Home Office

NON-TECHNICAL SUMMARY

Repair and protection of the retina and optic nerve.

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

Blindness, CNS disease, glaucoma, nerve regeneration, neuroprotection

Animal types	Life stages
Mice	adult
Rats	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?

To test new treatments for enhancing protective and regenerative machinery in the optic nerve. These treatments aim to treat loss of vision due to glaucoma, and to regenerate fibres in the injured or diseased central nervous system.

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?

The aim of this work is to treat disease or injury to nerve cells (neurons) in the optic nerve and retina. The intention is to develop new treatments for the degenerative eye disease, glaucoma. Additionally, the findings will be relevant to treating many neuronal diseases or injuries in the brain or spinal cord.

Glaucoma is one of the leading causes of blindness worldwide. Glaucoma is a neuro-degenerative disease which causes the nerve fibres (axons) between the retina and brain to deteriorate. This ultimately leads to the death of the nerve cells (neurons) in the retina which extend their axons towards the brain. Currently treatments to protect retinal neurons are not very good, and there are no treatments that allow regrowth of damaged axons back towards the brain. Adult retinal neurons are similar to neurons in the brain or spinal cord, in that they do not regrow after they are damaged by injury or disease.

The aims of this work are to identify and test new treatments that will (1) prevent retinal neurons from dying during glaucoma or after other injury or disease to the optic nerve, (2) enhance the regrowth of damaged fibres. Our work has shown that axons in the optic nerve, brain and spinal cord do not regrow after injury or disease because the cellular machinery for protection and regeneration is not abundantly there. We have discovered some treatments that allow this cellular machinery (components that trigger growth or survival) to be directed into the nerve fibres. This enhances the ability of nerve fibres to regrow, and prevents retinal neurons from dying. We have tested many treatments using models of injury and disease in cell-culture dishes, reducing the need for animal experiments, but ultimately, we need to test these treatments using animal models of retinal and optic nerve injury or disease, so that we can see whether they are worth developing to treat patients.

We also need to fully examine specific protective and regenerative molecules (cell components) into the optic nerve. We know about some of these molecules, but we need to understand associated processes much better in order to identify better treatments.

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

As with our previous work in this area, we will publish our results in high-impact peer-reviewed journals. Our latest manuscripts were published in broad-appeal and clinically relevant journals, meaning the work is read not only by clinicians interested in potential new therapies but also by scientists from many different disciplines interested in scientific advance.

We would also aim to identify potential treatments that are novel and unexpected and suitable for business development. Suitable therapies with high potential for clinical translation will be considered for patent applications.

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

Our research appeals to scientists investigating novel treatments for glaucoma, and repair of the injured or diseased retina and optic nerve. Clinicians in this field are also very interested in our work, keenly anticipating potential new therapies. The work is also of interest to the central nervous system (CNS) repair community. Interventions which enable axon regrowth in the optic nerve are usually efficacious in the brain and spinal cord, so researchers looking to repair the injured spinal cord are also very interested in our findings.

Ultimately, our research is aimed at developing new treatments for eye disease. The intention is for our work to benefit the vast number of patients worldwide who are suffering from loss of vision due to degenerative disease in the retina and optic nerve.

The work will also be widely disseminated to the scientific community through publications and the findings will be presented at local, national and international scientific meetings.

The data will also be used in support of future funding applications and potentially for patent applications for development towards clinical treatments and enterprise.

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

We collaborate with many scientists throughout the world and build on our findings through further experiments which are better performed by these additional experts. Our recent papers were published together with many labs worldwide, and we are part of global research consortia. In addition to publication in high-impact journals, the work is also presented at conferences worldwide, within the eye research community, CNS repair community, and at specialist cell biology meetings aimed at understanding relevant cell biological mechanisms. Papers are usually published as open access articles, and can include positive and negative (unsuccessful) findings.

Species and numbers of animals expected to be used

- Mice: 900
- Rats: 550

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.

This project will use mice and rats, which have not been genetically modified. The group has extensive experience with both species and all new ocular drugs require testing in a mammalian system..

Mice and rats are used because their eye anatomy is similar to that of the human eye, including the response to injury. We use two models of optic injury and disease. The injury model is well characterized in mice, as is the disease model in rats. Rats are required for the disease model, because the larger globe size is necessary to accurately model disease pathology. It is also useful to show that our novel protection and regeneration strategies work in more than one species.

These models need to be investigated in adult animals, because the response to injury is very different in young animals, and the disease we are modelling does not occur in young patients. The young central nervous system has a limited ability to regenerate after injury, whilst the adult central nervous system has almost none. It is the injured or diseased adult central nervous system that we are aiming to repair.

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

We have spent many years investigating the mechanisms that control the ability of nerves to repair after injury or disease, using cell culture models of injury, and we continue to generate new targets for repair using these models, which we make into new therapies. The animals used in our projects will typically receive an injection into the eye. This is a not painful, and is routinely used for many current therapies to treat patients with glaucoma and other eye diseases. The injection is intravitreal, which is a shallow injection through the white of the eye, to deliver a small amount of liquid into the vitreal humour inside the eye.

Animals are then left for a few weeks to allow for the treatment to work in the eye. The animal will then receive either an optic nerve crush (to model injury) or a laser injury to the eye, which results in raised eye pressure, mimicking disease. The optic nerve crush is performed under anesthesia, and involves moving the eyeball forward of the socket to expose the nerve behind. The nerve is crushed with fine forceps, and the eye returned to its usual position. The nerve does not sense pain, so the crush is not painful afterwards. Animals are left for some time (typically two to six weeks) for regeneration to occur. The animals are then killed, and we analyse regeneration in the optic nerve, and protection from cell death in the retina.

For the disease model, the animal is anesthetised, and a laser is used to cause a very small injury (similar to cauterising) to a specific area around the edge of the eye. This slows down the normal drainage of eye fluid, leading to a build-up of eye pressure, (similar to glaucoma). The injury heals, meaning that the pressure build up is transient, so after a week the injury is repeated. 42 days after the initial injury, the animals are killed, and the optic nerve and retina are analysed for degeneration,

regeneration, and protection from cell death. During the course of this disease model, eye pressure is measured regularly with a tonometer (which is like a blunt pen that is gently pressed against the eye), and eye function is measured by electroretinography ERG, a non-invasive assessment of retinal function using light and electrodes placed on the eye.

For ERG recordings an electrode is placed in contact with the surface of each eye, wire loops placed around the circumference of the eyes and an electrode placed into the tail or skin of the animal. This is done under anesthesia. During the same anesthetic, imaging may be done, using a camera to image through the cornea to observe the retina at the back of the eye.

Tonometer measurements involve touching the cornea with a small probe which provides an instant resistance measurement based on the eyes intraocular pressure. This does not require an anesthetic.

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

Neither the injury procedure nor the disease model are expected to cause adverse effects. The injections into the eye should not cause any complication, and the in life assessments are not painful and do not cause adverse effects. The group has several years' experience with these techniques and rarely encounter problems.

The eye procedures can cause temporary swelling and tenderness, but this should rapidly resolve itself. The animals will be very closely monitored after the procedures to ensure these effects subside, and if necessary, animals will be treated for pain or inflammation. Vision loss occurs rapidly after the optic nerve injury, and more slowly in the disease model. Loss of vision is limited to one eye, so the animals do not become blind from the procedures.

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)?

C57BL/6 (a wildtype mouse strain) – mild (50%) moderate (50%).

Wistar, Sprague-Dawley, Lister Hooded (wildtype rat strains) – moderate (100%).

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

- Killed

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 have worked for many years using cell culture models to study the molecules that control the regenerative capacity of the central nervous system (CNS: brain, spinal cord and optic nerve). We continue to use these systems, which have proven to be incredibly valuable for identifying new targets for stimulating regeneration after injury or disease, as well as protecting injured or diseased neurons from death. Our in vitro studies have discovered treatments that are effective at preventing cell death in the retina as a result of injury, and that they are very effective at stimulating regeneration of the fibres that connect through the optic nerve to the brain.

Whilst we have been very efficient at modelling CNS injury, the eye and optic nerve cannot be modelled in cell culture systems. In order to test whether our new gene therapy tools could be useful for treating patients, we need to find out if they can protect and repair in intact eyes and optic nerves. It is therefore necessary to investigate our new treatments using animals.

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

We use cortical neuron cultures to model CNS neurons, and we are developing new human neuronal culture systems using inducible stem cell derived neurons. We also plan to pursue other cell culture injury models, including scratch assays, where we use a needle to injure a "hedge" of axons and monitor regrowth afterwards.

These are all well respected models of CNS injury. To model eye injury and disease we are working with collaborators who are experts in the retinal explant model.

Our philosophy remains to conduct cell culture experiments to identify new treatments, and to only use animal models for confirmation of therapeutic benefits.

We additionally considered culturing retinal ganglion cells (RGCs) in dishes, but these are notoriously difficult to culture, and they do not behave similarly to RGC neurons in a real-life situation (in live animals).

Why were they not suitable?

Whilst we use cell culture models to study mechanisms of nerve injury and neuroprotection, these models are not sufficient for this project because the cells we are targeting are influenced by many conditions which are not represented in cell culture. For example, the retina and optic nerve contain many cell types in addition to neurons, including immune cells, and to investigate whether our new treatments are beneficial in a meaningful fashion, we need to understand the regenerative or neuroprotective effects in a complete biological situation, where the RGCs are also influenced by the many other cell types. None of the available cell culture models completely model the situation in the injured or diseased eye or optic nerve, but we will continue to use cell culture systems to model aspects of injury or neuroprotection as closely as possible, and all off the new treatments we test have been previously studied in cell culture models.

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?

Sample sizes are calculated from previous similar experiments and from pilot data to minimise group sizes required to demonstrate clinically relevant effects. Pilot studies are always carried out using a small cohort of animals (3-6 animals) before beginning a full study.

Testing of preliminary novel treatments are typically performed on group sizes of 3 to 4 animals due to the reproducibility of the procedure and results. Controls are needed in order to correctly analyses effect size. In the case of our novel therapies, these usually mock delivery systems.

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

For each of the procedures, there is a lot of prior experience within the group, and statistical advice has been obtained from the biostatistics unit. Experiments are conducted to the PREPARE guidelines, and will comply with the ARRIVE guidelines for publication.

Sham surgeries have previously been used to control for unexpected inflammation which may cause an unpredictable response, and are not required for acceptance as a published article. We are quite confident our surgeries only have the desired effect and are keen not to use excessive animals where possible to comply with the 3R’s.

Any new surgery, and any new Procedure Individual Licence (PIL) holder will undertake preliminary experiments to generate data relevant to the technique and research question. Additionally, doses will be calculated from effective non-toxic concentrations in tissue culture studies to minimise dose ranges needed to be testing in animals.

The minimum number of control groups will be used for each experiment in order to reduce numbers whilst allowing for the experimental data to be statistically meaningful. For new treatments, we know from previous studies that phosphate-buffered saline (PBS) injection and mock delivery injection can be used interchangeably (as results are almost identical) so only one type of control will be included alongside test compounds.

To ensure accuracy of data, we will also use blinded experimentation for the majority of studies whereby each animal is given a number and the investigating scientist is blinded to which procedures or treatments each animal has received. Unblinding only occurs after the final analysis of tissue and data.

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 maximise data by analysing as many parameters as possible, using as much tissue as possible. Whilst we may be aiming, for example, to analyse regeneration in the optic nerve, we can also retain complete eye and brain tissue for complete analysis of all the pathways potentially involved / affected.

We will list excess organs, where possible, on the animal facility mailing list. We have shared organs with other groups in the past and will continue to donate extra tissues.

Injury or disease procedures will only be performed on one eye per animal, with the other eye serving as the uninjured control. This also helps to optimise the number of animals per experiment, rather than using additional animals as controls.

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.

CNS axon injury model: - Mouse optic nerve crush injury.

The optic nerve crush injury is a widely used and widely accepted model of injury to the axons of the central nervous system, as well as being accepted as a model for measuring neuroprotective effects. Effects identified using this model are good indicators of targets or treatments for protecting and repairing both the injured and diseased CNS. Of the available models of CNS injury, it is one of the least severe, but returns many beneficial results.

The optic nerve crush procedure has been used for many years. It causes damage to the axons of the optic nerve which is highly reproducible, and leads to a progressive loss of RGC neurons in the retina, which again is highly reproducible. The optic nerve relays visual information from the retina to the brain. The injury process involves exposing the nerve behind the eye, followed by crushing with a pair of fine forceps for a defined period of time, usually a few seconds. The nerve and surrounding tissues are then replaced, and the injury site heals without assistance.

All of our work identifying potential treatments for repairing or protecting the injured or diseased CNS begin using cell models in a culture dish. The optic nerve crush injury is challenging, but is far less severe or invasive than other brain or spinal cord models of CNS injury. Use of the model allows validation of our treatments, whilst assessing regenerative and protective effects for the injured or diseased visual system. The optic nerve crush model is a highly beneficial system for identifying, validating and comparing multiple and varied treatments.

A laser intervention to raise intraocular pressure and model glaucoma. An established model in rats.

A laser is used to damage to the trabecular meshwork, the eye's drainage system. This causes a blockage which reduces aqueous outflow from the eye, causing eye pressure to build up transiently. This is reproducible, so has a high degree of success, and has been used extensively by the group. It is only successful in rats, and performs best in non-pigmented animals. The laser settings and parameters required have been standardised and optimised over many years. This will keep complications to a minimum, however if these arise they can normally be treated by maintaining eye lubrication.

The combination of these two models means that we can test potential therapies in both injury and disease scenarios, and in two different species. This is important to demonstrate that treatments can be moved towards developing treatments for humans, and for generating data that can be used for patent applications. For this it is necessary to use accepted pre-clinical models.

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

The objective of this work is to identify treatments for the adult human central nervous system, focusing primarily on repairing the injured or diseased visual system. Adult mammals are required for this work primarily because of the similarity of the visual system to humans, but also because of the comparative regenerative ability of the adult central nervous system (CNS). The regenerative ability of the mammalian CNS declines rapidly with maturity, so that infants have some ability for regrowth after an injury, whilst adults have very little capacity for regeneration. It is critical to use adult animals that exhibit this feeble regenerate ability in adults. Degenerative diseases such as glaucoma also mostly occur in adult and aged patients.

Because we are measuring regeneration, it takes a long time for effects to occur (weeks), so it is not possible for animals to remain under terminal anaesthesia. It is crucial that the injury and disease models have relevance to human disease, because the ultimate aim of the work is to identify strategies that can ultimately be developed to treat human patients.

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

This is a concise application, with minimal injury and disease models, which will be performed by a limited number of researchers. We will use a recently developed scoring system for recording surgical procedures, on a range of 1-10. Scores below 7 will be followed up with additional care post-surgery, and notes will be made of any particular areas of concern. Animals will be examined throughout the study for evidence of damage and given post-operative eye-gel, or antibiotic if required. Weight and behaviour of animals of concern will be additionally monitored for a 7 day period. Investigators will be required to maintain these records to improve their techniques, and confirm optimal care of animals.

Researchers train routinely on dead animals to allows them to perfect their surgeries and techniques (dead animals are sourced from excess stock in house, which were destined to be killed). This reduces variability in their studies and helps reduce animal usage through failed procedures and minimises suffering.

For injury or disease models, analgesia is provided during the procedures to limit suffering, and maintained for a day afterwards.

A shared spreadsheet will be maintained within the group which details the course of all animals through all procedures performed. This spreadsheet will record each animal, every procedure performed on that animal, the actual severity reached, additional comments to the study and how the tissues were used and stored. This system has worked very well in the past and we have improved our recording and monitoring of animal welfare.

We will ensure optimal communication between the researchers and the technicians in the animal facility, to educate facility staff and managers about the techniques we are using, so that abnormal or adverse effects can be rapidly identified and treated.

Our wider research group has extensive previous experience with the techniques and procedures in this licence application, and has a comprehensive list of potential adverse reactions that could potentially arise from either these or other procedures involving the eye. This will be made available to all the investigators training for or using the procedures, as well as technicians and researchers who are looking after the animals, in order to aid in identification of adverse effects and to minimise potential harms.

Animals will be housed in groups to enable social interaction and grooming. Animals used in these procedures do not normally fight after surgery and open wounds are not expected. We have experienced minimal signs of stress or discomfort from our procedures in the past and expect a good quality of life for the animals on this licence.

We are also developing new tools which allow for a reduction in the number of eye injections.

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

We will follow ARRIVE and PREPARE guidelines. Current publications on the LASA website will also be read. There are also best practice guidelines issued by ARVO, the Association for Research in Vision in Ophthalmology. They have issued a “Statement for the Use of Animals in Ophthalmic and Vision Research” which is available on their website. New researchers will be directed to these resources to encourage current best practice. Further resources regarding veterinary ophthalmology are available through BRAVO, the British Association of Veterinary Ophthalmology although these relate mostly to up to date approaches for treating animal eye disorders.

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

We have a strong record in cell based models of CNS injury, and will continue to develop these to be as relevant to human injury or disease as possible. In routinely keeping up to date with neuronal cell biology literature we will also be alert to new developments in cell-based neuronal models, with developments arising from studies using stem cell derived neurons, human "mini-brain" organoid type cultures, and patient-derived neuronal cell models of disease. We will also continue to monitor the

NC3Rs website for developments, as well as the Norwegian 3Rs website "Norecopa"and will incorporate new approaches into our research. Up to date surgical advice is available on the LASA website, as well as best practice guidelines from ARVO, the Association for Research in Vision in Ophthalmology, available on their website.

We are also collaborating with labs worldwide who use human and animal models of retinal disease, and will keep up to date with developments in their labs, and incorporate these in our collaborative projects.