Home Office

NON-TECHNICAL SUMMARY

Ageing and therapy in mouse models for neurodegenerative diseases

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

Ageing and neurodegeneration, Therapy, aSynuclein pathology, tau pathology, gut

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

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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 understand the processes that lead to nerve cell death in mouse models of Parkinson's and Alzheimer's disease and test interventions at early stages that decrease the severity of the disease and help the development of treatments

A retrospective assessment of these aims will be due by 02 August 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

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?

Our findings will enable us to identify pathological pathways leading to abnormal aSynuclein and tau accumulation, the proteins that cause dementia in neurodegenerative diseases like Parkinson's and Alzheimer's disease. Key steps in these pathways may serve as potential targets for therapeutic intervention.

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

Diseases that kill nerve cells and lead to dementia, like Alzheimer's disease (AD), or great physical and mental disability, like Parkinson's disease (PD), have no cure. Treatments are being sought all the time but for the most effective treatments we require an understanding of the core processes that cause the disease. We are trying to understand the earliest processes that go wrong because revealing how they work may point to new therapeutic targets that delay disease onset so that people can lead a healthy life for longer. By genetic manipulayion, we have created unique mice that develop core features of these diseases that are also found in humans. One line of mice develops abnormal protein clumps

(aggregates) made up of tau, the protein core of toxic fibres known as neurofibrillary tangles in Alzheimer's disease. Clumps of tau also cause other dementias, such as frontotemporal dementia, progressive nuclear palsy, and chronic traumatic encephalopathy, which develops in sports people exposed to repeated head impacts. The group of diseases that are caused by tau are knonw as tauopathies. Other lines of mice develop abnormal clumping of the protein alpha-synuclein, which forms the core of toxic Lewy Bodies in Parkinson's disease and similar dementias, known as alpha-synucleopathies. At the end of this project we will have acquired important new insights into how the abnormal protein clumps made of tau or alpha-synuclein are formed, and why they cause the death of nerve cells in the brain. We will also have determined how neuronal tau clumping causes insults to the surrounding supportive cells (known as glia) and how this leads to inflammation. Inflammation is a key aspect of both AD and PD that has been neglected until recently but now is a basic part of disease diagnosis. It has been proposed that PD begins in the gut and spreads into the brain through nerves that connect the two tissues. To test this hypothesis, we have created further genetically modified mice in which alpha-synuclein clumps begin forming in the gut, and eventually spread into the brain. By studying these mice, we will also understand the steps that lead alpha-synuclein to spread from the gut into the brain, and identify which species of the synuclein clumps are responsible for disease spreading and for the different symptoms seen in PD. We expect to test new compounds that inhibit alpha-synuclein aggregation into clumps and spreading to delay the development of pathology. Understanding of these processes may also benefit clinical diagnosis identifying new participants in the disease process and by identifying molecules in body fluids that can be used for early detection of the disease. Our findings will be made publicly available by publishing in open access journals, presenting our findings at open meetings, by collaborating with colleagues who use techniques that complement our range of methods that can add value to our findings, and with the pharmaceutical industry.

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

In the short term the research community will benefit from our outputs, by having new insights into mechanisms of disease progression and the molecules that can be targeted for novel therapeutic strategies. Through our work, we will have contributed to long-term societal benefits by improving the quality of life and well-being of the ageing population, since age is the highest risk factor for development of dementias and loss of mental functions. Our research will also benefit younger patients with inherited early onset diseases due to mutations in tau and alpha-synuclein that are incorporated in our genetically modified mice.

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

Outcomes of our research, including unsuccessful approaches, will be reported in conferences and webinars and in open-access, publicly available databases.

Several collaborations are in place and will be developed to obtain new tools to further the understanding of these pathological processes.

Species and numbers of animals expected to be used

- Mice: 5300

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.

One out of six individuals over 80 years old will develop some form of dementia (the inability to think and remember) like Parkinson's (PD) or Alzheimer's disease (AD) and, to date, there is a lack of treatments that effectively stop or delay disease worsening. These diseases share in common the abnormal clumping of proteins such as aSynuclein (PD) or tau (AD), which become toxic and ultimately lead to the death of brain cells, which is the cause of dementia and movement problems. In humans, diseases that involve tau and aSynuclein start many years before the disease reveals itself as an impairment. These hidden stages of disease are not well understood but it is thought that if they could be diagnosed at an early stage before clumping there is better chance of effective therapy. For the past 20 years, our lab has produced several genetically modified mouse models that develop clumping of aSynuclein and tau that replicate key aspects of human disease, such as loss of neuron cross-talk, death of neurons, movement impairment and memory loss. By studying how the disease develops throughout the lifetime of the mice we will acquire new insights into what causes these proteins to clump and become toxic. These insights will enable the study of the effect of potential cures that delay the beginning of these diseases and promote a healthy ageing.

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

Most of the PD animal models used in the studies covered by this licence will be aged until defined time-points (6-12-18-24 months) which correspond to different stages of disease; tissue from these animals may be used to study the pathways that lead to protein clumping. Brain fluid may be sampled once surgically using an implanted cannula, and blood may be sampled via the tail vein, to see if we can detect molecules that could be used to predict the disease in humans before it fully develops. The behaviour of mice will also be tested in some instances to see if they have developed a movement problem and whether their memory is impaired. At each time point, some mice will be killed under anaesthesia and tissues preserved so that the molecules identified can be matched with disease progression.

Once we have mapped how these molecules and behaviours appear during the aging process, some mice may be given drugs or treatments at early stages that may slow the clumping of aSynuclein and therefore delay disease progression. To monitor the effects of these treatments, the mice will be assessed using the same tests that we use in untreated mice, as described above. These treatments could potentially lead to new cures.

One idea is that PD develops first with aSynuclein in the nerves that line the gut, mainly the intestine (PD patients are often constipated) that then spreads to the brain. Mice with aSynuclein clumps in the gut will be tested for their gut function by giving them a small amount of a natural dye through a flexible tube inserted into the stomach and measuring the time for the dyed stool to be expelled during 4 hours. This experiment may be repeated in different groups of animals at 3, 6, 12 and 16 months. At each time

point, some mice will be killed under anaesthesia and tissues preserved so the whole route that the clumps follow from the gut to the brain will be analysed.

Regarding PD beginning in the gut, it is suggested that aSynuclein clumping is caused by an irritation, for example a high-fat diet or inflammation. In another series of experiments, we will model such an irritation by, for example, feeding the mice with a high-fat diet. The same behavioural and gut function tests and endpoints will be applied as described above.

In certain instances, mice will undergo one session of sampling of the brain chemical dopamine, because the neurons that produce dopamine are the ones that die in PD and cause the disease. In this experiment, a small needle is implanted into the brain a day before, and we let the mice recover. The next day, a small amount (a few thousandths of a millilitre) of brain fluid is collected over 3 hours without causing any pain to the mice, after which the mice will be killed under anaesthesia. The fluid is analysed chemically for dopamine content and the brains will be analysed to determine disease stage.

In our tau models, the aim is to uncover the events that lead to the clumping of tau, and how tau causes cell death. We will also study why tau triggers the immune cells in the brain to cause inflammation. For this purpose mice will be killed at various stages of disease as determined by measuring the gradual loss of movement. The brains and spinal cords will be analysed for molecules that change in the nerve cells and in the various types of immune cells that might be involved in the disease.

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

We do not expect adverse effects in the ageing protocols of PD animal models with regard to administration of a high fat diet or administration of therapeutic or labelling compounds. Animals undergoing surgical procedures will experience transient discomfort after surgery, however these animals will fully recover in less than 24 hours with no lasting effect on their general well being.

The tauopathy animal models will develop motor impairment beginning around 3-5 months of age and decline from there until the end point, which is 15% weight loss, or loss of movement of either hind limb, but with no pain or other signs of discomfort. Because of these symptoms, the protocol falls in the severe category. To study early stages of the disease, when some molecular events may start to take place with no impact on the motor system, we have set a moderate category. Animals under this protocol may have scruffy fur or a hunched posture but will otherwise lead a normal life, e.g. walk around the cage freely, associate with the other mice in the cage, and feed and drink normally.

All animals developing tumours as a result of advanced age 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)?

Mild 70%

Moderate 24%

Severe 6%

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

- Killed
- Kept alive
- Used in other projects

A retrospective assessment of these predicted harms will be due by 02 August 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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?

It is not currently possible to accurately re-create a functioning nervous system that mimics the complex interaction between different neuronal cell types, tissues and organs. Therefore, to understand the mechanisms behind disease progression and full therapeutic effectiveness of relevant drugs, an animal model is needed.

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

We are currently using cell culture technologies involving human cells that are allowing us to make great progress in understanding how aSynuclein and tau proteins are toxic to cells. This procedure involves taking human skin samples and converting the cells into cell types that reside in the brain in 2D culture systems. Currently, we are also generating organoid cultures that may help to further understand the involvement of blood supply, the immune cells in a 3D environment more closely resembling the complex cytoarchitecture of the human brain. We also use techniques that promote clumping of aSynuclein and tau in a test tube to test compounds that might intervene in this process but this also has limitations, as the clumps in the test tube do not accurately replicate of the clumps in the brain, and the cell responses that might be part of the toxic process is missing.

Why were they not suitable?

Cell culture technologies are very useful, however they complement but do not replace the study of animal models. It is impossible to recreate the 3D structure of the brain in a dish, given all its cell types, intraconnections, and influence of other tissues, most especially in the gut and the immune system.

A retrospective assessment of replacement will be due by 02 August 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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 several years experience in using these models of synucleinopathy and tauopathy. We have been reducing animal number year on year as we learn more about the models and understand the disease process better. The number of animals is kept to the minimum by carefully monitoring the colony size and breeding, and matching these to the demands of the experiments. By applying statistics to the experimental design, we will obtain meaningful results that will prevent the need for experiment repetitions.

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

For best experimental design we refered to the PREPARE guidelines checklist: https://norecopa.no/prepare/prepare-checklist, which considers the steps needed to ensure that meaningful results are obtained while using the minimum number of animals. Taking these into consideration, we carefully weighed the balance between harm and benefit based on several years of experience in using our models of synucleinopathy and tauopathy. We have been continuously improving our understanding of how the animals respond during the development of the disease process, allowing us to reduce animal use year on year through careful maintenance of colony size and breeding. By applying statistics to the experimental design, we ensured that we will obtain meaningful results that will prevent the need for experiment repetitions.

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 coordinate and share tissues between multiple users and generate a tissue bank from animals, maximizing the use of each mouse. For some experiments, we will culture cells or brain slices from the mice, generating multiple samples from a single mouse.

For the majority of the experiments, preliminary (pilot) studies have already been conducted previously so will not be needed in this licence and data from these pilot studies will be used to inform work

undertaken on this project.

For gut inflammation experiments, we will conduct a pilot study on a small cohort of animals to establish the technique based on previously published data in our transgenic model.

A retrospective assessment of reduction will be due by 02 August 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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 transgenic mice that model the most common neurodegenerative diseases, Parkinson's disease and Alzheimer's disease. Parkinson's disease is modelled by expression of the human protein alpha-synuclein in a form that clumps in nerve cells and causes their death; Alzheimer's disease is modelled by expression of mutant forms of the human protein tau, which forms clumps and tangles that cause nerve cell death and brain inflammation. We will take tissues from these animals after they are killed at different stages of the disease to study how the disease develops. In some cases animals will be treated with drugs and their brain function monitored under analgesia. When animals are used in an invasive, potentially painful procedure, analgesia and anaesthesia will be used to minimize suffering. Extra monitoring will be provided to those animals for at least 24h after the procedure and under close supervision of the establishment veterinary surgeon. Regarding embryo transfer methods, we hope to explore the non-surgical embryos transfer (NSET) method as a refinement over the course of the licence.

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

We are using mice because they have a nervous system that is sufficiently similar to that of humans and where the biology of neurodegeneration is almost the same. In addition, mouse behaviour is well characterized, and our mouse models mimic the behaviour in the human clinical condition. Furthermore, mice can be genetically manipulated, allowing molecular hypotheses about the mechanism of disease development to be tested.

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

We will continue to enrich the environment with nesting and tunnels. Induction of inflammation by diet change instead of chemically induced inflammation is a more refined and physiologically relevant method. As we come to understand the later stages of pathology, this will guide us as to what to look for at earlier stages of the disease, thus avoiding the development of the most severe clinical disease.

We have developed a traffic light scoring system to track the development of disease progression in one of our mouse models which we use to determine when the mice need mash and gel and softer nesting material to offset the increased disability. By weighing the mouse every day when they reach 10% weight loss we can more precisely define the humane end points. We will remove males from pregnant P301ST43 females to ensure that each female has only one litter so that she remains healthy during pregnancy and can take care of her litter until weaning.

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

We will follow the NC3Rs guidance notes and webinars (https://www.nc3rs.org.uk/welfare-assessment) and those from the Jackson Laboratories (https://www.jax.org/news-and-insights/jax-blog/2016/march/experimental-design-top-four-strategies-for-reproducible-mouse-research). Advice and notes provided by the LASA guidelines will be also consulted (www.lasa.co.uk/publications.html) and we will follow the ARRIVE guidlelines (Animal Research: Reporting of In Vivo Experiments). The guidelines provide a checklist of recommendations to improve the reporting of research involving animals to maximise the quality and reliability of published research, and enable others to better scrutinise, evaluate and reproduce it.

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

We will continue to routinely access the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) website for information and advice and spread this knowledge among all members of our group. We also follow Norway's 3R centre (NORECOPA) guidelines (https://norecopa.no/about-norecopa). For example, using a tunnel to pick up mice initially to reduce anxiety because picking up by the tail enables a hind limb clasping test without causing anxiety. (https://www.nc3rs.org.uk/mouse-handling-webinar). We will also refer to the RSPCA website (https://science.rspcs.org.uk/sciencegroup/researchanimals) for further information.

A retrospective assessment of refinement will be due by 02 August 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?