Home Office

NON-TECHNICAL SUMMARY

Regeneration in the nervous system

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.

Key words

nervous system, myelin, regeneration, stem cell, ageing

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 project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The processes of nerve cells (axons) are wrapped in layers of fatty membrane (myelin) which protect them and allow them to conduct electrical impulses very rapidly. Myelin in the central nervous system (CNS – which comprises the brain and the spinal cord) is produced by cells called oligodendrocytes. Oligodendrocytes and myelin are lost in diseases such as multiple sclerosis (MS). If myelin is damaged (demyelination) and not restored, the axons will not work properly and will eventually die. The loss of axons is irreversible, and its accumulation due to myelin regeneration failure accounts for the currently untreatable progressive phase of MS. Although stem cells in the brain can make new oligodendrocytes that restore myelin, this regenerative process declines with age. Currently, there is no therapy promoting myelin regeneration in the clinic. There are also rare but severe genetic myelin disorders affecting infants, in which, the stem cells fail to develop myelin, the individuals often die in early childhood, for which there is no treatment. To meet these urgent unmet clinical needs, it is necessary to know exactly what causes this failure and how to overcome it. The aim of the project is to study: (1) how ageing and disease hampers myelin regeneration in the CNS, by adult stem cells and to develop new therapies to promote myelin regeneration in demyelinating diseases and (2) whether the defective stem cells can be rectified to fulfill their functions in the context of myelin in the CNS.

A retrospective assessment of these aims will be due by 14 July 2024

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.

What are the potential benefits that will derive from this project?

The project will advance the understanding of 1) myelin regeneration in the nervous system, 2) the factors and conditions that influence this process, and 3) why myelin regeneration fails in diseases and ageing. Our discoveries may be harnessed to develop new regenerative therapies in the treatment of MS. In addition, the findings in gene manipulation experiments will provide pre-clinical data for genetic myelin disorders and benefit developing potential treatments for the devastating disease.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

This project will use rats and mice. We expect to use up to 13,700 mice (including breeding genetically altered mice) and 2,100 rats over a five-year period. The actual number of mice is expected to be lower

than the above figure because some of the mice bred under breeding protocols will be used in the experimental protocols on this licence.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

We will predominantly use animal models that involve creating a very small area of injury in the CNS. Most of these models require surgical procedures, which usually take 30-60 minutes under anaesthesia. More than 90% of the animals will recover spontaneously without showing apparent deficits in normal functions. A small proportion (<10%) of rats undergoing brain injury may exhibit a significant loss in movement control and balance, which will be killed humanely. A small number of animals will be used to create genetic model of myelin loss. These mice may potentially display difficulties in movement control from early age. The defects may be accompanied by other severe neurological signs such as seizures and tremors, which may cause early deaths. They will be closely monitored and killed if they reach the predefined level of harm (end points) such as incapability of voluntary feeding to minimise suffering. Some animals will be subjected to experimental interventions such as delivering drugs by injection or implantation of slow releasing minipumps, and imaging under a relatively long period of anaesthesia. These procedures likely lead to considerable discomfort, stress and even pain to the animals, indicated by signs such as reduced activity, subdued behaviour. Animals receiving certain substances (e.g. compounds to be tested) may suffer considerable weight loss due to toxicity and affected food and water intake. We will make sure the animals undergoing procedures to have adequate pain relief as standard, e.g. during and after surgery. If animals are identified suffering considerably from experimental procedures (reaching the established end points) indicated by e.g. loss of mobility, sharp reduction of body weight, signs of distress or pain not readily alleviated, they will be humanely killed. Some animals in this project will be subjected to calorie restriction or exercise or behavioural tests, which are expected to cause no more than temporary stress until the animals have acclimatised. Certain strain related defects may develop in genetically modified animals such as malocclusion, the overgrown of misaligned front teeth in rodents which affects normal eating. Although these animals may be maintained healthy by trimming the overgrown teeth, we will only carry out the procedure if we cannot obtain healthy alternatives to minimise stress and incidence related to the procedure. A significant proportion of animals will be maintained and used to old age which will exhibit functional decline associated with ageing such as ; any animals with clinical signssuch as tumours and rectal/vaginal prolapse will be killed without being used for experiments. All animals used in the project will be killed under anaesthesia or humanely using another method and at end of studies, tissues are harvested for further analysis.

A retrospective assessment of these predicted harms will be due by 14 July 2024

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 why you need to use animals and why you cannot use non-animal alternatives.

CNS (brain and spinal cord) myelin regeneration is a complex biological process involving many interacting cell types locally and also affected by factors of whole body (e.g. from blood). At present, there is no non-animal substitute that can accurately replicate CNS regeneration. Although non-protected animal alternatives (non-vertebrates) may be useful for studying fundamental cellular processes, they do not exhibit the same level of complexity in the CNS, i.e. their nerve fibres do not have myelin sheaths. For this reason, mammals are the only option from which we can gather enough information to meet the objectives of the project. We therefore use mainly mice for our work and a lesser number of rats.

There are some alternative techniques that can be considered as a partial substitute: using cultured cells isolated from animals; using human induced pluripotent stem cells (cells capable of generating any cell type in the body) and using synthetic fibres to replace axons. However, these techniques are still in their infancy and cannot completely substitute the use of animals. We will however, conscientiously explore the opportunity to develop and maximise the use of these techniques wherever possible.

A retrospective assessment of replacement will be due by 14 July 2024

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 you will assure the use of minimum numbers of animals.

We will ensure our experimental design is sound, and we aim to use the minimal number of animals that will give statistically meaningful data.

Experimental groups will be randomly selected to consist of animals with same age, sex and strain. The number of animals in each group will be determined by a statistic principle based on existing data from similar studies or early phase so called 'pilot' experiments. In addition to randomisation, proper controls and sample sizes, we will avoid the introduction of bias that may otherwise influence interpretation of results by “a blind” approach, so that the researchers are hidden from the information and identities of the animals (e.g. control or treated) during the experiments until all the data have been obtained.

Where possible, repeated non-invasive tests (e.g. imaging, behavioural tests) will be performed on the same animal to maximize the efficiency and robustness of data acquisition, reducing overall animal usage whilst simultaneously ensuring there is no increased harms to animals used. We will continue to use cell and tissue culture in our studies to obtain preliminary data before using animal models. This ensures only the most promising experiments are progressed and performed on animal models.

Where possible, we will use human tissue and cells with appropriate ethical permissions in our work which will reduce the use of animals.

Where possible, we will use advance gene editing techniques in our study instead of genetically altered animals, to minimise the breeding of genetically altered animals. The latter usually involves more animals ending up redundant.

We will ensure our materials, e.g. control tissues and data are shared with other researchers to eliminate the unnecessary repetition elsewhere of the same experiment using animals.

A retrospective assessment of reduction will be due by by 14 July 2024

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

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The animal models proposed were chosen because of the minimal functional loss that they induce. The toxin-induced rodent demyelination models have been demonstrated by many laboratories extremely valuable in addressing specific questions in regenerative biology and clinical translation research. Among the models available, we will always choose the one which is least harmful to the animals, gives the clearest results and is the most consistent.

In conducting animal work, we will always ensure most appropriate handling and housing, minimising the impact of single housing of animals (which is at times scientifically necessary) and other sources of stress. We will take appropriate measures (pain relief and anaesthesia) to minimise pain in the animals undergoing procedures. We will limit the numbers of procedures on individual animals to prevent cumulative and unnecessary harm. Whenever feasible and scientifically appropriate, we will choose the least harmful procedures for our tests.

Non-specific developmental defects or other abnormalities may occur in genetically altered mouse lines, such as retarded growth, or 'runt', and malocclusion, overgrowth of misaligned front teeth. We will always ensure proper selection of breeding animals to avoid the presence of such traits in the background based on their recorded health status in the ‘family tree’, so that the occurrence of the defects may be eliminated or minimised.

Before testing a new compound which not previously been used in animals we will perform small scale 'pilot' experiments with low numbers of animals to determine effective dosage and toxicity to minimise potential harm on larger scale studies.

We will continue to improve the method of detecting the impact of adverse effects of procedures on animals, using a consistent, objective measurable way to record pain, stress and functional deficits e.g. utilising 'scoring' systems where appropriate. For new procedures potentially leading to obvious harms we will always start with small scale pilot test first and create an suitable monitoring programme to minimise suffering.

We will ensure adequate training of all researchers who carry out animal work and continue to develop effective measures to reduce pain and discomfort for animals under procedures such as incorporating pain relief agents into food supplements after surgeries as an alternative to giving by injection, and work with animal technicians to strengthen our monitoring system.

A retrospective assessment of refinement will be due by by 14 July 2024

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?