Home Office

NON-TECHNICAL SUMMARY

Neuron-glia biology in neurodegenerative disorders

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Ageing, Alzheimer's disease, Inflammation

Animal types	Life stages
Mice	adult, neonate, juvenile, pregnant, embryo, 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 understand the role of glia and neuron-glia interactions in neurodegeneration, particularly in Alzheimer'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?

Alzheimer’s disease accounts for around two thirds of all dementia cases (around 33 million people worldwide) with a complex cellular response and no treatments that can stop, slow or prevent it. Understanding the biological mechanisms behind the disease is essential to finding ways to treat it.

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

The project comprises basic scientific research that will increase our understanding on the role of glia (support cells in the brain) in Alzheimer's disease and neurodegeneration in general. The outputs will primarily take the form of publications in peer-reviewed journals, but will also be disseminated at academic conferences and to the lay public via popular science initiatives. Materials, data and methods may, where appropriate, be disseminated online.

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

By understanding the role different brain cell types play in neuroinflammation we can begin to dissect out the different stages in Alzheimer's disease progression. In the short-term, the primary beneficiaries will be other scientists working in the field of neuroinflammation, ageing and Alzheimer's disease. In the medium term to long term, the basic science knowledge gained may have the potential for clinical translation. For example, understanding key pathways in disease progression may identify new targets for developing new pharmaceutical or therapeutic interventions in Alzheimer's disease.

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

We will share data, analysis tools and resources between other members of our research community and this will reduce the number of replicated experiments in the field and the number that we need to do in our laboratory. In addition, we will disseminate our research by publishing results in peer reviewed journals. We aim to publish all results, including those that do not confirm our hypotheses. We will also present our work to academic peers at scientific conferences (national and international), and engage with public partners to disseminate our results.

Species and numbers of animals expected to be used

- Mice: 5000

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.

We will do these experiments in mice because of the number of available mice that are genetically suitable and the comparison with previous studies in the scientific literature. Using transgenic mice has the distinct advantage that we can use mice that express diseases, such as Alzheimer's disease. We will use adult and aged mice, since these ages represent the most relevant for the study of age-related neurodegenerative diseases.

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

Some animals will be bred, and may have dyes injected to label the blood or cell types or drugs injected to alter the function of some cell types, and will then be humanely killed in order to study their tissues.

Some animals will undergo a surgery, where a small part of their skull is replaced with a glass coverslip to give us repeated optical access to the brain. Adverse effects after the surgery may reach moderate levels of severity for a short period of time, but all animals receive pain relief and are closely monitored until they recover completely. After that time, they will have a number of non-painful behavioural tests, some of which will also involve measuring from the cells in their brain using light. These tests may be repeated several times. At the end of these behavioural measurements, the animals will be humanely sacrificed and their brain tissue may be used for post-hoc analyses.

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

The animals will undergo surgery of moderate severity. This means that the mice will be quiet and move less for a day or two after surgery. The animals will be given painkillers after surgery. Animals might lose a bit of weight, but will typically regain that weight within two to three days. Some animals will also be allowed to age (up to 24 months) meaning they may experience certain discomforts associated with the ageing process. At the end of experiments, or if mice show signs of ill health, distress or suffering that are not improved or resolved within a timeframe approved by the veterinary surgeon they will be humanely killed.

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)?

30% of mice will experience a severity category 'Mild'

70% of mice will experience a severity category 'Moderate'

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

- Killed
- 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?

Mice will be used in this project. Studying the cellular mechanisms involved in dementia and AD is extremely complex and involves understanding the interactions between different systems in the body (e.g. nervous, immune, and vascular). It is very difficult to mimic such complex interactions ex vivo, and whole animal in vivo experimentation is therefore vital in order to obtain a greater understanding.

The proposed studies could not be undertaken in lower species because they do not show such similarities to humans (e.g. they do not have similar glial cells), and in vitro experiments do not allow the study of interactions between different body systems (i.e. immune and vascular), which are critical for this project. Thus, the questions and hypotheses to be addressed cannot be fully studied in vitro alone and require in vivo studies.

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

This project will be done in parallel to studies using non-animal alternatives such as

- Human post-mortem and brain biopsy studies.
- Induced pluripotent stem cells (iPSC)
- Brain organoids

Why were they not suitable?

The use of non-animal studies will provide us with useful information that can be used to design our animal experiments. However, in vitro experiments (e.g. using induced pluripotent stem cells or brain organoids) do not allow the study of interactions between different body systems (e.g. immune, vascular and nervous) which are critical for the understanding of the physiological changes occurring in Alzheimer's disease. The use of human post-mortem and brain biopsy tissue, while helpful, will only provide us with a snippet of the cellular states at the later stages of the disease, but in order to understand disease progression we need to be able to study the brain at multiple timepoints during disease development in a living 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?

The numbers of animals have been estimated based on previous experience when dealing with complex genetic crosses in mouse breeding as well as estimates on the number of experimental animals required based on expected effect sizes from the literature, past work, and in vitro pilot experiments.

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

Several factors lead to a reduction of animal numbers, including reducing variation and good experimental design involving the use of appropriate statistics. In particular statistical tests will be used to ensure that we use the minimum number of animals possible to reliably interpret our data.

We will design our mouse breeding strategies carefully to minimize the number of generations necessary to reach the desired endpoint (i.e. desired combination of transgenes/ mutations), and to cut down the number of unwanted offspring. Where appropriate, we will use otherwise unwanted offspring for negative control experiments.

In our experimental design, we always collect as much data as possible from each animal. With longitudinal experiments, we repeatedly measure the same structures or cellular activity over a long period of weeks. This approach allows us to directly compare the structural/functional plasticity in the same synapses, cells and animal before and after sensory deprivation or alterations in behaviour. Furthermore, in each animal, we can collect data from many cells, increasing the amount of data from each animal used and reducing the overall animal number necessary.

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

Efficient breeding and holding lines as frozen down embryos and sperm will be used to minimise the number of mice being produced for these studies. Genetically modified lines will be sourced from repositories to avoid remaking of lines whenever possible. Any excess stock will be offered to other researchers to minimise wastage. Pilot studies will be undertaken to ensure the correct time course and experimental paradigms for our experimental purposes before larger scale studies. Tissues sampled from the animals used in this project will be shared with other researchers and the data produced linked to that generated by the project, to maximise long-term utility.

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.

Rodents have been chosen for this work as the least sentient species to mimic the human nervous system well enough for our work to be relevant to understanding of human brain function and disease. Mice are essential because the experiments require the use of the latest and most refined transgenic technologies to identify and label cell subtypes, to allow cell specific knock-in and knock-out approaches, to express genetically encoded indicators and optogenetic modulators and to model human diseases (e.g. Alzheimer's disease). These mouse models are not expected to exhibit any harmful phenotype.

We will be using chronic imaging in combination with cranial window surgery preparations, which allows us to follow the same cells over a period months. This preparation is ideal for examining how the same cells are changing their structure and function over time during disease progression. Cranial window surgery is necessary to allow us to image individual cells at a resolution that will allows us to draw meaningful results. This protocol minimizes suffering, as the initial surgery is the only one in which any pain may occur. All imaging sessions afterwards will only require light anaesthesia and no surgical intervention. For the surgical step appropriate anaesthetic/analgesic regimens and post-operative care will be used to minimise pain. Any surgical procedures will undergo regular review to identify further refinements to minimise animal suffering including optimisation of implants and anaesthesia/analgesia regimens.

In addition to chronic imaging, we plan to extract tissue from mice for in vitro downstream applications. These terminal experiments will take place entirely under anaesthesia and therefore will have minimal suffering. We plan to use these experiments to test a number of potential mechanisms before testing them in vivo, which will help reduce the number of animals undergoing the in vivo paradigm and that may suffer as a result.

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

Rodents will be used for this project as they represent the least sentient species appropriate for this type of work. Other species which are less sentient (such as invertebrates) cannot be used because they do not show such similarities to humans (e.g. they do not have similar glial cells which form the basis of this proposal). Mice which are genetically tractable, allowing transgenic identification and manipulation of specific cell types and there are a significant number of well characterised transgenic mouse lines available to model disease.

Animals at a more immature life stage cannot be used because this work focuses on the cellular physiology of ageing and age-related disorders and as such, we need to work in adult animals.

A number of our experiments are done in animals that have been euthanised or are terminally anaesthesised, but in order to look at long term cellular changes throughout disease progression we

need to carry out some chronic experiments.

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

The animal facility staff run a comprehensive health-monitoring programme whereby animals health and welfare are checked and recorded on a daily basis. The animals will be maintained under conditions where their health status can be protected as far as is reasonably practicable. We use refined holding techniques for the animals, as well as group housing and enrichment. We use appropriate anaesthetic/analgesic regimens (including pre- and post-operative analgesia) to minimise pain and will refine these with advice from the NVS to ensure that we are using the best possible option given our experiments.

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

Resources hosted on the NC3Rs website, in particular:

- ARRIVE guidelines on experimental design and reporting results.
- PREPARE guidelines for planning animal experiments
- 'Procedures with Care': 'Aseptic Technique in Rodent Surgery'.
- Rodent housing and husbandry
- Rat and Mouse Grimace scales

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

We will stay informed about advances in the 3Rs from several sources:

- Liaison with our animal care staff and NC3Rs representative
- Technological advances in the published scientific literature
- The NC3Rs website