Home Office

NON-TECHNICAL SUMMARY

Examining new ways to understand and treat dementia

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, Alzheimer's disease, Memory, Inflammation, Renin-angiotensin system

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

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 what goes wrong in dementia and to test ways to improve the 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 syndrome (a group of related symptoms) associated with an ongoing decline of the brain and its ability to function. The main symptoms associated with dementia are memory loss and problems with language and understanding, but also many other changes in behaviour are seen in people with this condition. One in three people over the age of 65 will develop dementia and currently there are almost 1 million individuals in the UK affected. The most common cause of dementia is Alzheimer’s disease. The annual costs incurred by UK society associated with dementia are over £25 billion. Currently there is no cure for this condition or drugs that can significantly slow progression. Therefore, dementia represents a significant medical and social problem. If an appropriate treatment or a cure is not found, it is predicted that 1.4 million people in the UK will have the condition by 2050.

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

We will achieve a greater understanding of the biological events that underlie dementia. By doing this, we also hope to identify new treatments for dementia and show that they can reduce the symptoms of the disease.

These findings will be widely disseminated through publishing scientific papers and participation in conferences. We will make our data available to others to use in their studies.

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

The main benefits of this research will be the generation of new knowledge on what happens during dementia, and identifying new biological processes and biomarkers. We also hope to determine if we can alleviate the symptoms of this disease with new treatments. In the short term, our research will benefit other researchers, the pharmaceutical industry, and clinicians studying the development of dementia and Alzheimer's disease. In the long term, we hope that our research will help guide the development of future therapies and ultimately could provide the basis for new clinical trials for people with dementia and Alzheimer's disease.

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

We will publish our findings from these studies in respected, open access journals, present our data at leading national and international conferences, and utilise pre-print servers to maximise the dissemination of our research. We collaborate with other establishments, to assist with identification and development/repurposing of effective treatments.

Species and numbers of animals expected to be used

- Mice: 3000

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 use mouse models of dementia including those that are genetically altered and compare these to control mice (without the disease). These mice will be bred in-house and used as adults. Dementia predominantly affects the elderly, so we will need to allow the mice to age so they start to develop symptoms. Memory loss is one of the predominant symptoms in people with dementia and we can effectively measure memory in mice. In people with dementia one of the first problems they have is with recalling recent experiences and the mouse models of this condition also have similar issues that we can measures using specific tests. As in some studies we are aiming to test if treatments can reverse the symptoms of the disease, we therefore need these symptoms to be present before we start to treat the mice.

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

Mice will be bred and allowed to age until approximately 9-12 months to allow for their symptoms (e.g. memory loss) to develop. During this time blood might be taken and their behaviour (e.g. testing memory) assessed using tests that do not cause any harm or distress. For example, one test involves using the animals' innate curiosity to explore novel things. In this test, the mice will be placed in an arena containing two identical objects and the time they spend exploring the objects will be measured. The mice are then removed for a short period of time and then placed back in the arena where one of the objects has been replaced with a new one. Mice with a good memory will spend more time exploring the new object and those with poorer memory will not. Mice will be culled at various time points and tissues (such as the brain) taken to identify what changes are happening. In some studies, mice will be given treatments to try to reduce their symptoms and, the usual way we will do this is by giving them drugs in their diet, especially if we are going to treat for a number of weeks. However, in some experiments we might give drugs by injection for a short period of time. At the end of all experiments, animals will be humanely killed, and blood and tissues taken to assess modulator/mediators and pathological changes in more detail.

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

The mice that we will use develop the major symptom of dementia and Alzheimer's disease specifically memory loss. These memory changes only become apparent when measuring their performance in specific tests. Memory loss is one of the first symptoms to be detected in these mouse models (similar to humans). Otherwise, mice appear healthy and develop/grow the same as the control mice for time period we propose to keep them for. We do therefore not expect any major adverse effects.

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)?

Sub-threshold/Mild (70%)

Moderate (30%)

What will happen to animals used in 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?

Mice will be used in this project. Studying the mechanisms involved in dementia and Alzheimer's disease is extremely complex and involves understanding the interactions between several physiological systems (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. In addition to the pathological changes in the brains of Alzheimer's disease patients, the disease is characterised by deficits in learning and memory, and as such this behaviour is very difficult to model in vitro or assess in lower species (e.g. zebrafish). In addition, in vitro experiments do not allow fully 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?

We have considered using non-animal alternatives (in vitro models) that we routinely use in our work including culture systems using human induced pluripotent stem cell (iPSC)-derived neurons and cerebral organoids. We will use data from these in vitro models to help us design our experiments using animals, and therefore help to reduce animal numbers.

Why were they not suitable?

While these non-animal alternatives will allow us to study some aspects of the disease (e.g. amyloid beta accumulation; an abnormal protein produced in the brains of people with dementia) they not allow us to assess memory loss and whether we can improve this with interventions and we will therefore need to use animals for this part of our work. However, if any relevant non-animal alternatives become available during the project, we will implement these in our studies.

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 used our previous experience of performing experiments of this type to help us decide the best way to answer the questions we are asking while using the minimum number of animals. For example, estimates of animal use is based on i) previous work and experience using the relevant methodologies, the parameters to be studied, and specific mouse models; ii) the scope and objectives of the current project; and iii) careful consideration of experimental design.

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 (e.g. keeping the environment consistent), good experimental design (including the use of the NC3R's Experimental Design Assistant) and 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, and also so we can refine our questions to then design the most informative experiments. Whenever we get new data, we will always re-do our calculations in order to make sure we are still using an appropriate animal number to achieve our aims.

We will also assess memory over time in the same group of animals thus eliminating the need for separate groups and reducing animal numbers.

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

We will minimise the number of animals bred by using efficient breeding strategies and by using mice of both sexes. Most of the mouse models of dementia and Alzheimer's disease will be used as heterozygous, meaning they have only one copy of the altered gene (+/-). We will therefore breed heterozygous (+/-) mice with control (-/-) mice and all the offspring produced (50% +/- and 50% -/-) will be used. In the rare event that animals will not enter a protocol, tissue (e.g. brain) will be taken for use in other studies. In addition, we usually take several tissues from the animals at the end of the

experiments for multiple analyses (and sharing of tissue), which often leads to additional scientific questions. Whenever we perform analyses that leads to a large quantity of data (such as RNA-seq to analyse all genes) we will make our data freely available to other groups so they can analyse it to answer their own research questions.

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.

Models: There are no in vitro alternatives to study the pathological and behavioural changes that occur in Alzheimer's disease and dementia. Transgenic rodent models are currently the most relevant models available to study Alzheimer's disease and dementia (e.g. APPswe/PS1ΔE9 mice and APP23) or models of accelerated aging (e.g. SAMP8) and no surgery or injections are required to induce the disease. These mice naturally present with memory deficits and pathological changes in their brains over time, but otherwise they are healthy.

Measurements: For longer term studies, animals will be monitored over time for well-being (e.g. body weight checked) and they will be housed with other animals with enrichment. Animals might also undergo a series of behavioural tests. These tests are well described in the literature, and we have experience that these do not cause any lasting harm or distress for the animals. For example, we use tests that rely on natural behaviours in rodents (e.g. exploration), and we will not use any adverse stimuli or food restriction. However, throughout the project we will review the literature and engage with colleagues/collaborators to learn of any new refinements to the protocols that could be implemented. For administration of substances over time we will use the least stressful method where possible such as administering agents in the diet. Studies will stop as soon as we see a relevant effect (e.g. improvement or reduction in memory).

In some studies, we will require a cardiac blood sample followed by perfusion with fixative in order to best preserve the tissue for subsequent analysis, and this will be done under terminal anaesthesia.

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

Our objectives cannot be fully achieved using less sentient animals (such as fish/insects) or with very young (neonate) mice mainly due to the time taken for the disease to develop differences in terms of the pathology and cognitive changes, and adult mice are therefore needed. We have considered lower species (e.g. zebrafish) and where appropriate these could be used (e.g. injection of amyloid beta into the brain). However, zebrafish do not always show such similarities to humans and methods to assess memory and cognition have to be performed in adult fish (so also needs Home Office Licence

approval). These methods (e.g. to assess memory) in zebrafish are not as well established and/or reliable as in rodents, but we will continually review this during the course of this licence.

As most of our studies involve keeping animals for months and assessing cognition, terminal anaesthesia cannot be used.

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

Most of our studies will involve long-term maintenance of animals with intermittent behavioural assessments and sometimes the mice will be treated with agents that we hope will improve outcome. The mouse models of dementia we will usually use have already been well characterised and we know when memory deficits should be seen. We will therefore be able to plan our experiments appropriately and use animals as early as possible. If we see a desired effect of any substance at an earlier time point than anticipated, we will complete the study earlier than planned. For our behavioural studies we will not use any aversive stimuli and will use tests that assess the animals' natural behaviour.

For all studies and at all times, mice will be monitored frequently, handled appropriately by trained researchers (e.g. using tube handling for movement in and out of cages) and suitable home cage enrichment will be used.

In a minority of studies a small device (osmotic mini-pump) might be implanted under the skin of the mouse in order to deliver drugs that we hope will reduce the symptoms of dementia. In all cases appropriate post-operative care and pain management will be used in the short-term as this is a relatively mild procedure.

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

We will stay up to date with the literature, publications and recommendations from the most appropriate bodies such as the NC3Rs and LASA, as well being informed from communication with the NVS and NACWO and developments within the scientific community in general. We will follow the PREPARE guidelines (https://norecopa.no/prepare) for all our experimental work and design experiments so we can use ARRIVE guidelines (https://arriveguidelines.org/) for publications. In addition, prior to any animal studies we will prepare and submit a full experimental study plan to our animal unit to ensure all studies are carried out in line with best practices.

For refinements involving injections we refer to https://researchanimaltraining.com/articles/an-introduction-to-the-administration-of-substances/, and/or Morton et al 2001 "Refining procedures for the administration of substances" in Laboratory Animals (2001) 35, 1-41 and/or Turner et al 2011 "Administration of substances to laboratory animals: Routes of administration and factors to consider", J Am Assoc Lab Anim Sci 50(5):600-613.

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

We will at all times aim to implement any advances in techniques that adhere to the 3Rs and improve the welfare of the animals.

We will stay up to date of any advances through, for example:

-NC3Rs literature/newsletters and recommendations

-Establishment newsletters and seminars

-Discussions with colleagues

-Scientific literature

-Discussions with the NACWO