for example delivery to the dopaminergic cells bodies or terminals to model aspects of Parkinson's disease). For all models we will ensure we characterise the time course of the pathology and utilise multiple biochemical and pathological measures to ensure we have the most sensitive methods to detect changes. In all cases the animals will be monitored regularly by the scientist, the NACWO and the veterinary surgeon and any health problems dealt with accordingly.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Validation of an in vitro subcutaneous injection model</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Subcutaneous injection, in vitro replacement</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</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>Yes</td><td></td></tr>
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<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>The overall goal of these studies is to obtain hypothesis-driven data sets that will validate a recently developed in vitro system than models dynamic events at the SC injection site. We hope to establish a way to predict SC injection site events in patients and to provide an explanation for differences observed between rat and man for SC injection outcomes for some of these materials.</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>Primarily due to our lack of understanding of critical events that occur at the SC space that affect the fate of an injected biopharmaceutical, there is currently no in vitro or in vivo method to predict the bioavailability of these molecules following their administration from a particular formulation. Validation of our in vitro system would allow pharmaceutical scientists to logically select an optimized formulation, potentially improving the poor and/or highly variable outcomes observed for some of these formulations when they are tested clinically</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We have planned studies that will use a maximum of 218 rats over a 5-year period.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>All surgical procedures will be performed under terminal anaesthesia. The materials to be tested for SC bioavailability in different formulations have either been approved for human use by injection or administered safely to rats as demonstrated by previous peer-reviewed publications. Thus, there is no realistic concern that these animals will</td></tr>
</table>

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

	experience adverse effects.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Screening of potential biopharmaceutical formulations for SC injection are screened *in vivo*, typically using rats. Paradoxically, in order to validate an *in vitro* system to replace this common practice, we must use an *in vivo* model for this comparison. We will not be able to convince formulation scientists to use this *in vitro* model without this validated correlation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have organized our program to reduce this animal number based upon the outcome of certain early phase studies. Industry standards have established 4–6 animals per dosing group.
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.	While no animal model has been shown to be predictive for SC injection site events in man, we have selected the rat since this species is commonly used by scientists when testing biopharmaceutical formulations. Rats are the smallest standard animal that can accept formulation volumes of biopharmaceuticals that are comparable to those used in man. Further, rats are the smallest species that can be readily manipulated to specifically test our hypotheses to identify factors that control SC injection site outcomes and to correlate these events with our *in vitro* model.

Reproductive & developmental toxicology
Reproductive, developmental, toxicity, hazards

- Summarise your project (1-2 sentences)

  The project aim is the determination of adverse effects on reproduction and/or development in rodent and rabbit reproductive and developmental toxicity studies for submission to regulatory authorities and/or for safety assessment purposes.

- 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.

  This project is being performed so that regulatory authorities can make an informed decision concerning the known reproductive/developmental toxicity hazards associated with a drug and whether the drug should be approved for use on clinical or veterinary trials, or approved for marketing, and to prescribe conditions for its safe use.

  For chemical and agrochemicals, the project will enable regulatory authorities to make an informed decision concerning the known reproductive/developmental toxicity hazards associated with the substance and whether the substance should be approved for marketing, and to prescribe conditions of safe use and handling of the substance.

  This work is needed because the dosing period required, and the age of animals required, on general toxicity studies in rodents and/or rabbits does not cover reproduction or development from fertilisation of the egg to sexual maturation, and therefore these studies cannot be used to detect reproductive and developmental toxicity. Thus, specific study designs have been designed by the regulatory authorities to cover testing of all stages of the life cycle and these studies are performed on this project.

- Outline the general project plan.

  Studies are not required until after results are available from general toxicity studies, usually in rats and a non-rodent species.

  Dose range finding or preliminary studies are performed before a definitive study, to aid careful dose selection and so that only small numbers of animals may experience moderate adverse effects.

  For drugs, regulatory assessments of effects on reproduction and in utero development are performed first to support clinical trials. The assessment of in utero and post natal development is usually performed later as it is required for marketing approval.

  For chemicals being tested under EU REACH legislation, testing is tonnage based with regulatory screens for effects on reproduction and development performed before definitive assessment of effects on in utero development and reproduction/development across 2 successive generations.

For agrochemicals, studies of in utero development in 2 species and a definitive assessment of effects on reproduction/development across 2 successive generations.

Studies for effects on neonatal/juvenile animals for drugs, effects on the developing nervous system and effects on cholinesterase enzyme activity for agrochemicals are only performed following a specific request from the regulatory authorities.

- 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.

Dose range finding studies are usually performed before regulatory studies to have the highest prospect of achieving the desired scientific endpoints and also resulting in the least pain, suffering, distress or lasting harm in the animals. Dose range finding studies are performed on the basis of limited information and, consequently, there may be uncertainty regarding the severity of the response. However, the majority of animals would experience effects of only a mild to moderate severity, with a small number showing substantial effects. Animals showing substantial effects will be killed humanely as soon as possible or treatment will be discontinued. In the subsequent studies, the majority of animals would experience no effects or those of a mild or moderate severity.

The procedures performed include the administration of substances by various routes (e.g. oral or injection). In addition to the findings indicated above, occasionally effects may occur which are expected due to the nature of the test material (e.g. pharmaceuticals) but they are expected to be transient. Most of the dosing techniques, manipulations or investigations do not cause any lasting adverse effects but a small number of animals may show temporary moderate distress due, for example, to withdrawal of blood.

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

People will benefit from this project because their safety will be better protected. Regulatory authorities will be able to make better informed decisions when deciding whether or not to approve drugs for clinical/veterinary trials or marketing and when prescribing safe conditions of use of a drug. For chemicals/agrochemicals, regulatory authorities will be able to make better informed decisions when deciding whether or not to approve substances for marketing and when prescribing safe labelling or conditions of use of a substance.

- 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.

  It is estimated that approximately 100,000 rats and their offspring, 6,000 mice and their offspring and 8,000 rabbits and their offspring will be used during the 5 year life of this project.

  The rat is routinely used on all study types as it is the regulators preferred rodent species. The rabbit is used mainly on embryo-fetal studies as it is the regulators preferred non-rodent species.

  If justified, the mouse may be used as a replacement for either the rat or the rabbit.

  The regulatory guidance usually indicates the design and number of animals included in a definitive regulatory study; otherwise, the number used is the minimum to achieve the aims of the study.

- 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.

  There is currently no regulatory acceptable non-animal alternative to the use of animals in these studies.

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

  Regulatory studies have been designed by the authorities so that the objectives can be achieved with the animals experiencing the least possible suffering. The regulators only expect some minimal signs of toxicity in the high dose groups on all regulatory reproductive and developmental toxicity studies, and doses are therefore carefully chosen to result in the least possible suffering.

  Studies are not required until after results are available from general toxicity studies, usually in rats and a non-rodent. Dose range finding or preliminary studies are performed before a definitive study, to aid careful dose selection as described above and so that only small numbers of animals may experience moderate adverse effects.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Inflammation-induced pain mechanisms</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neuropathic pain, inflammation, electrophysiology, nociceptors, repetitive strain injury</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project will investigate the role of inflammation in neuropathic pain.

Neuropathic pain is a common, debilitating condition. Patients describe shooting, burning and aching pains as well as increased sensitivity in the skin. Unfortunately, current treatments are ineffective, due to our lack of understanding of what is causing the pain. For some patients with neuropathic pain, there is an obvious injury to the peripheral nerves (i.e. the nerves that allow the spinal cord to communicate with the muscles and skin). However, for many patients, there is often no obvious nerve injury on clinical examination. These patients are frequently diagnosed with conditions such as back pain, repetitive strain injuries or complex regional pain syndrome. In these patients, a very minor nerve injury may be sufficient to cause symptoms. Local nerve inflammation is such an injury.

The current project will investigate the effects of inflammation on peripheral nerves. It will focus on the function of axons that convey pain information. Axons are the “wires” that conduct the electrical signal within peripheral nerves. In previous animal studies it has been shown that inflammation of a peripheral nerve can cause some axons that convey pain information to switch on, sending pain signals up to the brain. In this study, we will use specific drugs (e.g. that affect inflammation) to</td></tr>
</table>

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

	examine how inflammation causes axons to switch on. These drugs will be tested on an animal model of local nerve inflammation. In conscious animals, we will also assess how inflammation affects their ability to sense touch and heat.
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)?	It is expected that this project will advance our understanding of the mechanisms that contribute towards the symptoms of neuropathic pain. If successful, it may lead to potential drug targets for the treatment of a number of common painful conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	It is expected that a maximum of 1000 rats and 500 mice will be required over the duration of the project to achieve the objectives.
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 animal model of local nerve inflammation produces a short-lived minor injury without any obvious adverse effects to the animal. Much of the work will involve using untreated animals that will be exposed under general anaesthesia to drugs or inflammatory components. All animals will be monitored carefully and immediate action will be taken if there are signs of distress.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Unfortunately, there are no alternative (non-animal) approaches to study mechanisms underlying neuropathic pain, because such mechanisms involve the immune system as well as the circulation that cannot be reproduced out of the animal or using computer simulations.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A number of measures will be taken to reduce animal use. In most cases we will be able to use the same animals for examining how drugs cause inflammation to switch on axons as well as examining how inflammation affects the ability to sense touch and heat. When tissue is required for microscopic examination, it will be taken from animals that have undergone these previous procedures. The experimental design has been previously discussed with a statistician. Groups of experimental animals will be compared to untreated animals or animals that have been operated on but no drug has been applied. We will always aim to use the least number of animals to provide a meaningful result.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The animal models in this study contrast from more commonly used models of neuropathic pain, in that the inflammation is a very minor injury that does not damage axons. From our previous experience, these procedures do not give rise to observable adverse effects. In contrast to other neuropathic pain models, our models are short lived, lasting approximately a week. Under this project licence, the species of choice is the rat, since it is the most widely used in the study of inflammatory models of pain. The techniques necessary to record from axons that convey pain have previously been undertaken on the rat with great success. Some experiments will also be carried out on genetically modified mice that have structural differences to their nerves. The doses and exposure times of the drugs to be used will be kept to a minimum.

Safety and efficacy in non-human primates
Safety, Efficacy, Pharmacology, Primate

- Summarise your project (1-2 sentences)

  This project will allow for both efficacy and safety pharmacology studies to be conducted in non-human primates. It will also be required for associated research and development studies to, for example, gain comparative data and critically evaluate new test methods, equipment and techniques.

- 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.

  Governments require, and the public expects, that substances/articles to which humans may be exposed are effective and safe and/or well-characterised. Therefore, new substances must be evaluated before they are made widely available for use; this is a mandatory legal requirement which requires the use of animals in studies to evaluate systemic exposure, efficacy and safety.

- Outline the general project plan.

  This project licence contains protocols that will enable pharmacological efficacy and safety tests of new pharmaceuticals to be carried out in non-human primates, and so satisfy specific regulatory requirements.

  For safety pharmacology, the tests are designed to evaluate the possible side effects of compounds following either short or longer-term administration by a route (e.g. oral, intravenous) that mimics the anticipated route of administration in patients (or accidental exposure route). Based on background data, a range of doses are selected up to a level that will provide a margin of safety for the compound, or a level where mild toxicity is anticipated.

  The main focus of the project will be to examine the possible short-term cardiovascular, respiratory and/or behavioural side effects of potential new medicines and, very rarely, other novel chemical entities, in conscious or anaesthetised non-human primates. A major aim will be to identify adverse changes in blood pressure, heart rate and the ECG (electrocardiogram). ECG assessments will in particular examine the potential for compounds to interfere with the rhythmic beating of the heart. Cardiovascular parameters will generally be recorded by telemetry to minimise disturbance to the monkeys.

- 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.

  Animals are dosed by the intended/likely route of human exposure, and observed regularly to monitor appearance, behaviour and clinical health. Typically investigations will focus on cardio/respiratory parameters to determine possible adverse effects caused by test compounds. Blood sampling at various times after dosing will also provide useful information regarding pharmacokinetic profiles of compounds (i.e. how the body breaks down the compounds over time), which may aid interpretation of study results. In the majority of cases these investigations will be conducted in

conscious freely moving non-human primates, however the licence also includes provision to use anaesthetised animals where appropriate.

Most animals are expected to experience no adverse effects, or only mild effects such as slight weight loss. A small percentage of animals may show more significant adverse effects indicating moderate severity, e.g. more marked weight loss or reduced activity.

Animals in surgical studies may, as a result of the surgical procedure, experience some adverse effects similar to those that might be experienced by human patients; for example, in the case of cardiovascular telemetry studies, animals may experience post operative pain/distress and possible infection. However, supportive treatments are given to eliminate or minimise these adverse effects, and all surgical procedures are performed under anaesthesia, with full peri- and post-operative analgesia (i.e. pain relieving medication).

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

  The overall benefit of this project is that it supports the development of safe, new medicines to improve the health and quality of life of patients by generating high quality data that is acceptable to regulatory authorities and enables internal decision making. Achievement of the objectives of this licence will enable safe candidate medicines to progress to the next stage of pharmaceutical development.

- 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.

  Regulations require that a non-rodent species is used for safety pharmacology studies, usually selected from either the dog, minipig or the non-human primate.

  Non-human primates will only be used when they are scientifically the only appropriate species for a particular study and no other species or non-animal alternative is available or acceptable to achieve the objectives of the study.

  Non-human primates, particularly Old World monkey species such as macaques, are phylogenetically very similar to humans and share many anatomical, physiological, pharmacological and metabolic characteristics. In the context of regulatory safety pharmacology testing, macaques (cynomolgus or rhesus monkeys) and marmosets (common marmoset) are the species that are accepted by the regulatory authorities,

  All experiments will be designed in order to achieve the scientific objectives using the minimum numbers of animals. For study types that are less well established and for which historical data within HLS may not be available, the literature will normally be consulted to help establish the group size. Alternatively, the Sponsor may have data to aid this process. The Department of Statistics and Data Management will usually be consulted particularly where the study type is not routine.

- 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.

  There are many validated non-animal or *in vitro* tests available to examine the effects of test substances on specific cellular processes. There are, however, as yet no non-

animal tests or *in vitro* test systems available that can fully replicate the effects of substances on the complex biological interactions that occur between the various cells, tissues and organs that constitute a living organism.
• Explain why the protocols and the way they are carried out should involve the least suffering. Animals are carefully monitored for clinical signs or other effects on their health and wellbeing, and in order to prevent unnecessary suffering, humane end-points are applied under appropriate veterinary guidance (e.g. treatment with the test substance will be stopped or palliative or therapeutic treatments will be given).

Multimodality cancer therapy and imaging
Cancer, imaging

- Summarise your project (1-2 sentences)

This project aims to develop novel techniques for the treatment of cancer.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

Around 1 in 3 people in the UK will be diagnosed with cancer of whom about 1 in 4 will die. This picture is similar throughout the 'developed' world. There is thus a clear need to develop new therapeutic strategies for cancer. The methods we are investigating involve (i) selective exposure of tumours to non-ionising radiation in such a way that they are killed instantaneously and (ii) studying how this treatment method may be combined with others to improve patient outcome. Before either strategy can be used to their full potential in the clinic, a number of optimisation, safety and efficacy studies are necessary.

- Outline the general project plan.

The work in this project is two pronged. Firstly we wish to understand the fundamental biology of the way the therapeutic ultrasound interacts with tissue in order to optimise the delivery of the therapy, prior to clinical testing. Secondly, we wish to investigate how coupling different cancer therapies could give significant clinical benefit in terms of long term survival of patients.

- 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 surgical aspect of this project will cause short term injury from which the animals are known to recover quickly. In addition, implantation of tumours in animals will cause harm. Both these processes can result in the deterioration of the animal's health, with associated discomfort and distress. Animals will be closely monitored for deterioration and their suffering relieved.

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

The ultimate benefits from the use of animals in this project will be the discovery and development of novel cancer therapy techniques which will give improved benefit to some cancer patients in terms of increased survival probability and reduced side-effects when compared to today's conventional treatments.

- 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.

- 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.

Before starting in vivo studies, we perform extensive studies on dead tissue (butcher's meat), and computer simulation of expected results. This gives us essential information which allows us to translate the most promising therapy strategies into animals, prior to clinical use. Animal experiments are needed for two reasons. Ethically, it is not warranted to proceed directly from the proposal of a potential cancer treatment based on laboratory evidence, to evaluation in clinical trials in human patients. Also, a therapeutic technique can only be properly evaluated under circumstances that mimic the clinical situation as closely as possible, including the physiological response to therapies. In our case, blood

flow within the body has a significant impact on the delivery of e.g. thermal cell killing caused by therapeutic ultrasound. Furthermore, by good experimental design, the numbers of animals used is kept to the absolute minimum. In in vivo studies, results will be analysed in full at the end of each phase, prior to moving on, thus ensuring that animal numbers are minimised. Where appropriate small series of pilot studies will be used to investigate the best treatment delivery techniques whilst minimising the number of animals required for the study. Mice are too small for the majority of this work, and so rats and rabbits are needed.

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

Animals will be anaesthetized for the shortest time possible to allow conduct of the procedure. They will undergo tumour implantation, a minor surgical procedure from which full recovery is rapid. Animals undergoing surgery or receiving therapy will be monitored closely in order to ensure provision of effective pain relief.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The Morphological Effects of Dietary Consistency</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>MicroCT; PET; Morphology; Dietary Consistency</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></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>~~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[10]</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>This project will investigate the effect of dietary consistency on the morphology of the developing skull and mandible in mice. It will use the latest computer imaging technology to visualise cranial and mandibular form in vivo at multiple points throughout ontogeny. Although previous studies documented cranial shape change correlated with dietary consistency, none were able to record shape data during the experimental period owing to the need to sacrifice the animal to measure shape. Thus, this work represents a major advance in this field.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This research will benefit scientists in many different disciplines. Understanding the effect of mechanical loading on the skull will be of prime importance to developmental biologists, many of whom routinely use mouse models, and to clinicians engaged in craniofacial medicine.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>This project will use animals because it aims to look at the total morphological effect of diet on the skull and mandible. As the bones and muscles are an integrated system, it is not possible to use tissue samples in isolation. Computer modelling will be used in the later stages of this research to study cranial biomechanics, but our understanding of how mechanical forces influence morphology are not sufficiently advanced to allow the entire project to proceed virtually. However, the number of animals used will be kept to the absolute minimum necessary for statistical power. The use of in vivo</td></tr>
</table>

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

	PET-CT in this project means that data can be recorded from each individual multiple times. Hence, the experimental cohort will be very small – just thirty mice, ten in each dietary group. Mice have been chosen for this project because their short lifespan will allow the entirety of cranial and mandibular development to be recorded in the same individuals, and only small animals will fit in the scanner.
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?	Three groups of mice will be reared: hard diet; soft diet; and soft then hard diet. Each mouse will be scanned using positron emission tomography-computed tomography (PET-CT) fortnightly from weaning to 16 weeks. Shape differences between dietary groups will be determined from CT using geometric morphometrics. PET will reveal areas of the skull undergoing remodelling. The bite force of each mouse will be measured prior to each scan. After the final scan the mice will be sacrificed and subjected to two microCT scans – one conventional and one with iodine staining. The stained scan will reveal muscle morphology, and the conventional microCT will be used to create 3D reconstructions of the skull for biomechanical simulation. No adverse effects are expected from PET-CT. Scan times, exposure times and the current will be kept to an absolute minimum to ensure radiation doses are well below levels detrimental to health. The soft diet should not cause any adverse effects as the nutritional content will be exactly the same as the hard diet. The lack of attrition associated with the soft diet may lead to overgrowth of the incisors, but this will be monitored and corrected with tooth clipping. Bite force testing is a well-established procedure that will result in no adverse effects.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Part of this project involves the creation of 3D computer models in which feeding will be simulated to study the effect of dietary-induced shape change on feeding performance. So, for this part of the project, animals have been replaced with virtual models. However, such replacement is not possible in the earlier part of the project because we do not yet have sufficient experimental data that could validate such a model. One of the aims of this research is to provide suitable experimental data so that more future work can proceed via computer simulation rather than the use of animals.
2. Reduction	The project has been designed so that each dietary

Explain how you will assure the use of minimum numbers of animals	group contains the minimum number of animals necessary to be able to distinguish between them statistically. The use of *in vivo* scanning techniques greatly reduces the number of animals necessary in this project, as each individual will be scanned at seven points throughout its life. Thus the quantity of data retrieved from each individual is dramatically increased. Furthermore, the same mice will then be scanned post-mortem with high-resolution CT to provide anatomical data from which to build computer models, and with contrast-enhanced CT to provide information on the masticatory muscles.
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.	This project will use C57B1/6 mice. This species is particularly suitable for this project as the short lifespan will allow the development of the skull and mandible to be studied in their entirety within the same individuals throughout the course of the project. The use of imaging techniques in this project will result in very little, if any, suffering to the experimental animals. In order to prevent any distress, the mice will be imaged under anaesthetic (with recovery). Scans will take place at two-week intervals to give the mice ample recovery time between anaesthetics. The soft-consistency diet may result in overgrowth and malocclusion of the incisors. As this is an expected outcome, we will check the mice for such problems on a daily basis and perform tooth clipping (with a high-speed dental burr under anaesthesia) where necessary. The mice will be weighed on a weekly basis to monitor any weight gain differences between the groups.

Rodent regulatory genotoxicity
Genotoxicity, Cytoxicity, Rodents

- Summarise your project (1-2 sentences)

The project aim is the determination of scientific and/or regulatory endpoints in rodent genetic toxicology tests for submission to regulatory authorities and/or for safety assessment purposes.

- 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.

Governments require and the public expects that substances (e.g. agrochemicals, industrial chemicals, pharmaceuticals, medical devices, microbes used as pest control agents (MPCAs) and oncolytic viruses used to kill cancer cells) that we are potentially, or actually, exposed to are safe or their hazards are well understood.

- Outline the general project plan.

The preliminary toxicity tests, micronucleus test, comet test and UDS assay in this project are designed determine specific genotoxicity, cytotoxicity or regulatory endpoints and/or for safety evaluation.

- 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 procedures performed include the administration of substances by various routes (e.g. oral or injection). In addition to the findings indicated above, occasionally effects may occur which are expected due to the nature of the test material (e.g. pharmaceuticals), but they are not expected to persist for longer than a 24-hour period. Most of the dosing techniques, manipulations or investigations do not cause any lasting adverse effects, but a small number of animals may show temporary moderate distress due, for example, to restraint, confinement and withdrawal of blood.

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

The information gained from the studies performed under this project can be used by medical, health and safety practitioners, and toxicologists etc. to assess the relative safety of the substances being used, abused or handled and therefore develop appropriate strategies for the treatment or safe handling of the substances.

In addition, the information can be used to assist in the selection of dose levels for repeat dose studies in rodents and non-rodents with a higher degree of confidence and therefore minimise animal use and the severity of findings in later studies. Study designs can therefore be developed that cause the least pain, suffering, distress or lasting harm and which have the highest prospect of achieving the desired scientific endpoints.

- 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.

There is currently no regulatory or scientifically acceptable alternative to the use of animals in these studies. Rodents are used as they are required and accepted by the regulatory authorities for these study types. Approximately 3500 rats and 2500 mice will be used of the 5 year duration of this project license. The regulatory guidance usually indicates the number of animals included in a study; otherwise, the number used is the minimum to achieve the aims of the study.

- 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.

There is currently no regulatory or scientifically acceptable alternative to the use of animals in these studies. Rodents are used as they are required and accepted by the regulatory authorities for these study types.

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

To prevent unnecessary pain and suffering to animals and refine the studies, a tiered approach to safety testing is generally adopted. All available information will be reviewed to decide whether testing is acceptable. If acceptable, a logical sequence to testing will be determined. The majority of animals on these studies would be expected to experience no effects or those of a mild to moderate severity during the dosing and/or observation phases of the study. However, in order to achieve scientific and regulatory objectives in the preliminary toxicity tests, some animals may show severe effects (such as overt clinical signs, effects on bodyweight) and/or mortality.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Molecular mechanisms of pain</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Nociception; pain; sensory neuron; ion channel; G protein coupled receptor</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[11]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><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>Pain relief is a major clinical aim from which everyone could benefit at some time in their lives. The most problematic are chronic pains which are most difficult (sometimes impossible) to treat with conventional analgesics. British Pain Society estimates that the UK healthcare costs associated with chronic back pain treatment alone reaches £5 billion annually. This project aims to investigate molecular mechanisms of pain within the peripheral nervous system. We would like to understand main mechanisms controlling excitability of peripheral nerves and to determine how these mechanisms change during the development of acute and chronic pain. Such understanding is necessary for development of better pain therapeutics.</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 believe that our studies may lead to the development of improved, novel means by which both acute and chronic pain can be controlled; these new means may reduce side effects of analgesia (e.g. CNS side effects). Thus, the ultimate impact of this research should be with patients suffering from acute or chronic pain. The BPS estimates a national cost of treatment of back pain alone to reach £5 billion annually. Enabling individuals to return to work more promptly, or indeed to avoid absences, through the development of new approaches to pain control, may have a tremendous positive impact on national economy and, therefore, the nation’s international</td></tr>
</table>

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

	competitiveness, which in turn should further enhance individuals' quality of life. A more immediate impact is likely to affect those active in the field of pain physiology i.e. researchers (both in academia and in industry) with an interest in physiology and regulation of sensory nerve activity.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use rats and mice. We estimate usage of approximately 300 rats and 150 mice p.a.
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 of the experiments (pain models) will be of moderate severity. Lesions to peripheral nerves or peripheral inflammation may result in moderate hyperalgesia and in some distress associated with it. At the end of each experiment animals will be humanely sacrificed using the Schedule 1 procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no adequate in vitro or mathematical models for pain; yet, pain management is an unmet clinical need as many types of pain (i.e. neuropathic pain) cannot be treated with current medications. Therefore the experiments with mammals are necessary. However, our programme does involve a large body of experiments with in vitro expression systems to replace animal tissue.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our aim is to reduce the number of animal experiments whenever possible. A large share of our experiments is done with cultured neurons. This is a very efficient way of animal usage since a culture from one rat usually provides enough cells for up to a week of experiments. In the in vivo experiments we will keep the group size to a minimum sufficient to detect significant changes between the groups. Power calculations suggest that group of 6 animals will generally be sufficient.
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 species of choice for this work are rat and mice. These species offer a well characterised model that is used widely in PNS research. Majority of the current literature within pain research area is based on the studies conducted on rats and mice and most of our previous data were also obtained in these species. Moreover, the organisation of peripheral nervous system between rodents and human is more similar than that of CNS. We will only use pain models that are well established in the field. In most cases in these models animals only experience relatively mild distress, close to the threshold of feeling discomfort. As animals are checked daily, signs of significant discomfort will result in immediate sacrifice of the animal with

	humane schedule 1 procedure.

Project Title (max. 50 characters)	Interneuron development in the mammalian forebrain
Key Words (max. 5 words)	Brain development, autism, schizophrenia, nerve cells, network function
Expected duration of the project (yrs)	5
Purpose of the project (as in Article 5)[13]	Basic research: Yes / ~~No~~ Translational and applied research: Yes / ~~No~~ Regulatory use and routine production: ~~Yes~~ / No Protection of the natural environment in the interests of the health or welfare of humans or animals: ~~Yes~~ / No Preservation of species: ~~Yes~~ / No Higher education or training: ~~Yes~~ / No Forensic enquiries: ~~Yes~~ / No Maintenance of colonies of genetically altered animals[14]: ~~Yes~~ / No
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our brains are fundamental to whom we are - governing processes such as learning, memory and language, and central to these actions is a huge array of cells whose diversity has proven to be an obstacle to our understanding of brain function and conversely dysfunction. One approach to resolving this conundrum is to investigate the rules that govern the developing brain as this lays the foundation for the amazing processing power of our brains. At present we define two categories of nerve cell in the forebrain: excitatory pyramidal cells and local, inhibitory nerve cells termed interneurons. Although the latter are only a minor component of the total number of cells in the brain, they are critical to normal function. Our research relies on the power of genetics to interrogate the contribution of interneurons to emergent brain activity and dissect when and how these cells go wrong in models of autism and schizophrenia. Our purpose: to gain an understanding of the early brain that will (1) establish a set of rules for the more complex adult brain; (2) provide the foundation for a better understanding of these psychiatric conditions. This approach has proven hard to pursue in the past due to the dynamic nature of the developing brain and the difficulty in targeting specific cells. To overcome this, we will make use of genes crucial for cell identity. Our recent findings have revealed that the fate of a cell is specified early on in the embryo in response to a genetic code, which acts through a cascade of checkpoints

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

	to generate the diversity present in the adult. Unfortunately from then on the story is still largely incomplete and we have a limited knowledge of how specific classes of interneuron become functional in the newborn brain. By resolving this further we will have the cornerstone to probe the newborn brain in more detail. Specifically, this project will identify when and how interneuron deficits trigger abnormal brain development in mouse models of autism and schizophrenia. This will significantly advance our understanding of these conditions and enable researchers, including ourselves, to design targeted therapeutic interventions that will have the capability to restore to some degree normal brain function in these model systems. Ultimately it is hoped that we can then translate these approaches to human developmental psychiatric disorders.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will identify when and how interneuron deficits trigger abnormal brain development in mouse models of autism and schizophrenia. This will significantly advance our understanding of these conditions and enable researchers, including ourselves, to design targeted therapeutic interventions that will have the capability to restore to some degree normal brain function in these model systems. Ultimately it is hoped that we can then translate these approaches to human developmental psychiatric disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	The project is reliant on a number of genetically modified mouse lines. These are currently the preferred model system for a number of groups across the world as they represent the lowest and best vertebrate group in which to study the cellular and network properties of defined neuronal populations. The nature of our research – tracking the emergence of the brain networks over a prolonged period of development means that we need to breed and use large numbers than would be expected when studying adult neurological conditions. We predict that we will use up to 3,200 animals for breeding during the 5 year period of this project and a further 2,550 for surgical techniques and tissue biopsies.
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?	To achieve our scientific goals we need to breed and maintain a number of genetically modified mouse lines that replicate human neuro-developmental psychiatric conditions. These exhibit some mild adverse effects – for example mouse models of autism display behavioural abnormalities similar to those observed in humans (e.g. withdrawn, antisocial behaviour). To probe these conditions we need to be able to alter the genetic make-up of these animals either to track/manipulate

	the cells of interest or trigger a potential therapeutic intervention. On occasion this will require us to inject embryos with DNA via a surgical procedure. This can lead to complications in pregnancy. Finally to judge the efficacy of our intervention we will need to perform a battery of tests on the brain of both normal and mutant mice. This requires us to take tissue samples from the animals under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We cannot yet replicate the complexity of brain development using other alternatives. We are exploring and working with other groups to develop both cell culture systems and computer models that can replicate some aspects of development. The aim being to replicate and better predict facets of this highly dynamic and intricate process. Ultimately these models could be used to interpret human imaging data.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The hope is that our development of cell culture and computer models will enable us to more effectively target our research and thereby greatly reduce the number of animal used in experiments. We currently use genetics to label cells of interest with a fluorescent tag such that we can selectively record from them in this highly complex structure. This refinement means that we obtain high quality data from far fewer animals then used by researchers prior to the advent of this technology.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The project is reliant on a number of genetically modified mouse lines. These are currently the preferred model system for a number of groups across the world as they represent the lowest and best vertebrate group in which to study the cellular and network properties of defined neuronal populations. Our use of developmental genetics allows us to very efficiently target the cell types we believe are responsible for autism and schizophrenia. For all of our protocols we have defined our anaesthetic regime, possible outcomes and humane end points. These have been scrutinised by the veterinary and research communities, to avoid the occurrence of unnecessary pain and suffering to our mice. We constantly strive to further improve our studies in the light of our observations as well as new developments in anaesthesiology and surgery.