Home Office

NON-TECHNICAL SUMMARY

Understanding disease mechanisms in frontotemporal dementia and motor neuron disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (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, Motor neuron disease, Inflammation, Microglia, Vascular

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult

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 investigate the mechanisms which cause damage to the brain in frontotemporal dementia (FTD) and motor neuron disease (MND), particularly the role of the immune system in disease. We also aim to investigate changes relating to blood vessels in FTD/MND.

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?

Frontotemporal dementia (FTD) is the 2nd most common form of young-onset dementia, typically affecting people in their mid to late 50s. It is a severe, debilitating and progressive disease causing personality changes, irrational behaviours, loss of inhibition, changes in social behaviour and emotional processing changes. Around 15% of people with FTD also develop motor neuron disease (MND). MND is a devastating disease which causes the nerves which control muscles to die, resulting in progressive muscle weakness, difficulty with speech, and paralysis which spreads throughout the body. MND is almost always fatal within 2-5 years of onset. Crucially, there are no effective treatments currently available for either FTD or MND, which is why research into these diseases is so important.

People with FTD and/or MND show signs of excessive inflammation in the brain. The brain’s immune system goes into overdrive, and immune cells like microglia produce inflammatory chemical signals which are thought to damage the brain in other diseases. However, this has been under studied in FTD/MND, and therefore we still don’t understand what causes the inflammation or how it affects brain function. An emerging body of evidence strongly suggests that excessive inflammation does contribute to the brain damage which causes symptoms of FTD and MND in people. If this is the case, it may be possible to treat FTD/MND in future using drugs which target the immune system.

We also know that vascular changes in the brain are important in other types of dementia like Alzheimer’s diseases, but again this has not been well studied in the context of FTD and MND. Understanding how vascular changes may contribute to FTD and MND disease progression may also lead to the discovery of new treatments.

We will use mouse models of FTD/MND to investigate both immune and vascular contributions to disease. This project will provide novel mechanistic insights which will be invaluable for development of new treatments for FTD and MND, which are urgently needed.

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

The primary outputs of this project will be greater understanding of the disease mechanisms underlying both FTD and MND, and possible identification of novel therapeutic targets for both diseases. We anticipate publication of several papers in reputable, open-access journals, and presentation of key findings at major national and international conferences throughout the project. A large dataset detailing changes in gene expression in FTD/MND will also be published, which may be used for further analysis by other groups in future to avoid use of additional animals. Finally, the project will generate novel genetically altered animals (GAAs) with altered immune function (details in Protocol 1), which will be useful for a wide range of research application in future (basic immunology research and research into many diseases beyond FTD and MND) and these will be made freely available once published.

There is high potential of findings from this project translating to clinic in future (beyond the duration of the project), as findings will direct future drug discovery research for FTD and MND.

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

Our own research group and collaborators will benefit from these outputs as they will progress our research interests and direct future our research. Other research groups will benefit from our published papers, datasets and conference presentations as we expect to make major contributions to the field which will inform future research directions.

Our ultimate goal is for FTD/MND patients and their families and carers to benefit from our work in the long-term. We hope outputs from this project will contribute to future drug discovery research in a meaningful way, improving the quality of life of those affected by these devastating diseases.

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

This project is highly collaborative, with experienced colleagues contributing technically and scientifically locally and at other institutions. This will maximise our scientific outputs.

Our findings will be published in reputable open-access journals in line with faculty guidelines and our primary funder’s policy (Alzheimer’s Society). We will also disseminate findings at conferences and in open dialogue with colleagues internally and externally and we welcome feedback, ideas and new collaborations.

Alzheimer’s Society also assigns 2-3 lay volunteers with personal experience of dementia to each grant holder to act as “monitors” for the project. We will engage regularly with our research monitors to ensure our research does not lose track of our long-term goal and stays relevant to the needs of people affected by FTD/MND. These meetings are usually a good reminder of the importance of our work and can be very motivating for early-career colleagues, which further maximises output.

Species and numbers of animals expected to be used

- Mice: 2400

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 have chosen to use mice because this is the only species for which all of the required genetically-altered strains exist, and in which the clinical and pathological features of human FTD/MND can be closely mimicked. Mouse is also the most appropriate species for the techniques required, such as behavioural tests (which will be used to measure the severity of cognitive deficits and movement problems) and imaging techniques such as MRI scans.

The project requires mice to be used as neonatal pups and maintained into adulthood. This is because FTD and MND are progressive age-related diseases where symptoms begin in adulthood and worsen over time. We will inject reagents directly into the brain of neonatal pups, to introduce a genetic alteration that mimics a known genetic cause of both FTD and MND in people. This method has been published previously and used by several other research groups successfully. After a single injection, mice develop problems with cognition and movement by three months of age, and these are more severe by six months. We aim to modify immune/vascular function in FTD/MND mice using drugs or genetic alterations, and investigate how these changes impact the severity of deficits, age of onset, or rate of progression. Therefore experiments must be performed in adulthood, after the onset of deficits. Since the deficits worsen with age, mice will only be maintained as long as necessary for each specific experimental aim.

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

Genetic alterations (GAs) are required to create mouse models which mimic the clinical and pathological features of human FTD and MND. These alterations will be achieved either by breeding stable GA mouse lines, or by injecting neonatal pups in the brain to induce the genetic change. Some mice will also have an additional GA to alter immune function, or else a drug (e.g. an anti-inflammatory drug) may be delivered via food, drinking water or by injection. The least invasive delivery method possible will always be used.

Animals will be aged (typically no more than six months), and those with FTD/MND-related GAs are expected to develop progressive cognitive deficits and movement difficulties during adulthood. We will measure these deficits using behavioural tests, for example maze tests to measure memory, or timing how long the mouse can balance on a rotating bar to test movement ability. The tests are not invasive. Several tests will be performed to ensure robust data is obtained, and this will usually be repeated two or three times throughout adulthood, for example at 3 and 6 months of age. This is to measure the severity of deficits, the age of onset, and the rate of progression. We aim to investigate whether any of these parameters can be changed by modifying immune function, which would inform future drug discovery research for FTD/MND. For example, could an anti-inflammatory drug administered in middle age delay the onset of FTD/MND symptoms, or could a drug administered after the onset of symptoms slow down their progression and improve quality of life?

A smaller number of animals will also undergo imaging procedures such as MRI scans, which will be done under general anaesthesia. We may also take blood samples (up to a maximum of six occasions

per animal, at volumes which will not cause adverse effects or distress) or measure blood pressure (usually weekly) in some mice as required.

All mice will be killed by humane methods once experiments are completed. This usually will be done under terminal anaesthesia, in a way which allows maximum collection of tissues and samples (e.g. blood, brain, spinal cord). This will allow us to perform additional experiments and maximise the data output from each animal.

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

Mice with altered immune function (induced by genetic alteration or administration of substances e.g. injection of anti-inflammatory drug) may have increased susceptibility to infection, although mice will be kept in a clean environment, so infection risk remains low. Mice with altered immune function may also exhibit sickness behaviours e.g. changes in food intake/body weight, grooming, nesting/burrowing behaviours. These adverse effects are expected to be mild in severity.

Mice expressing FTD/MND genes are expected to develop progressive cognitive and locomotor deficits reminiscent of human FTD/MND as they age. Based on published models, these may include memory impairments, altered gait and muscle weakness/movement difficulties. We expect these impairments to develop by approximately 3 months of age and continue to worsen with age. These adverse effects are necessary to investigate disease mechanisms in FTD/MND and are not expected to exceed moderate severity. Humane endpoints for locomotor deficit in particular will be observed throughout the project to ensure animals do not experience excessive distress or health complications, and that deficits do not exceed moderate severity.

The majority of our experimental aims involve understanding changes in brain function that occur early in disease, prior to the onset of cognitive/locomotor deficits, or investigating factors which impact the progression of disease. For example, we will investigate whether drugs which reduce immune function can delay the onset of clinical signs, reduce their severity, or slow their progression. As such, most experimental procedures will be performed on animals in the early stages of the disease. Since adverse effects will worsen over time, animals will not be aged any further than required for the specific aims of an experiment, to ensure adverse effects do not become more severe than is absolutely necessary.

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)?

Approximately 75% moderate and 25% mild or subthreshold.

What will happen to animals used in 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?

We need to use animals to monitor behavioural phenotypes as one of our key experimental readouts of FTD/MND disease progression and severity. We also require animals to investigate whole organs/systems which cannot adequately be replicated in vitro like investigation of blood-brain barrier (the protective barrier which keeps the brain separate from other organs, and prevents toxins getting into the brain from the blood) integrity and infiltration of immune cells into the brain.

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

We have an ongoing programme of in vitro work in this field which will continue to be used for any experimental aims which can be achieved in this way. However, the experimental aims of this particular project can only be achieved in vivo.

We also considered using unregulated animals such as C. elegans worms, Drosophila or Zebrafish larvae for this work.

Why were they not suitable?

In vitro methods would not facilitate monitoring of locomotor and cognitive function, or investigation of whole systems such as blood-brain barrier integrity or infiltration of peripheral immune cells into the brain.

We have previously been successful using worms and fish for investigation of motor phenotypes relevant to MND, and there are also published models using flies, although these models are less useful for investigation of cognitive deficits relevant to FTD. There are also conflicts in the published literature between mouse and zebrafish models of FTD/MND, with mouse models more closely mimicking the human disease.

In addition, the immune system has a great deal of inter-species variability, and therefore mammalian models are most suitable for this work. Furthermore, we require the use of in vivo imaging techniques which are not available in lesser species such as flies, worms and fish to study immune and vascular function e.g. PET scans.

Finally, mouse is the only species for which all the relevant GA strains required are available, as this project requires a combination of several different genetic modifications.

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?

We have consulted with a biostatistician with experience of in vivo behavioural testing to determine group sizes required for experimental cohorts, and estimated the number of experimental conditions and timepoints we will require to achieve our objectives.

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

We employed the NC3Rs' experimental design guidance and experimental design assistant (EDA) to plan our experimental design, practical steps and statistical analysis utilising the advice and support for randomisation and blinding, sample size calculations and appropriate statistical analysis methods. We will use the EDA diagram and report outputs to support experimental planning with animal users.

We have chosen models of FTD/MND with the least variability to reduce necessary group sizes for behavioural testing.

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

For any questions which could be answered using alternative methods such as immortalised cell culture, we will do this instead of using animals.

At the end of experiments, we will harvest as many tissues as possible at post-mortem. If we don’t need to analyse the tissues immediately, we will freeze them and make them available to other researchers working on similar questions. We will also share organs used for primary cell culture preparations with colleagues (e.g. thigh bones to isolate immune cells from bone marrow) to reduce the number of mice used overall between multiple groups.

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.

We will use several different GA models of FTD/MND, either stable lines or GAAs generated by injecting virus-like particles into the brain of pups to trigger expression of FTD/MND-related genes. In order to study FTD/MND, animal models which display clinical signs reminiscent of the human diseases are required. Therefore there is no way to avoid some degree of suffering, primarily through locomotor and cognitive deficits. Animals will be closely observed and humane endpoints will be followed to ensure adverse effects do not exceed moderate severity. Experimental procedures will always be performed at the earliest timepoint possible, to minimise progression of locomotor deficits.

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

The immune system has a great deal of inter-species variability, and therefore mammalian models are most suitable for this work. Furthermore, we require the use of in vivo imaging techniques which are not available in lesser species such as flies, worms and fish to study immune and vascular function e.g. PET scans.

Finally, mouse is the only species for which all the relevant GA strains required are available, as this project requires a combination of several different genetic modifications.

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

We will keep up to date on the literature regarding best practices and updated protocols for all procedures. We will continue to use resources such as the NC3Rs EDA and adhere to the PREPARE and ARRIVE guidelines.

The major concern for animal welfare throughout this project is progression of locomotor deficits, which may cause distress and limit an animals’ ability to access food and water. Mice developing locomotor defects will be closely monitored by trained technicians/researchers and once severity is considered moderate for a particular animal, more detailed welfare checks will be performed regularly with clear humane endpoints observed. Floor feeding and/or provision of food and water via mash and gels will be used if required to improve access.

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

We will use the NC3Rs EDA and PREPARE guidelines to assist with experimental design and study planning, and follow the ARRIVE guidelines when publishing our findings. We will also keep up to date with literature and resources on best practice including those shared by the BSF at our establishment and experienced colleagues and collaborators.

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

We will regularly check information on NC3Rs website and sign up to the NC3Rs newsletter, check internally distributed updates at our establishment, and attend relevant seminars and consult with colleagues and collaborators regularly.