



Home Office

NON-TECHNICAL SUMMARY

Discovery of novel compounds for the treatment of dementia.

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

Dementia, Neurodegeneration, Drug discovery, Alzheimer's disease, Parkinson's disease

Animal types

Life stages

Mice

adult, embryo, neonate, juvenile, pregnant

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?

Our aim is to identify and validate new mechanisms for treating dementia, to identify novel, drug-like compounds that work through these mechanisms and to demonstrate their efficacy in animal models of brain disorders such as Alzheimer's and Parkinson's disease.

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?

Dementia is a significant global health problem. There are already approximately 50 million people worldwide with dementia, and as the general population ages, this has been predicted to increase to 150 million by 2050. Dementia causes an increasing burden to sufferers, their carers and to society as a whole through the cost of healthcare.

There are currently no treatments available that stop or reverse the progression of dementia. Our project aims to identify chemical compounds that have the potential to be developed as drugs for treatment of this disorder.

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

Outputs expected at the end of this project

Information on potential targets for drug discovery.

Our first objective is to identify novel "drug targets". These are the molecules in the body which drugs interact with to exert their effects. We need to validate drug targets by demonstrating that interfering with their action causes the appropriate changes in the animal, including reducing the symptoms of disease.

- A successfully validated drug target may be the subject of a drug discovery project for further investigation as part of our internal program.
- Occasionally a drug target may be successfully validated but does not fit within our strategic aims or objectives. In this rare scenario we aim to partner with other organisations or publish the data to generate interest in the wider scientific community.
- Validation of a particular target may be unsuccessful but our data may indicate that the target molecule forms part of a pathway which may include other possible drug targets.
- Where target validation is unsuccessful, this information will also be made publicly available through publication, particularly where there is broader interest in the biology, for example in other disease areas.

Demonstration of compound efficacy in disease models

The second objective is to discover drug-like molecules that may interact with the drug target and to show that these molecules reduce disease symptoms in animal models of dementia.

- If a drug discovery project results in a novel drug-like compound with efficacy in a relevant model for dementia this will initiate efforts to further develop the compound towards clinical testing in humans, for example through partnership with a pharmaceutical company.
- Compounds that do not achieve efficacy, but nonetheless have appropriate drug-like properties may be repurposed for use in other disease areas or as tool compounds for research.

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

Data on new drug targets may be available in the short term and will benefit the our Institute through feeding into more long-term objectives or may benefit the wider field on neurodegenerative disease. We collaborate with other groups working in the same field and may share data, samples, reagents and animal models with these groups. (1-5 years).

Favourable data on compound efficacy that leads to clinical research will take longer to generate, but may have a major impact on drug discovery for dementia, due to the current lack of research in this area within the pharmaceutical industry. (3-10 years).

It is our intention that compounds we develop will eventually reach approval as drugs for dementia. The likelihood of this occurring is relatively low but the benefits to society would be considerable. (10-15 years).

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

Our institute is an academic group with expertise in preclinical drug discovery methodologies used within the pharmaceutical industry and applying these in an academic setting. We work with a wide network of collaborators in the academic sector to investigate and validate interesting and novel drug targets and have strong connections within the pharmaceutical industry to facilitate the eventual development of these ideas into drugs to benefit patients suffering from neurodegenerative disease and dementia.

As an academic group we seek to publish our findings whenever possible.

Chemical structures of molecules originating in our laboratory and the properties of these molecules may be published in the form of patents. This is necessary to protect intellectual property, but also provides a valuable source of information on the viability of drug targets and chemical structures and activity to the wider pharmaceutical industry.

Where projects are unsuccessful or no longer of interest to us, we will seek every opportunity to disseminate the work at scientific and public meetings as well as publish data in scientific journals.

Species and numbers of animals expected to be used

- Mice: 20,000

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.

All of the procedures on this project will be carried out using adult mice. Some of the mice used in our studies will have genetic modifications. In the majority of cases this will not have any impact on the health and wellbeing of the animals. In some cases we will need to use mice that have been genetically altered so that they develop symptoms of neurodegenerative disease.

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

There are several different scenarios which a mouse may experience, the following are examples:

Example 1: In many cases mice will be given a single dose of a compound by injection. After an interval of up to 48 hours the mouse will be given a terminal overdose of anaesthetic and a blood sample withdrawn from the heart.

Example 2: Genetically altered disease model mice will have their diet reduced at 6 weeks of age and be tested in touchscreen apparatus daily for three weeks. This comprises an automated testing system for learning, memory and other aspects of cognition in which mice are taught to respond by touching an image on a screen in return for a food reward. The mice will then be dosed with a virus to induce a genetic change in the brain. This will be done through a vein in the tail while the mouse is anaesthetised. The mice will be tested again in the touchscreen apparatus once a day for five weeks. At 15 weeks of age they will be given an overdose of anaesthetic and killed by perfusing a substance through the heart to preserve the animal's tissues for microscopic examination.

Example 3: At six weeks of age mice will undergo surgery under general anaesthetic to inject a substance directly into the brain. The mice will be allowed to recover for two weeks, and then will be dosed with a substance once a day using a feeding tube through the mouth. Over the same period, behavioural tests will be carried out once a week to test their ability to remain on a rotating cylinder and to cross a narrow beam. During this time the mice are expected to experience a gradual deterioration in their movement abilities, experiencing tremors and impaired coordination. At the end of the dosing period the mice will be given an overdose of anaesthetic and killed by perfusing a substance through the heart to preserve the animal's tissues for microscopic examination.

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

Dosing either by injection or through a feeding tube and blood sampling will cause mild pain and distress to an animal, but this will be short-lived.

The techniques used for behavioural testing in this project are not expected to cause pain or distress other than transient stress due to being handled. For some behavioural tests the animals' food is

restricted. This will lead to weight loss, but the general condition of the animals is not expected to be affected in other ways.

Surgical techniques involve a greater degree of pain and distress which may last for a few days after surgery. Pain relief will be given routinely to animals undergoing surgery. Occasionally (less than 2%) a surgical wound may fail to heal properly, in which case the named veterinary surgeon will be consulted. One attempt may be made to re-close the wound if this is within 48 hours of surgery, otherwise the animal will be humanely killed.

Most genetic alterations to animals used in this project do not result in any apparent change to the wellbeing or behaviour of the animals. In some of the disease models that we use, genetic alterations do lead to a progressive impairment in the animal's ability to function normally, especially where these lead to impairments in the animal's movement (loss of balance and coordination, tremors). Typically these symptoms worsen over a number of weeks (depending on the model). Once symptoms appear, mice are monitored at least three times per week and the mice are humanely killed when specified limits are reached.

Dosing of substances to induce disease symptoms may result in similar changes to the genetically altered models mentioned above. We will monitor these animals in the same way, and apply the same limits before humanely killing the animals.

Some of the substances we dose will be completely novel, and may have potential unknown side effects. These substances will be dosed at low doses initially and the animals will be closely observed for any adverse effects before we proceed to higher doses.

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)?

The expected severities are mild (approximately 75% of mice) and moderate (approximately 25% of mice).

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

- Killed

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?

When investigating novel drug treatments for disease it is necessary to understand how manipulation of the target for the drug impacts on the biology of the whole animal. There may be unwanted effects of

drug treatment in other tissues or organs which would indicate a risk of side effects if the drug were to be dosed in humans. Furthermore, we need to gain a thorough understanding of what happens to the drug when it enters the body, including the rate it is absorbed from the gut into the bloodstream, where it is distributed in the body and how quickly it is eliminated.

When considering the effects of drugs or manipulating drug targets in the brain it is important to recognise the unique complexity of this organ, with many different cell types and networks of connections between nerve cells which cannot be fully replicated outside a living animal. Furthermore, if we want to understand how potential drugs may impact on symptoms of dementia we need to be able to test their effects on learning and memory in mouse models of disease.

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

We have considered, and already use many non-animal alternatives wherever possible. These include assays using cells grown in culture for testing the activity of potential drugs and understanding the basic biology underlying their mechanism of action. In addition we make extensive use of computer based modelling of drug properties to understand how the structure of drug molecules may affect their interactions within the cell and how they are likely to be absorbed and cleared from the body.

Why were they not suitable?

These approaches are suitable for much of the work that we carry out. However, in order to fully evaluate novel drugs and their targets, which may have the potential to progress to studies in human volunteers, it is necessary to gain a thorough understanding of how they behave in a whole organism.

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?

This number is based on estimates of:

- the number of projects that we anticipate running
- the expected number of studies to validate and test potential drugs for each project
- the number of animals needed per study, using the minimum number of animals to give a statistically valid result

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

In designing experiments there are several factors that we will use to reduce unwanted variability in our data, this will reduce the number of animals needed to show that a result is not just due to chance:

- The number of animals used per treatment group will be carefully worked out using data from previous studies to carry out these calculations
- Randomisation when assigning animals to treatment groups to avoid unwanted bias from factors like cage differences, time of treatment
- When appropriate, use of baseline data when assigning animals to treatment groups to ensure that baseline measurements between groups do not differ
- We have many years of experience in study design and statistics within our group and in addition we will draw on support from colleagues who are specialists in biostatistics.

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

Other steps we will take to reduce the number of animals used in our project:

- Using animals from the same colonies, or if possible the same litters for comparison, to ensure that genetic variation between animals is kept to a minimum
- Pilot studies will be used to determine the optimal conditions for experiments, e.g. the appropriate dose levels of drugs, the length of treatment time, the age at which experiments should be started in mice
- Where possible the same individual will carry out procedures, collect and analyse the data within a single experiment, and this person will be blind to the treatment which each animal receives
- Wherever possible we will collect multiple samples from each experiment to avoid unnecessary repetition of studies, these include taking samples to determine drug efficacy, measure drug levels within tissues and investigate possible side effects all from the same experiment
- Where possible we will share information, biological samples and mouse strains with other researchers in the field.

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.

Wherever possible, wild type mice will be used in studies.

When genetically altered mice are used we will take a number of steps to avoid harm to these animals:

- In some cases the genetic alteration can be "switched on" by giving a drug either by injection or in the food. This ensures that any harm resulting from the genetic modification is experienced only during the experiment, rather than for the lifetime of the animal
- Genetically altered mouse models of disease show a progressive worsening of symptoms with age. When using these mice we will use models with the mildest symptoms and at the earliest age that are compatible with our scientific objectives.
- When using animals that have been dosed with a substance to induce disease we will carry out pilot studies to determine the minimum doses and length of time necessary for treatment that are compatible with our scientific aims.

Surgery to dose a substance directly into the brain will only be carried out if the substance cannot be dosed non-surgically by a different route, and all surgery will be carried out under general anaesthetic using aseptic techniques to minimise the chance of infection. Medication will be given for pain relief and to prevent infection.

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

Adult mice will be used in our project. Mice are regarded as a good experimental substitute for humans as much of our genetics, anatomy and physiology are closely replicated to this species. Furthermore, there are existing mouse models of neurodegenerative disease which replicate many of the features of these disorders that are seen in human patients. Adult mice need to be used as the brain does not complete development until adulthood.

We already carry out studies in lower animals (zebrafish) which contribute to this program of work. This is carried out through a collaboration on a separate licence.

It is not possible to carry out our experiments in anaesthetised animals as due to the length of time the studies typically last.

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

Examples of refinement in our experimental procedures include the following:

The rTG4510 tauopathy transgenic line shows a tendency to be hyperactive, a phenotype characterized by them running inside the cage. This behaviour can occasionally cause injury through the bedding becoming tangled around the leg of the animal. We will use a softer bedding type to prevent injury. We will also investigate providing running wheels for these mice.

In some of our studies, agents require subcutaneous implanted mini-pump delivery. We will adopt as standard practice the soaking of these pumps in saline overnight prior to implantation so as to reduce adhesion formation on the skin of the animals. All surgery is completed using aseptic techniques. Local

analgesia is applied and post-surgery monitoring in a separate recovery room is also carried out to ensure animals return to normal activity before being transferred back to normal housing. Following surgery and re-housing, they are monitored for activity, weight and wound healing for up to 7 days. This is recorded on welfare monitoring sheets.

Compounds are also delivered via oral gavage - a process requiring a flexible tubing for delivery. The tip of the tube is also dipped in a sweet solution before starting the procedure to make it more palatable for the mice to swallow.

In transgenic lines that do show disease signs, such as the Huntington's mice, welfare is regularly monitored. Since a major phenotype indicative of disease progression in these animals is weight loss, we use this to monitor welfare. They are weighed at least weekly, increasing to daily as the disease signs become clear. Before weight loss reaches 15% of maximum body weight animals are humanely killed.

When administering drugs in the animals' diet it has been found that the texture and composition of the modified diet is different from the regular diet which the mice usually eat. This has sometimes led to aversion to the modified diet and reduced food, and drug intake. When dosing drugs in the diet, we will acclimatise the mice to the modified diet without the drug for a number of days before switching to the diet with the drug.

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

PREPARE guidelines checklist for designing and planning experiments

ARRIVE guidelines to ensure reporting quality of data is optimised

LASA Guidelines for Aseptic Surgery - https://www.lasa.co.uk/current_publications

NC3Rs - <https://www.nc3rs.org.uk/3rs-resources>

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

Regular awareness of developments via websites of organisations such as NC3Rs, LASA.

Communications from University Biomedical Services and the UBS 3Rs research tool and consultation with named individuals within UBS