Home Office

NON-TECHNICAL SUMMARY

Axon degeneration and repair

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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Axon degeneration, Motor neuron disease, Alzheimer's disease, Parkinson's disease, drug targets

Animal types	Life stages
Mice	embryo, neonate, juvenile, pregnant, adult
Zebra fish (Danio rerio)	embryo, neonate, juvenile, 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?

We aim to identify genes that influence the survival, degeneration and repair of nerves and understand their roles in human 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?

There are very few disease-modifying treatments for neurodegenerative diseases (progressive degenerative diseases of the nervous system such as those mentioned below). A disease-modifying treatment is one that halts, or even reverses the course of the disease, in contrast to most current treatments which block only the symptoms. Neurodegenerative diseases include common disorders such as Alzheimer's disease, Parkinson's disease, peripheral neuropathies (common pain disorders associated diabetes, cancer chemotherapy and genetic conditions) and glaucoma, some moderately common ones such as motor neuron disease and multiple sclerosis, and many rare disorders such as hereditary spastic paraplegia (a type of motor neuron disease characterised more by spasticity than paralysis) and spinal muscular atrophy (a childhood onset, very severe form of motor neuron disease). Around half the population suffer one or more of these disorders at some point in their lives. Some molecular mechanisms recur in many of them so blocking such a mechanism could have profound implications for healthcare. One such mechanism, into which our research has played a leading role, is known as 'programmed axon death'. This leads to loss of axons (nerve fibres) with consequences including pain, paralysis, spasticity, blindness and memory loss, and it is triggered by a wide range of causes. Blocking programmed axon death is protective in animal models of disease, and in some circumstances rescues nerves for the entire lifetime of a mouse. We have pioneered understanding of its roles in human disease and here we aim to find more disorders to which it contributes. This will enable drug companies aiming to prevent programmed axon death to direct clinical trials to disorders in which they are most likely to work.

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

Our plan is to publish research papers with new information on which human diseases involve programmed axon death and which specific people are most at risk. We may also carry out testing in cell culture of drugs that subsequently enter clinical trials.

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

Ultimately, it is patients with neurodegenerative diseases, and their families, who will benefit because some of these diseases will be prevented, slowed down or even reversed. Individual people in families known to be at risk will also be able to determine their genetic makeup, if they wish, in order to understand their personal risk and any lifestyle modifications they may wish to make as a consequence. In the more immediate future, as well as longer-term, pharmaceutical companies and their employees and owners will benefit from commercial use of the knowledge we generate. Most immediately, our outputs benefit the research community by advancing their research and careers, and they benefit the funders of this research by helping them achieve their aims of advancing knowledge and medicine.

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

Our papers are all 'open access' (not behind any paywall) to maximise their accessibility to readers. This will include publication of unsuccessful approaches to avoid unnecessary duplication by other researchers. We also regularly discuss our research results with other scientists, both in academia and in the Pharma industry, to maximise its uptake in future research. This includes activities such as conference presentations, seminars and collaborations.

Species and numbers of animals expected to be used

- Mice: 25,000
- Zebra fish (Danio rerio): 4,050

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 use mice because they have an approximately 1:1 correlation of their genes with those of humans, because their genome (their complete genetic material, or DNA) is fully sequenced and well-characterised, and because there are existing genetically-altered strains of mice that are appropriate for our research. We use newborn mice or embryos because these enable us to grow neurons in cell culture effectively (such methods do not work well with older cells). However, this requires us to have an older stock of mice to breed, both to obtain newborn mice for these cells and to maintain the genetic lines.

We use zebrafish embryos before five days post-fertilisation because the ability to render these fish transparent makes them highly useful for imaging and characterising the growth, survival and repair of nerves. This too requires us to maintain a stock of older zebrafish in order to breed them and they become protected animals.

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

Adult mice with harmless genetic alterations will be mated to generate newborn mice, or embryos. Humane methods of killing will be used to obtain embryonic or newborn mouse tissue for cell culture.

Zebrafish with harmless genetic alterations will be used for breeding to obtain embryos for nonregulated procedures before reaching the free-feeding stage.

Zebrafish gametes may be collected using a mild procedure involving gentle pressure or stroking, causing only transient discomfort.

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

There will be no adverse impacts of the experiments themselves, apart from transient discomfort during biopsy or gamete collection. Health problems from non project-related causes will be assessed and if necessary the animal 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)?

The expected severities in mice are subthreshold (90%) and mild (10%).

The expected severities in zebrafish are subthreshold (60%) and mild (40%).

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?

We are modelling normal biology and disease processes and this requires the use of living cells. Sometimes we need these to have a specific genetic makeup that is present in genetically altered mouse strains, and always we need the cells to closely resemble those in the living animal.

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

We have already moved all of our ongoing experimental work to mouse neurons in culture and tissue obtained from humanely-killed mice. We have also considered (and increasingly use - see below) both human stem cell (iPSC)-derived neurons and cell lines.

Why were they not suitable?

We are in the process of moving all our research away from animals to focus it exclusively on iPSC (stem cell)-derived neurons and human genetics. However, optimising these techniques, in particular adapting them for the higher throughput we can already attain in mouse neurons in cell culture, takes time, and ongoing projects that were begun using mouse cells in culture must continue to use them because to change the experimental system in mid-project would introduce unquantifiable variables that make accurate interpretation of the data impossible. Cell lines are not useful for most of our work because cell lines acquire major genetic artefacts (known as 'chromosomal abnormalities') when propagated in culture for long periods. We do use them for some work that is not substantially affected by this but they are not a solution for most of our work.

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?

A typical experiment using cells in culture derived from mice requires a group size of up to 10 for statistical comparisons and may have three variables we are testing, so it requires 30 newborn or embryonic mice. The number of experiments is in practice limited by people’s time and resources (equipment, space, funding) because there is always a logical next question to ask, even within this specific area of research. Thus, the following calculation is based on what is a feasible level of throughput for the time and resources we have. We do around three experiments per week, so with 30 newborn or embryonic mice per experiment this brings the total to 90 per week, giving a theoretical total of 23,400 (90 mice x 52 weeks x 5 years) in five years if the work was continual. In practice, however, holidays, sickness, and the time needed to interpret data and design new experiments will lower this number, while the need to keep additional adult mice to generate newborn and embryonic mice and maintain the strains raise it. Thus, the estimate of 25,000 is based on this starting point of 23,400 which is then modified by both of these effects.

Similarly, a single experiment using zebrafish typically requires up to 10 fish per group and has three variables, so 30 fish. The frequency of these experiments is lower, around one every two weeks. Thus, over 5 years we require 30 x 52/2 x 5 = 3900 zebrafish. After similar adjustments to above for holidays, sickness, planning/interpretation time, and adult breeding, the estimated number is 4,050.

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

We minimise experimental variables, which would otherwise increase the group sizes needed to obtain statistically significant results, by using standardised protocols and performing experiments in parallel whenever possible. We only retain the required number of adults to ensure maintenance of the strain and the ability to breed enough young animals for our work. We also follow guidance on the NC3Rs website https://nc3rs.org.uk/3rs-advice-project-licence-applicants-reduction and the PREPARE guidelines https://norecopa.no/PREPARE.

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

We breed homozygous lines where possible (lines where all offspring are identical to the parents), to avoid excess generation of animals that will not contribute to our conclusions. Occasionally, breeding of homozygous lines would involve adverse effects on health so as a Refinement measure to prevent this, we breed those specific animals as hybrids instead, which do not have this problem. We regularly use pilot studies to optimise experimental design and identify potential problems, before deciding whether to scale up a particular line of work. We select our experiments carefully based on sound knowledge of the relevant scientific literature and extensive internal and external discussion before proceeding.

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.

All our experimental work is in cell culture or zebrafish embryos. Thus, apart from the need for breeding and maintenance of mice and zebrafish to generate these, we have replaced animal experiments entirely in our work. Apart from minimal tissue biopsies to determine genetic makeup of individual animals, there will be no pain, suffering, distress or lasting harm resulting from the project.

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

We do use immature life stages and humane methods of killing. We cannot use less sentient species because we need to use mammals for a 1:1 relationship with human genes, and vertebrates to retain representation of peripheral and central nervous system. There are fruit fly geneticists working in this research field, and this is a very powerful experimental system, but the results need to be validated in vertebrates and in mammals. Thus, we collaborate with several such groups to do this. There would be

no point for us to duplicate their work, especially when we have less expertise in those methods, while no-one does the necessary validation in vertebrates and mammals.

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

We will carry out all our experimental work in cell culture or in zebrafish embryos. Where it is necessary to breed a genetic alteration that is harmful in homozygotes in order to generate cells for culture, we will maintain the line using heterozygotes (hybrids) where the alteration is not harmful. For the regulated procedures of adult breeding and maintenance needed to produce these embryos and young animals, we use enriched environments, optimal handling methods and minimise single housing of animals to limit stress

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

We regularly review the NC3Rs website for this guidance https://www.nc3rs.org.uk/3rs-resources, the most relevant sections of which pertain to mouse https://nc3rs.org.uk/3rs-resources/breeding-and-colony-management and zebrafish breeding and maintenance: https://nc3rs.org.uk/3rs-resources/zebrafish-welfare. We also consult specific guidance or position papers from the Laboratory Animal Science Association, (LASA) https://www.lasa.co.uk/current_publications/.

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

We routinely attend conferences and discuss with other scientists at seminars and in collaborations. We will also consult with the NVS and NACWO for new advances, and journals and web resources such as ATLA (Alternatives to Laboratory Animals) Journal: https://journals.sagepub.com/home/atla and the LASA Guidelines: https://www.lasa.co.uk/current_publications/. As we have shown, when a new 3Rs method becomes available (e.g., hiPSC-derived neurons), we use it.