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NON-TECHNICAL SUMMARY

Nervous System Injury 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.

Key words

gene therapy, regeneration, spinal cord injury, stem cells, traumatic brain injury

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

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?

Both brain and spinal cord injury are crippling conditions due to the severance of nerve fibres that connect the brain with the spinal cord and body. Injury to the brain or spinal cord can be acute, as in stroke or traumatic brain and spinal cord injury, or chronic as in neurodegenerative diseases, leading to loss of motor and sensory functions, potentially resulting in paralysis and/or loss of sensation. The number of patients living with paralysis due to brain and spinal cord injury is growing in both the developing and developed world.

Repairing nervous system damage in a rodent model involves inducing cut nerve fibres to regenerate across the injury and to make connections below it. Alternatively, or in addition, undamaged nerve fibres remaining after injury can be made to return some function through stimulation of plasticity (fibre sprouting), bypassing the lesion.

The goal of this project is to develop and test treatments to repair damage to the nervous system by promoting nerve fibre regrowth from injured fibres (regeneration) and/or fibre sprouting from existing or uninjured fibres (plasticity). In addition, we will evaluate the robust growth response which occurs in the visual and peripheral nervous systems (PNS) as well as the immature/developing central nervous system (CNS) relative to the minimal growth response occurring after adult CNS injury to further our understanding of these differences and determine how they may be utilised to enhance CNS repair.

Our treatments for nervous system damage aim:

(1) to block the degeneration process around the lesion and/or to inhibit the gradual loss of cellular function in chronic neurodegeneration,

(2) to repair the lesion, by inducing nerve fibre regeneration or reactivating plasticity/sprouting in the brain and/or spinal cord.

This project will explore the normal injury responses anatomically within the growth-poor CNS and growth-rich PNS to better understand endogenous malfunctions contributing to the lack of repair. This project will also examine different strategies for repair and protection of the brain and spinal cord after injury focusing on modification of endogenous cells through gene therapy (through non-toxic viral vectors) and cell replacement therapies (including stem cells).

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?

Results from the proposed experiments described in this project will have direct benefits for scientists in the near term but will also have potential benefits for human patients living with brain or spinal cord injury in the future as we work toward viable treatments for repairing the dam-aged nervous system. As such, these experiments will add to our fundamental knowledge of nervous system injury and impaired regeneration as well as the basic biological processes and connections in the nervous system. Furthermore, we will publish our findings in high impact peer reviewed journals to inform other scientists working in similar fields.

The studies included in this proposal will provide prospective treatments which one day may be suitable for patients suffering from nervous system damage such as spinal cord injury, traumatic brain injury or stroke. In addition to validating our novel CNS repair treatments, we will combine these with therapies already used in the clinic such as rehabilitation and therapies close to clinical trials in order to move forward translation of viable and novel therapies towards application in human patients.

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?

The proposed experiments will be performed in rats and mice, in which the biology of the nervous system is similar to humans. Up to 500-600 rats and 500-600 mice will be used yearly during the 5 year duration of the project for experimental studies. Additionally, up to 300-400 rats and 400-500 mice will be used yearly for the support procedures (breeding and obtaining tissue) required for the project.

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?

The overall goal of this project is to investigate the molecular and cellular mechanisms regarding why some parts of the nervous system do or do not regenerate while also implementing strategies to enhance the regenerative capacity of the nervous system specifically after injury and within areas that do not regenerate. The types of injury that we perform to the brain, spinal cord, or peripheral nerve will be in the form of direct, physical lesions (cutting or crushing of the nervous tissue) or chemically-induced (injection of a chemical) lesions.

The majority of lesions used in this licence (brain and spinal cord) are non-paralysing lesions. Our lesions are of moderate severity, whereas within a week post-surgery, animals will have returned to a near normal condition similar to pre-surgery condition with very mild deficits only being apparent through specific behavioural and anatomical analysis. In lesions that affect dorsal roots or peripheral nerve, there may be dragging of the affected hindlimb which may be associated with hypoesthesia (dorsal root injury or peripheral nerve injury) or paralysis (peripheral nerve injury). The animal will still be able to use the paw for certain movements including mobility. Likewise, gross feeding and drinking ability should not be compromised.

For animals undergoing surgical procedures, our models of brain and spinal cord injury will be performed under general anaesthesia, with additional analgesia being given peri-operatively (pain relief administered at the time of surgery) to minimise suffering during and in the days and weeks following the surgical procedure. Specifically following surgical procedures, post-operative observations will be performed continually until the animal regains consciousness, following that, the animals will be observed several times in the first few days following surgery as well as at any other

stages in the experiments that pose a higher risk of adverse effects. Once the animals have stabilised, post-operative care and observations will be performed daily at a minimum.

The animals will be humanely killed at the end of the experimental procedures, and tissues will be collected for analysis. Specific humane endpoints will be used to ensure that adverse effects do not go beyond the minimum required to achieve the scientific objectives and the numbers of animals will be minimised by careful experimental design. On the rare occasion of post-surgical complications, such as animals which exhibit signs of pain, distress, or have difficulty eating, drinking, or moving about as normal will be humanely killed.

In addition, approximately 100-120 animals per year will be used for tissues only and not undergo surgical procedures. In these cases, animals will be humanely killed to obtain the necessary tissues.

Replacement

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

Much of the development of our treatments is performed with extensive tissue culture analysis (on cells) prior to moving to an animal model. Once we have interrogated these treatments fully in vitro and in order to determine whether these treatments are likely to help human patients, it is vital that they are then evaluated in animal models. For this aim, we will carefully design our experiments so as to use the fewest numbers of animals possible to achieve significance in our results.

The basic concepts and treatments for nervous system injury repair are worked out using tissue culture models. Concepts developed in tissue culture have to be tested and refined in an animal model where the complex environment of the adult nervous system is present, and where functional recovery can be measured. No treatment for nervous system injury repair could be tested in human patients without extensive prior validation in animal models.

Reduction

Explain how you will assure the use of minimum numbers of animals.

The number of animals used in these experiments will be kept to a minimum whilst ensuring power in our experimental design and ensuring that we can adequately address and answer the questions we propose. We will obtain behavioural and anatomical (and in some experiments electrophysiological) data from animals following our injury and repair procedures in the nervous system. Our experiments allow for multiple types of analysis on one animal (behavioural and anatomical) which effectively reduce the total numbers of animals required to reach our outcomes whilst not compromising animal welfare.

No animal experiments are performed until a well-developed treatment concept has been developed using tissue cultures. By making very repeatable lesions we achieve minimal variation between animals, making it possible to use smaller experimental groups. Animal group size is determined based

on previous experience as well as reference to statistical readouts, so that the number of animals is sufficient to achieve statistically significant results. For example, in studies using neurohistology (tract tracing) as a readout, 3-4 animals per group is required as we and others have found that there is extremely low variability in these types of experiments. On the other end of the spectrum is in studies using behavioural testing as a readout. In these cases, because there is larger variability amongst animals, 8-12 animals per group are required.

Small pilot studies will be used for new studies to assess feasibility and outcome measures of the experimental paradigm, mainly regarding new treatments. The number of animals included in these pilot studies will be kept to a minimum (usually 6-10 per experimental treatment group) followed by analysis through statistical tests. Prediction of numbers of animals needed for experimental design will be based on our 17 years of experience with these surgical models and results from the pilot studies. When possible, we will attempt to further optimise these pilot studies and reduce the number of animals used, in addition to seeking statistical advice from experts within the applicant's research establishment.

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.

Project Goals and choice of animal models

The overall goal of this project is to investigate the molecular and cellular mechanisms regarding why some parts of the nervous system do or do not regenerate while also implementing strategies to enhance the regenerative capacity of the nervous system specifically after injury and within areas that do not regenerate. We use rats and mice in our studies as both have beneficial characteristics vital for nervous system research and both are accepted animal models in nervous system research.

When rats are used in experiments, it is because they have a nervous system that is sufficiently similar to that of humans and the biology of axon regeneration and plasticity is almost the same. Rats are also capable of complex behaviour and skilled paw use, making it possible to achieve good behavioural outcomes with only subtle changes in behaviour stemming from the small circumscribed lesions that we use in our experiments.

When mice are used in experiments, it is because they can be genetically manipulated, allowing molecular hypotheses to be tested. Their behaviour is almost as good as that of rats however in certain behavioural tasks such as skilled forepaw reaching (one of our main behavioural assays), mice do not perform this task well enough to obtain usable data. Likewise, some of the molecular and cellular responses to tissue injury in mice differs substantially to the human response, whereas rats have a very similar molecular and cellular injury response to that of humans.

Surgery, post-operative care and humane endpoints

Animal suffering will be kept to an absolute minimum by ensuring necessary post-operative monitoring and care including the administration of peri-operative analgesia for all surgical procedures. We minimise suffering by developing and/or using behavioural outcome tests of high resolution that pick up deficits in fine movement control. Therefore, it is not necessary to make large and disabling nervous system injuries, and although we study nervous system injury, the majority of our lesions do not paralyse the animals. In these cases, animals recover sufficiently to show normal behaviour within their home cage within the first week post-surgery. In certain cases, including peripheral nerve or dorsal root injury, animals may experience dragging of the affected paw due to decreased sensation (dorsal root injury) and/or reduction in motor function (peripheral nerve injury). In these cases, only one limb will be affected and it will not gross affect mobility in terms of the animal's ability to move around their cage nor will adversely affect eating or drinking behaviour.

Post-operative observations will be performed continually until the animal regains consciousness, following that, the animals will be observed several times in the first few days following surgery as well as at any other stages in the experiments that pose a higher risk of adverse effects. Once the animals have stabilised, post-operative care and observations will be performed daily at a minimum. Our monitoring of post-operative animals will include analyses of mobility, body condition (piloerection, hunching), facial expression (https://www.nc3rs.org.uk/using-facial-expressions-pain-animals) and weight. Loss of up 20% of body weight will be taken as a humane endpoint. This monitoring protocol will ensure that any animal showing signs of paralysis or other adverse effects will be picked up immediately so as to limit suffering as much as possible. Any animals exhibiting signs of distress will be closely monitored and advice from the local NVS will be sought.

In our experience, by choosing well-established lesion models that have been extensively used in my previous studies, we are able to ensure a high rate of reproducibility with our lesions (consistent size and outcome), leading to less adverse effects in animals and overall lower numbers. Likewise, our behavioural tests build upon well-established protocols for which the adverse effects are known and preventative measures will be taken to avoid them. Furthermore, we continue to refine our surgical methods to ensure reproducible results.

If we observe evidence of distress, measures will be taken to alleviate these symptoms as described in the adverse events sections for the Protocols. If animals show signs of distress for which a cause cannot be identified, we will seek advice of the local NVS. If animals are anticipated to be close to a defined endpoint, they will be monitored more closely. From experience, very few (<5%) animals experience or show signs such that humane end points may be reached.