Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 2

Projects with a primary purpose of: Translational and Applied research – Human Nervous and Mental Disorders

Project Titles and Keywords

1. **Microdialysis to profile novel drugs**
    - Drugs, CNS, Mode of action

2. **Schwann cell development and function**
    - Schwann, regeneration, myelination, schwannoma, nervous system

3. **Novel Treatments for CNS Disorders**
    - Neuropsychiatric; neurodegeneration; models; treatment; neurochemistry

4. **Modelling and therapeutics for neurodegeneration**
    - Motor neurone disease, Gene therapy

5. **Investigations into Disorders of Movement**
    - Parkinson's, Dystonia, neurodegeneration, movement, dyskinesia

6. **Development of Novel Analgesics**
    - Analgesia, Inflammatory Pain, Neuropathic Pain

7. **Understanding cerebral folate metabolism**
    - Hydrocephalus, brain development, folate

8. **Mechanisms and Treatments for Neuropsychiatric Diseases**
    - Schizophrenia, Cognition, Brain Imaging, Pharmacology

<table>
<tr><td>PROJECT 1</td><td>Microdialysis to profile novel drugs</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Drugs, CNS, Mode of action</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></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>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>Psychiatric, neurological and metabolic disorders are a major problem for sufferers, healthcare providers (including friends and family) and society. It is clear that there can be a considerable overlap not only between psychiatric conditions, e.g. depression and anxiety, but also between neurological and psychiatric disorders, e.g. severe depression in 25% of Parkinson’s disease patients. There is an enormous unmet need for more efficacious and safer drugs to treat these disorders compared to those currently available. The main purpose of this project license is provide highly specialised preclinical services to the pharmaceutical and biotech industry to evaluate the efficacy, mode of action and side effects of novel drugs and novel pharmacological targets for the treatment of CNS disorders. There is a real demand</td></tr>
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	for these services from pharmaceutical and biotech companies due lack of appropriate expertise or laboratory facilities in-house and/or capacity issues.
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)?	Work carried out under this project licence will identify more efficacious and safer compounds to treat psychiatric, neurological and metabolic disorders. The substances tested are being considered for development into new medicines for the treatment of human diseases where there is clear unmet need for improved (efficacy/safety) medications.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 2500 rats and 750 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All of the animals used under this proposed project licence will undergo surgical implantation of one or more dialysis probe/probe holders into the brain and in some cases a jugular or femoral catheter, all carried out under anaesthesia. It is possible that surgical and post-surgical complications may occasionally arise, e.g. anaesthetic overdose during surgery or probes may be damaged during the experiment but the likely incidence of this is rare (<1% overall). Animals tolerate this surgical intervention well and no adverse effects on their overall condition are expected as a consequence of the procedures carried out. As all animals will undergo surgery, the likely/expected level of severity of the license is moderate. At the end of procedures animals will be humanely killed to allow post mortem examination and collection of tissues for analysis if required
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although in vitro techniques, e.g. the use of cell-lines, cells and tissues, provide essential characterisation of the pharmacological mode of action and pharmacokinetics/pharmacodynamics of drugs/compounds, the information obtained will always relate to part of, rather than a whole animal.

	There are currently no available in vitro alternatives to the in vivo model of microdialysis described in this licence as it is used to assess the integrated behavioural and/or physiological/pharmacological responses of the whole animal to the different treatments. The use of living animals for PK/PD studies is a Regulatory Authority requirement for the development of new candidate molecules.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used will be kept to a minimum by:- i) careful experimental design specifically to target the experimental objective and obtain the maximum information from each animal used and ii) important regard for suitable controls (i.e. the use of positive controls and the design of experiment to allow the most suitable statistical analysis to be performed). To assist in this process, we employ a fully qualified, highly experienced biostatistician who will advise on experimental design.
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 have been chosen as details about their central nervous system have been well-documented and they are the lowest form of mammal that can provide meaningful data about man. Most studies will employ normal adult healthy animals. Occasionally, genetically-altered animals may be used to model specific CNS disorders or provide proof of concept for novel targets for treatment of CNS disorders. Animals displaying adverse phenotypes will not be used. The technique of brain microdialysis is a well established approach for elucidating changes in the synaptic concentrations of neurotransmitters due to physiological mechanisms, e.g. firing rate, and/or pharmacological effects, e.g. reuptake inhibition, induction of release. In comparison to in vitro and ex vivo techniques, microdialysis is performed in the brains of intact, freely-moving animals. Thus, the neurotransmitter changes in the specific brain region under investigation are subject to the normal physiological regulatory influences of a complex neuronal matrix where pharmacological action is modulated by firing rate, autoinhibitory control,

	feedback neuronal loops and excitatory and inhibitory neurotransmitter systems. Crucially, function is also not compromised by factors such as anaesthesia and as sequential samples are taken, it is possible to determine the profile of drug action on neurotransmitters and/or their metabolites, e.g. time of onset, magnitude of effect and duration of action; all of which are essential data in the evaluation of new centrally-acting drugs/compounds. Substances will be given by the least severe route of administration. If substances have not been given to animals before, pilot studies will be performed. Suitable anaesthesia/ analgesia will be used and all animals will receive the highest possible standard of post-operative care. The project is supported by a dedicated animal husbandry and technical support team. Studies will be conducted by staff highly-experienced in animal handling and surgical techniques. Animals on study will be monitored closely and veterinary advice will be promptly sought should it be needed. Consideration will always be given to ways to minimise any welfare costs to the animals.

<table>
<tr><td>PROJECT 2</td><td colspan="3">Schwann cell development and function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Schwann, regeneration, myelination, schwannoma, nervous system</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[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 colspan="3">Our nervous system is divided into two parts, the central nervous system (CNS), the brain and spinal cord, and the peripheral nervous system (PNS). The nerves of the PNS carry information that allows us to move our muscles, breathe and sense our environment. Our size means that information has to be carried quickly over long distances and this is achieved by insulating nerve fibres in the PNS. This job of insulating or myelination is carried out by specialised cells called Schwann cells. Malfunction of Schwann cells occurs in many common clinical conditions, such as Charcot-Marie-Tooth peripheral de-myelinating neuropathies, Guillain-Barre syndrome and even leprosy. Approximately 50% of patients with diabetes will develop diabetic neuropathy leading to loss of both sensory and</td></tr>
</table>

	motor functions of the PNS. Abnormal proliferation of Schwann cells causes tumours seen in patients with neurofibromatosis types 1 and 2. There are currently no effective treatments for any of these conditions. Another role for Schwann cells is in the repair of the PNS following trauma. Although the PNS can repair itself, in practice this rarely leads to a full functional recovery; there is almost always some sensory or motor deficit.
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)?	We aim to understand better the biology of Schwann cells, their role in development, PNS repair and in tumours of the PNS. Our aim is to move this basic understanding quickly into the use of potential disease modifying treatments and therapies for these debilitating conditions. Our specific aims are: 1. To characterise the signalling in Schwann cells that governs their normal function in the PNS. 2. To identify changes in Schwann cell function that underlie demyelinating neuropathies, sub-optimal repair of the PNS and tumour formation in the PNS. Pathological changes in the PNS that affect Schwann cell function are relatively common eg. peripheral neuropathies such as Charcot-Marie-Tooth occur at 1 in 2500 people; dysfunction of the PNS occurs in 50% of patients with diabetes. Currently there are no treatments for such conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	For mice, we would plan to use approximately 1200 per year and for rats approximately 300 per year. For about 50% of the rats used, these would be for the preparation of cell cultures from neonatal animals. For the genetically modified mice, about 50% of these will have no abnormal phenotype and will be used as controls and the rest will have a mild phenotype.
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	During the breeding and during experiments with animals, the animals are monitored daily by staff with extensive training and expertise in animal care. In the unlikely event of an infection or significant discomfort, the named veterinary surgeon will be

happen to the animals at the end?	notified and the animal(s) will be given antibiotics and analgesia under veterinary supervision. If symptoms persist >24 hours, then the animal will be humanely killed. A significant proportion of the animals used for cell culture experiments (30% approx.) will be killed humanely without any experimentation. Some animals will suffer mild discomfort due to disruption or injury to a nerve on one leg, which is achieved by an operation using general anaesthesia, pain relief is used perioperatively. A proportion of the mice will have altered function of the PNS due to genetic changes the effects of which are generally mild and not detectable without detailed examination of nerves themselves, although some changes may be visible in those mice that have undergone nerve injury. In some experiments, simple measures may be used to assess the behaviour of the animal in tests that measure balance, footprint analysis or sensitivity to touch to measure any possible impairment. At the end of the experiments, animals are humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	A significant amount of the work we do relies upon using cell culture systems, but the development and repair of the nervous system involves a complex interaction between many different cells types and therefore cannot, unfortunately, be accurately modelled in vitro. We can get useful information from the cell culture experiments but the validation of these results needs to be performed using animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of replicate experiments in our work is always kept to a minimum. In the case of cell culture experiments the maximum amount of PNS tissue is taken from each animal, expanded in culture as far as is possible and cells frozen for later use. Pilot in vivo experiments (2 or 3 animals of each genotype) are performed and, if positive, numbers expanded to n=4 or 5 to ensure statistically significant results. Precise n numbers

	used for each experiment will depend upon the scatter of the data and the appropriate statistical test(s) used to analyse the data set; advice will be taken from medical statisticians within the University where appropriate. Strict training and supervision of PIL holders will also ensure that procedures carried out are reproducible and consistent for all work carried out. Where possible, the strategy for breeding of transgenic knockout mice will be set to minimise the numbers bred to ensure the maximum number of mice bred are of the correct genotype.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use mice and rats for these studies as there are a large number of tools (antibodies, growth factors and molecular biology methods) available for these two species; far more than for any other species. Furthermore, mice and rats have a nervous system that is much more similar to that of a human compared to other model organisms such as fruitflies, fish or worms.

<table>
<tr><td>PROJECT 3</td><td colspan="3">Novel Treatments for CNS Disorders</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Neuropsychiatric; neurodegeneration; models; treatment; neurochemistry</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 section 5C(3)[5]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">For a number of disorders, like schizophrenia, depression, Alzheimer’s disease and Parkinson’s disease, current therapy is unsatisfactory in terms of the level of undesirable side effects, lack of efficacy or delayed time for onset of clinical effects. Moreover, as these diseases progress they can 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 will hopefully relieve their symptoms as well as slow the progression of the disease.</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</td><td colspan="3">It is hoped that the project will increase further our understanding of the role of key biological targets and pathways in the brain responsible for controlling brain function in normal animals and in models of disease. The work will lead to improved models that better reflect the human disease, and</td></tr>
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project)?	we will test new drugs, that if successful, could advance into clinical trials and lead to new drugs for treating psychiatric or neurodegenerative diseases. Improved treatments would allow people to be more independent, live longer and healthier lives, as well as reduce care burden and nursing home costs.
What species and approximate numbers of animals do you expect to use over what period of time?	It is estimated that about 1000 rats and 1500 mice will be used per year over the course of the project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Many of the procedures used in the project will involve surgery, and when animals are subjected to surgical techniques, these are always carried out under general anaesthesia with perioperative analgesia and close postoperative monitoring. Very occasionally (<0.05% incidence), administration of novel compounds can result in unexpected adverse effects, for instance seizures or respiratory distress, that might require an animal to be immediately and humanely killed. At the end of a study, animals will be humanely killed and tissues may be obtained for subsequent biochemical and histological assessment, for instance, to understand a particular model, or the effects of drug 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	Compounds are tested first using *in vitro* and *in silico* methods, and only a relatively few of these progress to require testing in living animals. However, the processes that give rise to chronic neurodegenerative conditions occur over a long period of time, involve multiple neurotransmitter and signalling pathways, and the interaction of deposited proteins in discrete brain regions, which is not possible to replicate *in vitro.* In addition, for evaluating potential drug candidates, compounds have 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. Where new surgical models are being developed, smaller "pilot" studies will be carried out to optimise experimental conditions and protocols. For studies requiring brain pathways or brain regions to be lesioned, we will endeavour to perform these unilaterally (on one side) so that the other hemisphere, or side, can be used as a "within animal" control. In the majority of experiments we will measure multiple ex vivo or in vivo (e.g. neurochemical or biochemical) end-points and therefore generate multiple data sets from the same animal. Based on previous experience it is unlikely that the numbers stated will be used. The models in the Licence are very labour intensive and their use will depend on Company priorities and the number of compounds coming through from initial *in vitro* drug screening. However, to allow for alterations in research priorities, the figures for projected usage cited in this document are based on maximum possible usage for each individual test.
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 will be the species of choice for all protocols described in the Licence in order to align with other *ex vivo* and *in vivo* models utilised in the Eli Lilly drug discovery process. Rodents have been utilised for many years to model behavioural, biochemical and pathological changes relevant to neuropsychiatric and neurodegenerative disorders. Both rats and mice have a gross functional neuroanatomy that is broadly comparable to that of man. For example both have a nigrostriatal projection pathway, which can influence movement and can be lesioned with neurotoxins as a model of Parkinson's disease. Both have hippocampal and cortical structures that are known to play important roles in learning and memory in a similar way to humans. In some studies, it may be necessary to use animals that have been genetically altered to remove, modify, under-express or over-express a particular protein target implicated in the disease.

	The analysis of the neurochemical phenotype of transgenic mouse strains may provide a way of investigating disease related gene products and may provide a model against which to test potential new medicines. 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 4</td><td colspan="3">Modelling and therapeutics for neurodegeneration</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Motor neuron disease, Gene therapy</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 section 5C(3)[7]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[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 colspan="3">Virus carriers modified to remove all their harmful properties are being used for human clinical trials. The same carriers can also be used to generate animal models of human diseases. Our ultimate aim is to develop novel therapeutic strategies for diseases of the brain and the spinal cord (e.g. motor neuron disease). To achieve our ultimate goal our strategy is to:

1) develop novel models of neurodegeneration using virus carriers;

2) investigate how alteration of genes associated with human disease cause cell (motor neuron) death. This can help identify novel therapeutic targets;

3) obtain neuropathological, biochemical, behavioural and neurological landmarks of disease</td></tr>
</table>

	progression in transgenic and other mutant rodent strains in which genes linked to the pathogenesis of neurodegeneration are altered. 4) utilise virus carrier systems to test therapeutic strategies in our transgenics and newly created animal models.
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)?	1) The current widely used models have been generated using a method called transgenesis, a long process which requires various procedures and backcrossing of several generations (F1 to F7). Obviously this process requires the use of large animal numbers and few years to complete. Virus carrier methodology would speed up the process and allow reduction of animal use. 2) Animals will be generated when needed. We don't intend to generate colonies for long term breeding and maintenance. 3) The use of animal models will allow a greater understanding of the molecular and cellular mechanisms by which neurons selectively degenerate and die in in these devastating diseases. 4) Such information will reveal new targets for therapeutic intervention in which we will test in preclinical studies. There are no effective treatments for motor neuron diseases. It is thus important to test new therapeutic approaches and this involves assays in cellular and animal models of these diseases. By performing such studies, we will be able to determine whether a particular approach is likely to be useful in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats Estimated numbers of animals: 4275
In the context of what you propose to do to the animals, what are the expected adverse	Modelling will be achieved using virus carriers to manipulate genes in wild type mice. The animals liable to develop neurodegenerative disease will be

effects and the likely/expected level of severity? What will happen to the animals at the end?	allowed to develop some clinical signs of motoneurone disease, eg tremor, abnormal gait, partial limb paralysis, but will be killed humanely before the development of complete paralysis of any limb. Our protocols cause the least pain, suffering, distress or lasting harm consistent with achieving our scientific objectives. Some therapeutic studies require the use of protocols of substantial severity. Mice suffering from paralysis we monitor them closely and optimize their housing conditions to make them as comfortable as possible. We are actively developing methods to detect very early, subtle markers of disease onset and progression. We hope that these tests will ultimately replace experiments that currently use mice with a significant burden of disease. Modelling protocols are limited to no more than moderate severity. All animals will be killed after completion of each study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have carefully considered the extent to which these experiments could be replaced by studies involving use of cells in dishes. It is important to consider the complexity of the brain and spinal cord and the connections and interactions that motor neurons make with other cell types in the spinal cord, axon shaft and at the neuromuscular junction (connection between nerve terminal and muscle). It is impossible to replicate this *in vitro*, even using primary cell cultures. We have also considered using zebrafish for our in vivo modelling. However, this was not possible because of the low efficiency of our virus carriers in zerbrafish.
2. Reduction Explain how you will assure	The numbers of animals used will be minimised as follows:- i. Our general approach is to test hypotheses in *in*

the use of minimum numbers of animals	*vitro* systems (cells in dishes) prior to more formal testing in rodents. We plan first to teste the efficiency of our virus carriers in cultured cells and having observed a positive effect, only then move on to transgenic approaches. ii. By using virus carriers for modelling the disease in rodents we will generate only the number of animals that we need for our studies. This strategy will minimise the numbers of animals by excluding the breeding and keeping large transgenic colonies. iii. Our studies will usually be staged with the aim of obtaining key pilot data on the efficacy of our approach, and in order to perform power calculations to determine an appropriate sample size for subsequent investigations. iv. We aim to design experiments that maximise use of animals for data collection. Thus we aim to use the same animals for behavioural/neurological testing, and biochemical and pathological studies where possible. v. We will continuously monitor our experimental data and refine the design of experiments and the number of animals that might be required to provide statistical relevance. Where necessary we will consult biostatisticians at the University of Sheffield for confirmation that our approaches use the minimum number of animals necessary. As part of good laboratory practice, we will write a protocol for each experiment including: a statement of the objective(s); a description of the experiment, covering such matters as the experimental treatments, details of the experimental material, and the size of the experiment (number of groups, numbers of animals/group); and an outline of the method of analysis of the results (which may include a sketch of the analysis of variance, an indication of the tabular form in which the results will be shown, and some account of the tests of significance to be made and with the treatment differences that are to be estimated).
3. Refinement	Our protocols cause the least pain, suffering, distress or lasting harm consistent with achieving

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	our scientific objectives and are limited to no more than moderate severity. We wish to use rodents for these experiments as evidence suggests that the genetically altered models (mouse) that have been generated and wild type animals are best suited to model the aspects of neurodegenerative disease that we wish to investigate. There are no other suitable vertebrate models that are available to us, which will be suitable for our proposed investigations. Although we have also considered using zebrafish but this option has been dropped because of low efficiency of our virus carriers in this model. Close monitoring will be in place for animals under our studies. We will observe the animals for body weight, morbidity, mortality, injury and intact of food and water supported by close monitoring of body weight. Any animals observed to be in poor health will be identified for further monitoring and possible anticipated study termination. Where any animals show signs of poor health or distress the NACWO and/or NVS will be informed and consulted. We work actively to minimize suffering. We have developed close links with the animal care staff at our facility and we actively involve them in decision-making. We use mice that behave in a very predictable way, allowing us to use fewer. For surgical techniques we will use appropriate anaesthesia and analgesia.

<table>
<tr><td>PROJECT 5</td><td colspan="3">Investigations into Disorders of Movement</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Parkinson’s, Dystonia, neurodegeneration, movement, dyskinesia</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)[9]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">There are a number of movement disorders which are poorly treated and for which there is no cure. The most common of these are Parkinson’s disease (PD) and Dystonia, both of which involve changes in the activity of the areas of the brain that control movement called the basal ganglia.

This project aims to find new treatments to slow the progression, treat the symptoms and reduce the incidence of chronic side effects of the existing treatments in Parkinson’s disease (PD) and dystonia. PD affects approximately 1 in 500 of the general population and 1-3% of individuals over the age of 60. At any one time there are approximately 120,000 individuals afflicted by this disorder in the UK. PD is primarily due to the slow and progressive loss of nerves in specific areas of the</td></tr>
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	brain including the substantia nigra resulting in progressive loss of control of movement as well as other symptoms including anxiety, depression, sleep disturbance and constipation. Although less common, dystonias significantly affect quality of life of sufferers from children to adults, and the contorsions that result are often painful and debilitating. The symptoms of both PD and dystonia can be treated, however, the treatments of both are associated with side effects, some of which are irreversible. Therefore, there is an unmet clinial need for treaments for these disorders that are not associated with undesirable side effects, and this is the first objective of these studies. In addition, if the progression of the PD could be slowed, then the quality of life of the sufferer would significantly improve and the burden of care to family and society will be reduced. Thus the second objective of these studies is to find new treatments that can slow the progresson of PD.
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 benefit of this work will be measured in the improvement of the treatment of Parkinson's disease and dystonia. We aim to find new treatments that can better treat the symptoms of the diseases without side effects, and to find drugs that will slow or stop the progression of Parkinson's disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice (including genetically modified mice). We expect to use less than 1000 per year.
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?	During these studies the animals may experience mild to moderate discomfort as they will undergo procedures that induce the symptoms of diseases, such as uncoordinated movement. In addition they will experience some of the side effects of the treatments for the disorders. This may include increased or decreased movement, abnormal involuntary movements, weight loss or altered gut function resulting in constipation or diarrhoea. These will only be mildly or moderately

	uncomfortable. Anaesthesia, pain killers and unilateral lesions will be used where appropriate to reduce the pain associated with surgery and the severity of the incapacity. We have introduced stringent limits for the frequency of injections, blood sampling and behavioural assessment that any one animal can experience. The majority of our studies will be short term (less than one week of treatment), however, we are searching for new treatments for long-term disorders, and we will perform extended studies to investigate the long-term effects of drug treatment. Overall, the severity of this license is expected to be moderate. At the end of the experiments the animals will be humanely killed and tissues may be investigated biochemically.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will continue to perform studies using cell culture models of PD derived from human and rodents to investigate the effects of drugs and toxins. Similarly, we will continue our analysis of drug activity using isolated tissues. However, brain function is extremely complex and neurodegenerative diseases such as Parkinson's disease and dystonia present an equally complex neuropathology with associated motor and non-motor symptoms. It is, therefore, vital to confirm the positive effects that may be apparent in an in vitro situation in a whole organism. Searches on www.frame.org.uk confirm that there are no alternatives to the use of animals for the investigation of these complex disorders of the brain. We will continue to use rodent models of PD and dystonia as these are well validated and predictive of the efficacy of therapeutic treatment. These studies provide a vital link in the progression of treatments from the preclinical to clinical environment.
2. Reduction Explain how you will assure the use of minimum numbers	For these studies the appropriate group size will be determined by power analysis allowing robust and statistically significant results. Expert advice will be obtained from statisticians as necessary, and

of animals	appropriate statistical analysis will be used. We have considerable experience in the design and performance of these types of experiments and have published extensively in peer-reviewed journals. Overall numbers of animals used has also been reduced through the use of cell culture techniques described in the previous paragraph.
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 neuropathology induced by the toxins we will use reflects that seen in PD and dystonia and therefore provides a basis for studying complex biochemical and behavioural changes. With respect to PD, the animal model that best recapitulates human Parkinson's disease is the MPTP-treated primate, however, it is unacceptable to use this model at early preclinical stages of therapy. Hence, rodent preclinical models are accepted as the most suitable for the investigation of symptomatic and neuroprotective treatments.

<table>
<tr><td>PROJECT 6</td><td>Development of Novel Analgesics</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Analgesia, Inflammatory Pain, Neuropathic Pain</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)[11]</td><td>
<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[12]</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>The identification of novel agents for the treatment of inflammatory and neuropathic pain.

According to the British Pain Society, almost 10 million Britons suffer pain almost daily, and 100 million Americans suffer chronic pain. About 20% of American adults (42 million people) report that pain disrupts their sleep more than two nights per week and more than half of all hospitalised patients experienced pain in the last days of their lives. 50-75% of patients die in moderate to severe. The TUC reported that British businesses lose an estimated 4.9 million days to employee absenteeism through work related back pain and the British Pain Society estimates that the cost of back pain to the UK exchequer is estimated to be in the region of £5 billion per annum.</td></tr>
</table>

	In addition, it is estimated that approximately 4 million people in the United States are currently suffering from painful diabetic neuropathy or post-herpetic neuralgia, two of the most prevalent types of neuropathic pain. The treatment of both inflammatory pain (in which the pain is caused by tissue damage) and neuropathic pain (where the pain is due to damage directly to nerves) is currently poor and is considered a major area of unmet need. Despite drugs being available for the treatment of these conditions, the majority of patients do not achieve effective pain management. Existing therapies have major side effects (sedation, nausea, vomiting) which can limit their effectiveness and also discourages patient compliance. Clinicians generally agree that even small improvements in safety and/or tolerability would be a good thing. Any novel therapies would be expected to have better profiles than existing treatments.
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 inflammatory and neuropathic pain and an increased understanding of the mechanisms responsible for these conditions
What species and approximate numbers of animals do you expect to use over what period of time?	6000 mice and 1200 rats over the five year lifetime of the licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of the animals used under this licence would be expected to experience moderate severity. The experiments we plan to perform mostly involve producing an insult in the animal which we will reverse using novel agents. We have expertise in running the models on this licence and the incidence of adverse events has been reduced to an absolute minimum such that now they are rarely, if ever, seen. However, should any unexpected effects be seen

	we will humanely euthanize the animal to minimise unnecessary suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Preliminary studies will be carried out in a range of in-vitro cell assays. Any substance which is selected for testing will have been examined in a number of these in-vitro tests to ensure that it has the required selectivity at the target site and has the desired affinity for the target. Furthermore, for the majority of substances there will be a high degree of confidence that the pharmacokinetic profile will be suitable for investigation. Where available, comparisons will be made with data obtained from other substances in the same class to determine which better satisfies the criteria for development. However, pain is a highly complex process requiring an input from many parts of the nervous system and so, for this reason, in-vitro testing alone is not enough to determine if new medicines will be effective analgesics. The use of animals is the only way to determine if novel medicines produce an analgesic effect.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All studies are examined by a qualified statistician who approves the study design. These designs are regularly reviewed to ensure best practice. This will ensure that minimal animal numbers are used and the correct analysis is carried out to give the best possible interpretation of the results. Statistical analysis will be used to show significance of the results and we will use statistical experts in order to ensure the correct analysis is used. Statisticians will also be consulted, where appropriate, regarding the design of studies, and the most powerful type of analysis that should be employed to analyse results. Current experience indicates that group sizes of 7-10 in rats will be used. The numbers of animals used in studies in which mice are employed are generally slightly higher, due to the larger variability found in this species, and will therefore usually be between 10 and 15. In order to control bias and variation

	animals are sourced from one supplier. Only one sex of animals is used enabling us to control, as far as possible, between experiment variability. Drug treatments are randomised based on pre-dose readings by dividing animals into relevant groups with approximately equal scores. Measurement of treatment effects are carried out with the operators blind to treatments in order to minimise bias.
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.	<u>Choice of species</u> All the studies carried out under this licence will be in rats and mice, predominantly the latter. There is a wide literature supporting their use in models of chronic pain, such as those outlined here, which will be used to provide a background to the studies prior to instigating any work. Whilst most studies will be carried out in mice, having the ability to also test in rats will allow us to provide information e.g. efficacious doses which can be used in future studies. Such studies will be required following the selection of candidate drugs and are carried out in rats. We may also use genetically modified mice in all procedures e.g. receptor knockout mice. Animals such as these will enable us to develop a better understanding of pain pathways and also provide information as to the selectivity of test substances. <u>Choice of models</u> The models we have chosen to use in this licence include those which we believe are the minimally invasive to the animal yet will provide us with most information. We have consulted with clinicians within the organisation in order to pick the most appropriate models where possible and intend to use those which model important features of the disease states. They have been extensively studied in the literature and have been validated using compounds which are in clinical use for the treatment of inflammatory and neuropathic pain.

	Minimising suffering All of the models in this licence are designed to mimic some of the symptoms observed in the human conditions and so some level of pain is inevitable. However, experience gained by the project licence holder has shown that the level of pain (indicated by hyperalgesia) experienced by the animals is not such as to cause any major changes in the welfare of the animals. For example food and water intake are normal, animals show normal growth curves and the general observed health and behaviour of the animals is unaffected by the treatments. Tests have revealed that the level of locomotor activity seen in treated animals is also no different from that observed in untreated animals. The insults used to induce any inflammation/ hyperalgesia have been assessed to be the minimum which can be carried out to cause a significant reproducible biological effect without causing excess adverse events. In all new studies undertaken the level of insult will be assessed prior to conducting further studies.

<table>
<tr><td>PROJECT 7</td><td>Understanding cerebral folate metabolism</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Hydrocephalus, brain development, folate</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)[13]</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[14]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Periconceptional maternal supplementation with folic acid has demonstrated benefits in reducing the incidence of neural defects (spina bifida) but has no clear reported benefits on other neurological condition. Many neurological conditions have now been identified with a folate problem affecting the brain but not the rest of the body. Furthermore, one of these, hydrocephalus, responds to natural folates but not the unnatural folic acid with a decrease in incidence. Understanding the precise nature of how the brain is supplied with and handles folates will allow the development of a maximised effective treatment of such conditions with a cerebral folate issue.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>With knowledge of how the brain is supplied with, and handles folates, a combination folate therapy can be defined that compensates for cerebral folate</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	insufficiency or imbalance and which can be used to maximise normal brain development in humans and animals. Too much of any folate can push the balance too far in specific directions leading to different problems so the proper dose and combination to provide a balance is essential.
What species and approximate numbers of animals do you expect to use over what period of time?	The best characterised model for human foetal-onset hydrocephalus is the hydrocephalic Texas (H-Tx) rat as well as the less well characterised hydrocephalic (hyh) mouse. These will be used to investigate the natural history of the brain folate problem underlying the development of the condition. The mouse is important as it is driven by a single gene mutation that gives hydrocephalus as opposed to the multi-gene susceptibility in the H-Tx rat. The consequence of fluid drainage problems should be the same so the mouse will give the important opportunity to test this. This will require maintaining a colony of both animals as well as using normal rats and mice as controls to see how the system develops in them. A maximum of 4000 animals from the two colonies (3000 from protocol 1 used in protocol 2) will be used as well as significantly fewer normal animals (max 2000 wild type bought in) over the course of the study but hopefully far less since we will analyse data as we go and stop experiments as we reach significance or we see them not producing significant data.
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?	Minimal adverse effects are expected from the experimental program and we therefore expect a mild or moderate severity limits. All animals will be sacrificed for brain analysis at the termination of experiments. Any unused animals produced from the hydrocephalic colonies will be processed into a brain bank for future study or supply to other researchers. Any animal showing any adverse effects likely to exceed mild-moderate levels, will be humanely killed. The H-Tx rats have a susceptibility to hydrocephalus which ranges from 20 to 100% of

	any particular litter dependent on the mother and her conditions. Stress leads to a greater incidence. Each individual neonatal rat pup is either born unaffected or affected with the degree of hydrocephalus measured by the obvious doming of the head that occurs as fluid accumulates within the brain. Hydrocephalus itself is not associated with any pain or distress but as fluid accumulates and pressure rises within the head neurological signs and symptoms indicate possible distress. These signs occur following skull plate fusion at postnatal day 10 and subsequent fluid accumulation and rising intracranial pressure. All affected animals will be culled on or before postnatal day 20 at which point they will have no more than mild clinical signs including doming of the head and some weeping and sunsetting (partial closure) of the eyes due to pressure on the occulomotor nerves. Similarly all affected hydrocephalic mice would also be culled by postnatal day 20. It is important to sample animals after day 10 as this is the point when the skull plates fuse and raised intracranial pressure is first detected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to investigate a complex condition and functional outcomes and this can only be achieved with the special colonies and by comparison to normal rats and mice. The H-Tx rat is the most well characterised model equivalent to human foetal-onset hydrocephalus and has proven to be an ideal model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will analyse data as we collect them and stop experiments as we reach significance or it is clear the experiment is showing no differences to controls or untreated animals.
3. Refinement Explain the choice of species	The objectives require a close model to the human condition and the H-Tx is widely regarded as the

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.	best model. The experimental plan involves minimal harm to animals and any possibility will be minimised by proper training of personnel.

<table>
<tr><td>PROJECT 8</td><td colspan="3">Mechanisms and Treatments for Neuropsychiatric Diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Schizophrenia, Cognition, Brain Imaging, Pharmacology</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[15]</td><td colspan="3"><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[16]</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 colspan="3">The aims of this project are to understand how genetic and environmental risk factors for neuropsychiatric and neurodegenerative disease impact on brain functioning to increase the risk of developing these disorders, and to develop preclinical rodent models that can help in the development of improved treatments for the symptoms of these diseases in patients.

Gaining a better understanding of how genetic predisposition and environmental factors contribute to causing psychiatric disease is crucial to helping patients lead a more normal life. Identifying novel compounds for the treatment of psychiatric diseases relies on the use of cell culture and other in vitro systems. Ultimately, however, these drugs need to be tested in relevant animal models before their efficacy can be further tested in clinical trials.

This project will look at what happens to behaviour, brain and neurotransmitter system functioning in rodent models relevant to neuropsychiatric and neurodegenerative disease. The effects of drugs currently used to treat patients with these disorders, along with potential new drugs, will be assessed to determine which aspects of the dysfunction seen in these models are reversed by these treatments. The data collected will be used to judge whether</td></tr>
</table>

	the certain compounds are worth developing further for clinical trials in man. Ultimately this work will be of potential direct benefit to patients since the therapies will treat those aspects of the disease that will assist in patients returning to a normal lifestyle.
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 work will provide insight into the mechanisms through which genetic and environmental risk factors for psychiatric and neurodevelopmental diseases impact on the brain. Thus identifying how the brain is dysfunctional in these disorders in human patients and identifying novel therapeutic targets and mechanisms. This work also aims to identify and validate novel drugs that may be used for the treatment of these disorders in humans, once assessed through clinical trials. These novel drugs will improve the quality of life for patients and their carer's and would reduce the societal and economic impact of these disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	This work will use both mice and rats. Over the 5 year period of this project it is estimated that the work will use 4200 mice and 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?	Animals will undergo test procedures that cause minimum stress and we will use minimal effective doses of drugs to minimise the side effects, such as sedation, experienced by animals. This is important as we are assessing subtle, clinically-relevant changes in brain function and behaviour. The behavioural tests that we utilise are unlikely to have adverse effects for the animals. The modern brain imaging protocol used is minimally invasive but may have mild adverse effects associated with tracer injection. The drug treatment protocols also used in these studies involve administration by injection. Animals will be humanely sacrificed at the end of experiments and their tissues examined.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The impact of genetic and environmental risk factors of brain connectivity, neurotransmitter system functioning and behaviour can not be adequately modelled in non-mammalian animal systems. Determining the ability of drugs to restore these abnormalities also necessitate the use of animals. Novel compounds with therapeutic potential for the treatment of neuropsychiatric and neurodegenerative diseases ultimately need to be tested in the animal models prior to work conducted during clinical trials. Rats and mice are the lowest vertebrate groups that can be used to reproduce the complex neurobiological and behavioural

	deficits, particularly the cognitive deficits, of the neuropsychiatric and neurodegenerative disorders.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The proposed experimental designs and methods of analysis of the results have been discussed with statisticians to ensure that the minimum numbers of animals are used to gain sufficient and reliable scientific insight. Where possible preliminary drug evaluation tests are conducted using *in vitro* assays. Only those compounds that show significant activity *in vitro* will be investigated *in vivo*. Wherever possible, behavioural, brain imaging and neurochemical studies will be carried out using the same animals to minimise the numbers used. When possible, neurochemical measures will be undertaken in brains from animals that have undergone behavioural tests. This approach both increases the value of the information obtained as well as reducing the number of animals required for the project overall. The numbers of animals used in these studies are reduced substantially as compared to other approaches, since a large amount of data (both neurochemical and behavioural) are generated from each animal thereby strengthening our understanding of the relationship between behaviour and brain activity.
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 rodent models we will use in this research have direct translational relevance to the psychiatric and neurodegenerative diseases we will investigate in our studies, based on the established genetics and neurobiology of these disorders. Mice offer the most refined mammalian species in the context of genetic alterations, as they are most amenable to genetic manipulation. The genetically-modified mouse strains used in this project are likely to exhibit only subtle behavioural deficits evident only under specific testing conditions. In our studies we employ a modern, less invasive method of functional brain imaging that reduces the suffering of animals. When drugs are being tested the dose tested will be the lowest required to cause effects, while minimising potential side effects/toxicity. This will minimise any potential discomfort. In any case where there is little available information on the *in vivo* effects of a compound pilot studies will be employed using incremental doses and very small group sizes prior to the initiation of a full experiment.