Home Office

NON-TECHNICAL SUMMARY

Towards mechanistic understanding and improved treatment of nervous disorders

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

neurological disorders, psychiatric disorders, genes, brain, behaviour

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To enhance understanding of how genetic alterations pertaining to human nervous disorders affect brain structure/function and behaviour.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

A wide variety of conditions come under the umbrella term 'nervous disorders' because they impair the normal functioning of the brain and affect behaviour. Neurodevelopmental disorders, such as autism and learning disability, affect the development and structure of the brain. Epilepsy is characterised by unpredictable epileptic fits or seizures. Psychiatric disorders, such as schizophrenia, alter behaviour, perceptions and mood. Neurodegenerative disorders, such as Alzheimer's disease, are characterised by the death of nerve cells in the brain. Although these disorders have different characteristics, the first sign that something is wrong is often a change in behaviour. For example, an infant with learning disability may not begin saying simple words at the expected age, or an elderly person with the first signs of Alzheimer's disease may get lost walking to the shops.

Nervous disorders can be difficult to treat effectively with currently available medications, which at best provide only symptomatic relief - not tackling the underlying cause of the disorder. For some patients, symptoms may be alleviated by the prescribed treatment, but the disease may still gradually get worse (known as disease progression). Treatments often have unwanted side effects (e.g. tiredness, tremors), which themselves may affect patient well-being, causing some patients to stop taking their medication - which could lead to symptoms returning. For other patients, symptoms cannot be alleviated at all with current treatments. There is thus an unmet need for improved treatments for nervous disorders.

It is important to undertake work to achieve the aim of this project because patients with nervous disorders often have changes in their genes compared with healthy individuals. Through the use of DNA sequencing, scientists are able to identify genetic changes that are likely to contribute to the patient's condition. In this way, we are beginning to gain an understanding of what is causing the brain to function abnormally. For example, my research group recently identified an altered gene as the likely cause of a form of learning disability. Better knowledge of what causes the onset of each nervous disorder, and the possible worsening of symptoms, will help scientists to develop better treatments.

Given the strict limitations upon invasive approaches in clinical studies, it is difficult to do the necessary experiments in human patients. Unlike taking a blood sample or a skin biopsy, it is hazardous to a patient's health to take a biopsy from their brain. Therefore, we need to consider alternative ways to study how disease-associated genetic changes cause the brain to function abnormally. One way is to introduce the same genetic change found in human patients into cells grown in the lab. This cell culture approach may tell us how the genetic change affects aspects of the health of cells grown in a Petri dish, but it cannot tell us how the genetic change affects a whole brain made up of millions of cells, nor how it affects behaviour. A more informative approach is to introduce the same genetic change into mice, and study its effects on their brain and behaviour. This is a valid approach because mice and humans share all but ~1% of each other’s genes, the brains of all mammals have the same basic components and

structure, and mice exhibit a range of behaviours that can be measured and are similar to those exhibited by human beings (e.g. learning and memory, anxiety, social interactions).

What outputs do you think you will see at the end of this project?

Outputs in the form of published scientific articles and presentations to scientific conferences and patients' groups at the end of this project are expected to emerge from new information gained on how genetic alterations pertaining to human nervous disorders affect brain structure/function and behaviour.

Who or what will benefit from these outputs, and how?

In the short-term, these outputs would benefit affected families through genetic counselling and carrier screening, and facilitate the genetic diagnosis of other patients. The latter would allow affected families with the same genetic diagnosis to contact each other and provide mutual support, and allow doctors to prescribe (and patients to receive) existing treatments known to be beneficial in other patients with the same genetic diagnosis. Other beneficiaries in the short-term would be scientists trying to understand the molecular mechanisms of nervous disorders, and those trying to develop better treatments. It may also benefit scientists studying the normal biological functions and development of the brain, because a useful way of understanding how something works is to find out what happens when it goes wrong. In the longer term, patients and their carers would be obvious beneficiaries of the development and availability of better treatments.

How will you look to maximise the outputs of this work?

I would look to maximise the outputs of this work in multiple ways.

(i) By collecting and archiving tissue from the brain and other organs of genetically altered mice and wild-type littermates, so that collaborators and ourselves can continue to study the effects of genetic changes after the mice have died.

(ii) By collaborating with other scientists who use techniques (under separate authority) in which my research group has little expertise.

(iii) By video recording mouse behavioural tests so that they can be analysed offline to look for subtle effects not detected initially.

(iv) By using non-animal approaches, such as differentiated neurones, to corroborate research findings.

(v) By publishing successful approaches in high-impact journals, but also attempting to publish less successful approaches.

(vi) By disseminating research findings to patients' group and clinicians.

Species and numbers of animals expected to be used

- Mice: 6000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

For several reasons, the project will use mice.

(i) There is extensive knowledge of their neuroanatomy, physiology, and genome.

(ii) They have a similar genetic mark up to humans, sharing all but ~1% of each other's genes.

(iii) The ability to manipulate the mouse genome permits the introduction of genetic alterations pertaining to human nervous disorders.

(iv) The techniques we intend to use have been designed for mice and shown to be successful, including a variety of behavioural tests that allow us to assess nervous disorder-related behaviours.

Given the strict limitations on studies with human patients to find out how genetic changes affect their brain structure/function and behaviour, we will use genetically altered mice that harbour genetic changes shared with nervous disorder patients. There is growing recognition that manifestations of many human disorders most likely emerge from an underlying developmental and cellular biology shared in large part with other species.

When we are using genetically altered mice to study neurodevelopmental disorders that begin early in life, such as autism, we will observe juveniles and conduct experiments on young adults. When we are using genetically altered mice to study neurodegenerative disorders that begin later in life, such as Alzheimer's disease, we will observe young adults and conduct experiments on mature adults and aged mice to determine how the disease progresses with age.

Typically, what will be done to an animal used in your project?

If only one copy of a genetic change is required to cause the human nervous disorder under study, we will breed mice harbouring one copy of the genetic change (known as heterozygous) with mice harbouring two copies of a normal gene (known as wild-type); we would expect 50% of the offspring to be heterozygous and 50% to be wild-type. If two copies of a genetic change are required to cause the human nervous disorder, we will breed heterozygous males to heterozygous females, and would expect 50% of the offspring to be heterozygous, 25% to be wild-type, and 25% to have two copies of the genetic change (known as homozygous). Typically just after weaning at 3-4 weeks of age, each pup will have a notch of tissue clipped from its ear to identify it and for DNA analysis to determine its genotype, i.e. whether it is heterozygous, homozygous or wild-type.

Typically, adult mice will be habituated to handling for 7 days prior to behavioural testing comprising a battery of up to 8 tests, including no more than one test involving electric shock, one test involving food restriction, and one test involving water restriction. The duration of an individual behavioural test can

vary from 5 minutes to 2-3 weeks, if a test requires extensive training of the mice. The behavioural testing would usually be completed within 3 months. Occasionally, to assess disease progression, 4 of the 8 tests may be repeated no more than twice, after a period of at least 3 months, such that any one animal will be subject to a maximum of 16 behavioural tests in their lifetime.

Occasionally, a substance (e.g. an experimental drug treatment for the nervous disorder) may be injected into the mice prior to the commencement of behavioural testing.

At the end of behavioural testing, the mice may be humanely killed (Schedule 1), and have their brain and organs collected, or may be retained for conventional breeding (Protocol 1 or 2) or ^{14}C-2-deoxyglucose functional brain imaging (Protocol 7) if they have not been subject to administration of substances or surgery.

Occasionally, mice may be subject to a surgical procedure for a variety of reasons, including implantation of electrodes into the brain (to record electrophysiological signals during sleep and seizures); to facilitate collection of fluids from discrete parts of the brain; or to implant a mini-osmotic pump (for continuous infusion of drugs).

What are the expected impacts and/or adverse effects for the animals during your project?

Most of the genetically altered mouse lines bred for use in this project do not show any adverse effects (*a mild rating*). However, three of the genetically altered mouse lines show reduced weight gain compared with wild-type littermates (*a moderate rating*). In one of these lines, homozgous mice also exhibit unusual repetitive hindlimb jumping in the home cage. Heterozgous mice in another of these lines exhibit stress-induced seizures in the home cage (*a moderate rating*), which are occasionally fatal (*a severe rating*). When an adverse effect is caused by the genetic change that the animal harbours, it would typically be lifelong.

Most of behavioural tests used in the project do not have adverse effects (*a moderate rating*). However, one of the protocols includes administration of a convulsant substance to evaluate seizure susceptibility. At the lowest dose, the convulsant is not expected to induce seizures in wild-type mice, but it may induce seizures lasting a few minutes in mice with increased susceptibility to seizures (*a moderate rating*). At a 1.75 times higher dose, the convulsant is expected to induce seizures in wild-type mice (*a moderate rating*).

Three of the protocols include use of surgical procedures (*a moderate rating*). The following adverse effects are expected during surgery: corneal drying (occurs regularly under anaesthesia), respiratory depression (occurs routinely), decreased body temperature (occurs routinely), and pain (occurs routinely). However, these adverse effects will be ameliorated and controlled using appropriate measures (e.g. use of appropriate analgesia).

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mice

Mild: 50%

Moderate: 47%

Severe: 3%

What will happen to animals at the end of this project?

- Killed
- Kept alive
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Biochemical, anatomical, physiological, pathological and behavioural assays all contribute to understanding the functional consequences of a genetic change. Behavioural abnormalities, such as memory loss or convulsions, are the primary symptoms and diagnostic indicators of nervous disorders. Behaviour is an emergent property of brain function, involving coordinated activity both within and external to the CNS. Changes to genes and their expression may affect brain functioning, and hence behaviour, at multiple levels.

Unlike taking a blood sample or a skin biopsy, it is hazardous to a patient's health to take a biopsy of their brain. Given the strict limitations upon invasive approaches in clinical studies with human patients, we need to consider alternative ways to study how disease-associated genetic changes cause the brain to function abnormally.

Whilst *in vitro* model systems, such as cultured cells, have enhanced our knowledge of the cellular mechanisms through which genetic changes act, they have very limited utility for determining how genetic changes confer risk for nervous disorders, primarily because cells do not exhibit behaviours. As behaviour can only be studied in intact living animals, it is necessary to use animals to achieve the aim of this project.

Our approach is to introduce the disease-associated genetic change into the mouse genome, and find out how it affects the mouse's brain and behaviour. This approach is valid because humans and mice share all but ~1% of each other’s genes, the brains of all mammals have the same basic components and structure, and mice exhibit a wide range of behaviours that can be measured and are similar to those exhibited by human beings (e.g. learning and memory, anxiety, social interactions).

Which non-animal alternatives did you consider for use in this project?

We recently began collaborating with a colleague to develop non-animal alternatives for the study of nervous disorders, namely 3-dimensional cultures called organoids. Genetic changes identified in patients with nervous disorders are introduced into human stem cells grown in the lab, which can then be turned into nerve cells and ultimately cultured into brain organoids (lab-grown 'mini-brains'). As human brain organoids are reported to recapitulate some features of the human brain, they could be used as alternatives to mice for studying the effects of genetic changes on brain development and structure.

Why were they not suitable?

Human brain organoid models are not currently suitable for use in this project because the technology is still at an early stage and too limited for organoids to replace animals. In future, *in vitro* investigations using organoids may provide some information on how genetic alterations pertaining to human nervous disorders affect brain structure/function, but not behaviour.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimated number of mice used in the project is based on the following considerations.

(i) The number of genetically altered mouse lines to be used.

(ii) The length of the project.

(iii) The number of mice per experimental group (i.e. sample size) required to detect the effects of genotype (homozygous/heterozygous vs. wild-type) and sex.

(iv) The number of mouse lines bred from heterozygous males and heterozygous females (i.e. intercrossing).

(v) The number of mouse lines bred from heterozygous males and wild-type females.

(vi) The typical number of tests to which each mouse will be subject.

(vii) Previous experience of running similar projects under two previous project licences.

(viii) The likelihood of obtaining further grant funding.

These considerations have determined our overall plans for mouse breeding, maintenance and experimentation.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

General strategies that reduce the number of animals in each experimental group necessary to obtain statistically significant results will be adopted whenever appropriate. We will ensure reduction by writing a protocol for each experiment, which will include statistically designed sample sizes (by power calculations) and by searching the literature to ensure experiments are not unnecessarily duplicated.

Typically, sample sizes are estimated using statistical methods which result in group sizes of 12 being necessary to achieve satisfactory results. Such calculations have determined our overall plans for mouse breeding, maintenance and experimentation.

To avoid the necessity of breeding new cohorts for each behavioural test, each cohort will be subject to a battery of tests, rather than a single test. Battery testing has the advantage of reducing the number of animals required. Although this approach may lead to different results compared to naïve cohorts, large differences are unlikely. Hence, although this approach has limitations, the benefits outweigh the costs.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Breeding protocols will be designed to ensure that only the required number of animals are bred, to minimise wastage. The number of new mice born within each genetically altered line will be monitored on a monthly basis to ensure that the supply of mice for experiments or line maintenance is appropriate. If the supply of mice is excessive, the number of breeding animals will be reduced by Schedule 1 killing.

When studying a homozygous genetic change, mouse numbers could be reduced further by breeding independent cohorts of wild-type and homozygous mice for comparison. However, to minimise variation, we will instead intercross heterozygotes to generate sex-matched littermate controls for our experiments, which is standard practice for behavioural studies.

To avoid the necessity of maintaining animals solely for conventional breeding, animals that have been subject only to non-invasive behavioural tests, without administration of substances or surgery, may be maintained for conventional breeding.

To avoid an excess of post-weaning mice of unknown genotype on the shelf, we will strive to ear notch mice and identify genotypes as soon as possible after weaning. In this way, mice of a particular genotype and sex that are not required for experiments or breeding can be killed (Schedule 1) before reaching sexual maturity.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Medical conditions that come under the umbrella term 'nervous disorders' typically impair the normal functioning of the brain and affect behaviour. Behavioural abnormalities, such as memory loss or convulsions, are often the primary symptoms and diagnostic indicators of nervous disorders. Patients with nervous disorders often have changes in their genes compared with healthy individuals. Through the use of DNA sequencing, scientists are able to identify genetic changes that are likely to contribute to the patient's condition.

The ability to manipulate the mouse genome permits the generation of mice with the same genetic change as in human patients. The genetically altered mice are studied to determine how the genetic change affects their brain and behaviour. This is a valid approach to understanding more about human nervous disorders because mice and humans share all but ~1% of each other’s genes, the brains of all mammals have the same basic components and structure, and mice exhibit a wide range of behaviours that can be measured and are similar to those exhibited by human beings.

To measure nervous disorder-related behaviours in mice, they will be subject to a series of behavioural tests that are not expected to cause any lasting pain or distress. The techniques we intend to use have been designed for mice and shown to be successful. During the last project licence, it was realised that the active avoidance test, which includes administration of electric shocks over a 5-day period, could be adequately replaced by the less-stressful passive avoidance test, which we were already using. Similarly, it was realised that the forced swim test, which is classified as severe, could be adequately replaced by a 'water exposure' test in which the mouse is placed in a container of water that is sufficiently shallow to permit the mouse to touch the floor with its hind paws, such that the mouse is not forced to swim.

If a genetically altered mouse line exhibits behaviours that replicate abnormalities exhibited by human patients, we may subsequently use it to test prophylactic or therapeutic strategies. Dosing with drugs by injection will cause only transient pain.

To quantify the degree to which genetic changes found in epilepsy and related nervous disorders affect susceptibility to seizures, mouse lines harbouring these genetic changes will be subject to procedures that may induce seizures. However, the seizure-inducing procedures that we have selected are designed to induce seizures only in mice with an increased susceptibility to seizures, not in wild-type control animals.

Why can’t you use animals that are less sentient?

Behaviour can only be studied in awake, living organisms, so animals that have been terminally anaesthetised would be unsuitable. Although epilepsy, fragile X syndrome and other nervous disorders can be studied in lower model organisms, most notably *Drosophila* fruit flies, their nervous system is quite different from that of mammals, with less similarity to humans. Moreover, relative to lower model organisms, the wide range of behaviours exhibited by mice (e.g. learning and memory, anxiety, social interactions) has a greater degree of similarity to human behaviours.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Before behavioural testing, mice will be handled to reduce the stress of human interactions. Behavioural tests in general do not cause pain and suffering, but there will be a limit on the total number of tests and the number of aversive stimuli given to any one animal. Tests involving electric shock will only be used at the end of the test battery if milder tests of learning and memory are either inappropriate or do not achieve the desired outcome. Moreover, mouse lines with visible neurological defects due to the genetic variant they harbour will not be subject to behavioural tests involving electric shock.

Good handling and injection technique will minimise any minor/brief distress associated with the systemic administration of test substances. Where multiple daily injections are given, the injection site will be changed to minimise pain and potential inflammation.

For surgical procedures, suitable anaesthesia and analgesia will be administered in consultation with the Named Veterinary Surgeon; any sign of suffering will be discussed with the NVS for immediate advice.

There are multiple established tests to assess seizure susceptibility in rodents. However, the methods that we have selected are the least invasive and most refined because each procedure (including an observation period) can be completed within 1 hour and is designed to induce seizures only in mice with an increased susceptibility to seizures, not in wild-type control animals. For example, in the water exposure test, the mouse is placed in a container of water that is sufficiently shallow to permit it to touch the floor with its hind paws; consequently, unlike in the forced swim test (which is classified as severe), the mouse is not forced to swim.

Planned future work includes the use of home cage video-monitoring to assess the behaviour (including incidence of spontaneous seizures) of genetically altered mice, which may eventually lead to fewer behavioural tests being conducted in this project.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

To ensure experiments are conducted in the most refined way, best practice guidance in the following publications will be followed:

(i) *Handbook of Laboratory Animal Management and Welfare*, Third Edition. September 2003. S. Wolfensohn & M. Lloyd. Blackwell Publishing Ltd. https://onlinelibrary.wiley.com/doi/book/10.1002/9780470751077

(ii) *Guiding Principles for Behavioural Laboratory Animal Science*. Edition One: November 2013. LASA. https://www.lasa.co.uk/wp-content/uploads/2018/05/LASA_BAP_BNA_ESSWAP_GP_Behavioural_LAS_Nov13.pdf

(iii) *The Design and Statistical Analysis of Animal Experiments*. March 2014. S.T. Bate & R.A. Clark. Cambridge University Press. https://doi.org/10.1017/CBO9781139344319

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The licence holder has a track-record in the application of 3Rs in their programme of work, including current NC3Rs funding for a replacement strategy and sitting on an NC3Rs grant panel. To stay informed about new advances in the 3Rs relevant to this project, we will utilise the PubMed and NC3Rs websites. If refinements to experimental procedures are published that will improve animal welfare, we will conduct pilot studies to determine whether they are suitable for adoption in this project.