Home Office

NON-TECHNICAL SUMMARY

CNS white matter development, plasticity and 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.
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants.

Key words

Myelin, Regeneration, Stem cell, Glia, Neuronal circuit

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
- Required at inspector’s discretion

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?

Half of the human brain is white matter, which rapidly transmits information between the ~100 billion neurons (the nerves or 'wires' of the brain) situated in the grey matter (the other half). Unlike the grey matter, the white matter continues to develop long into adulthood. The function of white matter depends on oligodendrocytes, a brain cell type called a glial cell, that wrap myelin (a fatty insulating substance) around neurons to provide fast transmission of impulses or 'signals' to coordinate neuronal activity and maintain neuron function - similar to the insulation around an electrical wire. Alterations in myelination are increasingly being implicated as a mechanism for learning, and when myelin is damaged in disease it leads to both cognitive (conscious intellectual activities like memory recall, learning or thinking) and physical disability.

A large proportion of the human brain is made up of glial cells (astrocytes, oligodendrocytes and microglia cells), which are the 'supporting cells' of the brain, supporting neuronal function. The understanding of their role in the function of neuronal networks within the central nervous system is limited. Conventionally, research into neurological diseases has been extensively grey matter-focused, due to the clear loss of neurons and loss of their function. However, current knowledge on glial cells, and myelin within the brain implies a more systemic failure in the brain as a source of neurological disease than solely a neuronal problem. Thus, to untangle neurological diseases, focused research into neuron-glia interactions is needed.

The overall aim of this project is to gain insight into how myelination is regulated throughout life and in disease. We will focus on how glia cells affect formation of neuronal networks in the brain, how they are involved in memory and learning, the effect of ageing and how myelin regeneration can be enhanced in neurological diseases.

The work set out in this license ranges from fundamental questions about the biology of glia-neuron interactions, neuronal circuit development, and myelin damage, to studies more focused on finding new potential drug targets for medicines. For example, testing whether new approaches emerging from these fundamental discoveries have pro-regenerative efficacy (i.e. can new myelin be formed? or can loss of brain function be saved?) in suitable animal models.

A retrospective assessment of these aims will be due by 05 February 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?

Multiple sclerosis (MS), a disease where the primary cause is damaged myelin, affects around 2.5 million worldwide, with 130,000 people affected in the UK, costing the UK society nearly £4 billion per year. Currently there are no fully effective treatments available. In recent years progress has been made in modifying the immunological aspects of the disease, but as of yet no myelin regenerative treatments exist.

In addition to MS there are a number of other white matter diseases, such as cerebral palsy or post-infectious encephalomyelitis (inflammation of the brain and spinal cord), where myelin regeneration therapy could be suitable. Moreover, white matter damage can occur either as an unwanted side effect of other treatments, such as cancer (i.e. 'chemo brain'), post-vaccination encephalomyelitis or secondary to neurological damage (spinal cord injury and stroke) or HIV-1 infection (which if untreated can lead to AIDS). Therefore, the direct benefit of understanding the mechanisms regulating myelin regeneration is enormous and could lead to the identification of novel ways to augment myelin regeneration therapeutically for a number of white matter diseases.

Recent data indicated that myelin, and glia cells, are involved in multiple diseases that were previously considered 'neuronal' such as dementia, schizophrenia, attention deficit disorder, autism and bipolar disorder. Thus, understanding how glia affect development and changes occurring throughout life will have a significant effect on understanding the development, and mechanisms underlying, a number of brain diseases.

This work will advance our knowledge of how the brain develops and functions throughout life and the mechanisms underlying the spontaneous regeneration process that can occur in the central nervous system white matter. New insights may be gained that could lead to new strategies for treating neurological disorders: (i) developmental brain disorders; such as cerebral palsy (~17 million affected in the world), and leukodystrophies (genetic diseases which affect formation or maintenance of the white matter, often appearing in early childhood); (ii) acquired disorders such as schizophrenia and multiple sclerosis (~2.5 million affected in the world); and (iii) age-related disorders such as dementia (~50 million affected worldwide; including Alzheimer's Disease, frontal-temporal dementia and vascular dementia).

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 licence covers use of rats and mice.
Over a 5 year period we expect to use approximately:

(1) 12,640 mice (these include genetically altered mice bred under this licence).
(2) 5,450 rats (these include genetically altered rats bred under 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?

A variety of different procedures will be performed as part of this license, in most cases animals will recover quickly and without incident (mild severity) or occasionally will be non-recovery, where animals are anaesthetised for the procedure and are killed under anaesthesia. Procedures that fall under the mild category include; injections, drug administration in food or water, blood sampling, breeding and maintenance of genetically altered animals where the alteration is not harmful, behavioural tests, or labelling (with dyes or fluorescent markers) the central nervous system under non recovery anaesthesia. We use drugs or dyes, either injected or fed to the animals, for several different reasons; to activate genetic modifications, to provide pain relief before or after procedures, to label certain cells so that we can identify them, or to change the responses of certain cells to different stimuli e.g. so that we can boost or inhibit myelin formation or maintenance. Once procedures are completed animals will be humanely killed, the methods used to do this will vary but will be carefully considered to balance the experience of the animal with the usefulness of the tissues that we can obtain after death.
Some of the work under this license will lead to moderate adverse effects, these include genetic alterations which can lead to brief seizures or tremors, maintaining animals as they age, eye injections (under anaesthesia) which could cause irritation, brain injections (under anaesthesia) and neural implants (under anaesthesia) which can lead to temporary functional difficulties such as low level problems with walking or head tilts. Injections into the eye are used because the optic nerve is normally fully myelinated with a well known timeline of myelination and this makes it an ideal model system that allows us to investigate how myelin is normally made and ways in which it might go wrong. Eye injections are safe and are routinely used in human patients. The eye injections take place under general anaesthesia (human patients receiving eye injections generally just have local anaesthesia) to ensure that the distress of the animals is kept to a minimum. We use neural implants to either shine light into the brain to activate certain cells, to take pictures of the brain, or to have a slow release of drugs into the brain over a long period of time so that we don't have to do lots of repeated injections into the brain. We use injections into the brain for 3 main purposes; to change the activity of the brain cells, to label specific cells, or to generate a small demyelinated injury (or lesion, like in multiple sclerosis). The most severe work involves causing damage to the brain so that we can see how it heals, damage is initiated by injecting a chemical into the area of the brain that we want to have the injury; in most cases the worst clinical sign is a head tilt and some difficulties in balance when moving around that resolves in 72 hours or less. In less than 10% of cases this damage to the brain can lead to spontaneous rolling of the animal or rolling when stressed, or the animal being temporarily unable to right itself. Animals that continuously roll will be humanely killed immediately, and those that cannot right themselves and show consistent reactive rolling will be killed if they do not improve within 8 hours. Painkillers will be provided to animals so that they are not in pain from any of these procedures, and during the recovery period they will be kept warm and in a low stress environment, we will also help them to eat and drink if they need it by giving them easily accessible soft food and long nozzled water bottles. Animals will be closely monitored at all times and will be checked on with increased frequency during the recovery period.

Surgical procedures will be performed under general anaesthesia and sometimes multiple separate general anaesthesias will need to be used, typically after anaesthesia animals are somewhat

disorientated but will be receiving painkillers to minimise any post-operative pain. Animals will only undergo multiple general anaesthesias if there is a scientific need for a second surgery, for example, in one surgery we may need to inject a substance that makes certain types of cells in the area respond to light, allow the animals to recover and the substance time to work, then perform a second surgery in which we make an injury then shine light onto the cells to see if that leads to faster healing. Animals will only be used for multiple surgical procedures if they recovered quickly and completely from the first procedure. In general recovery from anaesthesia should be quick and animals can return to normal behaviour without much intervention. The cumulative effect of any procedures will be considered and kept to the minimum required to yield statistically meaningful data.

All animals used in this license are cared for by dedicated staff who follow detailed instructions regarding their care. Humane endpoints have been set throughout this license to minimise suffering, ultimately all animals will be humanely killed and their tissues harvested for further analysis. Procedures are continually reviewed and refined whenever possible to improve the overall welfare of the animals.

A retrospective assessment of these predicted harms will be due by 05 February 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.

The experiments proposed are to advance our understanding of how the brain develops during normal life and in disease and how glia affect this process, including in repair. Whenever possible culture experiments, using human central nervous system cells derived from skin cells, will be conducted in specific cell culture petri dishes in the laboratory to reduce animal use. We will also use tissue from dead animals for culture assays to reduce the invasive procedures as much as possible. However, cell culture based assays do not always represent an appropriate alternative to animal use, thus animal models are needed, especially to study complex environments with different cell types interacting, or to study behaviour, and are essential to understand complex disease mechanisms such as myelin regeneration and changes in the central nervous system with ageing. Existing data show that for most purposes the rodent nervous system is a good model of the human one, both for normal function and for the disease processes that we are investigating in this project. Thus, making it our best choice to investigate these complex interactions

A retrospective assessment of replacement will be due by 05 February 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.

In all experiments, we will ensure that the minimum number of animals will be used by optimising the experimental design and by using appropriate statistical methods compatible with obtaining scientifically and statistically significant results. We will ensure that researchers receive extensive training before they start experiments on animals and will share tissues between researchers wherever possible thus minimising animal numbers used overall. Sample sizes are based on statistical calculations using pilot experiments (where a small version of an experiment is run to test whether a full experiment is likely to work) and data from the literature. All experimental analysis will be carried out 'blinded ' i.e. the person analysing the data does not know what animal a specific data set belongs to, so there is no 'bias' or influencing of results based on the researcher anticipating what they expect the data to show; we will randomly allocate animals to different experimental conditions and for analysis, this reduces bias in our analysis and ensures that our conclusions will be valid.

A retrospective assessment of reduction will be due by by 05 February 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.

Myelination only occurs in vertebrates and so non-vertebrate species are inappropriate for understanding myelination and myelin regeneration. There are no myelin regenerative models that have been developed in birds, reptiles, amphibians, or fish. Thus, we use rats and mice because they are the least sentient species that can model glia-neuron interaction, myelination, cognition and behaviour in neurological disorders. The brain circuitry implicated in many neurological disorders is highly conserved between rodents and humans.

The brain lesion models proposed for inducing myelin damage have been chosen in part because of the minimal behavioural and locomotor (movementdeficits that they induce. For example, our primary model does not result in functional deficits, i.e. the animals are able to move freely and perform normal physiological functions, like feeding, drinking, grooming and movement after the initial surgical recovery period. Our brain lesion model is carried out by performing an injection under anaesthesia into a specific area of the brain where a lesion does not cause any lasting symptoms, animals get better over time and recover back to their pre-lesion state of fitness within a couple of days, though the symptoms immediately post lesion surgery can be severe and distressing to the animal. Causing lesions in other

parts of the brain could cause lasting symptoms either to behaviour such as social interaction, or to normal physiological functions such as movement - e.g. causing paralysis.

In undertaking these procedures, we are continually assessing how the procedures can be refined in order to minimise the discomfort that the animals may experience and seeking to replace *in vivo* procedures with *ex vivo* procedures where possible. Some of our previous refinements have included: the administration of post-surgical pain relief in edible jelly rather than by injection, thus reducing handling stress in recovering animals; numbing cream on the ear bars of stereotactic frames (used to keep the head still during surgery) to further minimise discomfort for animals during their recovery; and improved monitoring/grading systems so that any signs of distress can be readily and consistently recognised and recorded by all researchers and animal unit staff and appropriate action carried out to reduce that distress at the earliest available opportunity. We are testing to see if we can use an injection into a vein (such as the tail vein) rather than an injection into the brain for some of our experiments, made possible by new modifications to what we need to inject, to reduce the number and severity of the procedures that the animal goes through. We are also trialling the use of neonatal (from birth to 10 days) animals for some of our brain injection surgeries as this procedure is less invasive (no drilling of the skull) and requires fewer injection sites in order to get wide coverage of the brain.

We take the welfare of the animals very seriously and routinely use pain relief and provide appropriate supportive care during surgical procedures, we familiarise animals to handling for procedures where handling is necessary, and increase monitoring of animals that have undergone invasive procedures so that we can quickly identify and treat or minimise any adverse effects arising.

A retrospective assessment of refinement will be due by by 05 February 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?