Home Office

NON-TECHNICAL SUMMARY

Using light to control mammalian physiology

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

neuroscience, photobiology, retinal degeneration, opsins

Animal types	Life stages
Mice	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 develop new ways of remotely controlling cells and tissues using pulses of light and to explore the potential of this technology to restore vision in patients with retinal degeneration.

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?

A technique called 'optogenetics' is one of the most exciting new technologies in biomedical research and may soon be used to treat a variety of diseases. In optogenetics, a light sensitive protein (or proteins) is introduced to a cell that is ordinarily not responsive to light. Flashes or pulses of light can then be used to switch that light-sensitive protein on and off, allowing remote control over the physiology of the cell. Because all neighbouring cells may remain insensitive to light and because light can be focussed to a point and switched on and off very rapidly, optogenetics allows scientists to determine the consequences of changing the biology of one specific type of cell in an intact organ such as the brain. This is a very helpful in experiments aimed at understanding how human physiology is regulated. It also has lots of potential for new treatments for disease. One particularly exciting possibility is that optogenetics can be used to recreate the light sensitivity that many blind people suffer.

The basic technology of optogenetics is well established but there is an ongoing need for new, more refined, light sensitive proteins. The nature of the cell’s response to light is determined by the type of photosensitive protein employed. The light sensitive proteins that allow animals to see are called opsins and are especially interesting options for optogenetics because they naturally activate aspects of cell biology called G-protein signalling pathways that are very widely used by human cells and are targeted by >30% of approved drugs. If we could reliably design opsins to target particular G-protein signalling pathways, that would allow us to better understand how they contribute to cell physiology and also to use them to recover function in disease. In this work we will study how opsins work to photosensitise cells in mice to develop opsins as new tools to understand mammalian biology and to treat disease. We will particularly trial the ability of different types of opsins to restore vision in mice that have the same types of blindness that are common in humans.

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

Over the course of the licence we will 1.) determine rules by which the structure of light sensitive proteins determines what wavelengths of light they respond to, how reliably they can work in different cell types, and what types of response(s) they can ellicit when activated; 2.) develop new types of light sensitive protein that can be used to render different types of cell activity responsive to light. The light sensitive proteins have the potential to be used to address mechanisms contributing to many disorders but in the first instance we will work to develop them as a potential therapy restoring vision in people who have lost sight because of damage to their retina. We will publish our work in scientific papers and make our data available for others to use in their studies. We will submit patents to support further clinical development of our approaches. We will share protein sequences in suitable public repositories or make them available to researchers on request. In the long term (primarily beyond the lifetime of this licence) we hope also to use our findings to inform design and commission of clinical trials of new therapies in patients.

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

In the short and medium term, our work will benefit scientists looking for new ways to change the activity of cells in experiments to understand human and animal physiology and as a step to develop new treatments.

In the long term (primarily beyond lifetime of this project), people suffering from retinal degeneration (by some estimates 1 in 3500 people in industrialised societies).

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

We will publish our work in on open access journals and make our data available for others to use in their studies. We will share outcomes at scientific meetings. We will submit patents to support further clinical development of our approaches. We will share opsin sequences in suitable public repositories or make them available to researchers on request.

Species and numbers of animals expected to be used

- Mice: 3500

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 will be using adult mice for these experiments because they are by far the most popular model for biomedical research in general and as preclinical research on retinal degeneration. This means that our experiments can build upon a wide and deep base of physiological knowledge and take advantage of the wide array of tools developed for this species (including well characterised mutants and genetically modified organisms). It also means that our new optogenetic tools will be validated in the species to which they will most extensively be applied.

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

Animals will receive injection of a suitable gene delivery vehicle (usually a virus that does not itself produce a disease) under anaesthesia. These injections may be into the eye or brain. For brain injections, a small hole in the skull will be cleared to gain access to the brain. Animals will experience the induction of anaesthesia either by gas or an injection and experience distress and transient pain. They may experience some discomfort after surgery that may result in reduced activity or appetite and will be treated with analgesics. The virus we use is safe and used routinely in human gene therapy applications.

They will then be maintained under standard housing for several weeks while gene expression builds up. Many animals will then be killed at this point and body parts collected for laboratory analysis. In some cases we will make some recordings under terminal anaesthesia, in which case animals will experience the induction of anaesthesia and distress but no pain.

Some others will be exposed to different light conditions (including some days in constant darkness) and may be singly housed or will be presented with visual images/movies and their behaviour monitored. Mice are nocturnal, light-avoiding animals and do not show obvious signs of distress in constant darkness. They may experience distress from being singly housed.

A final group will undergo a second surgery under anaesthesia to implant a fibre optic light guide and/or recording electrode into the brain. This will likely use the hole in the skull created to inject the virus but will additionally attaching tiny screws to the skull and securing everything with dental cement before closing the wound. This surgery will typically last 2 hours. Animals will experience the induction of anaesthesia and may briefly experience distress and transient pain. They may also experience some discomfort after surgery that may result in reduced activity or appetite and will be treated with analgesics.

Some days after recovery of surgery the animals may be briefly restrained while the fitted fibre optics and/or electrodes are attached to a longer fibre optic and/or a wire. The weight of the fibre/wire will be supported to ensure that the animal has free movement. Light may then be applied via the fibre optic while we monitor activity in an experimental arena and/or record the activity of the brain. The animals will then be briefly restrained again while the fibre/wire are detached. This procedure may be repeated so that we can record on multiple times from the same animal. Animals will briefly experience distress and no pain from the fibre/wire attachment/detachment. They may experience transient distress but no pain upon release into the experimental arena which may lack bedding and environmental enrichment. The final procedures will be undertaken under non-recovery anaesthesia where the animals will only be aware of the anaesthetic being administered and may briefly experience distress and no pain.

We will also breed genetically modified mice, which may experience transient pain and distress while we take an ear notch for genotyping.

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

We expect transient distress and pain following eye injections or cranial surgery. Cumulative severity will be moderate and adverse effects will be self-resolving.

We expect mild distress for ear notching.

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)?

Mouse: 20% mild, 30% moderate, 50% sub-threshold.

What will happen to animals used in 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?

Light sensitive proteins represent an exciting opportunity to manipulate cell physiology for therapeutic and experimental purposes. They have their most powerful applications in animal studies where they can reveal how intact physiological and neural systems work in the whole organism. This is also the step by which new therapies will be trialled. We can, and will, do a lot of work to establish how light sensitive proteins work in cells in the laboratory. However, intact animals are much more complex than any cell that we can work with in the laboratory: they are influenced by neighbouring cells in ways that we can not recreate and respond to the changing circumstance of the animal; neurons form circuits and pathways that span the nervous system and that cannot be recreated in the laboratory. We therefore need to do final experiments in animals in order to establish how light sensitive proteins perform in the more complex cell environment of the intact organism and how activating an opsin in one cell type can engage biological processes (e.g. vision) that inherently rely upon communities of cells often extended across the body.

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

During our ongoing work in this area we have extensively explored non-animal alternatives to achieve our goals. We have been working in this field since its inception, meaning that we have an excellent knowledge of the options available to achieve our goals, and supplement these with regular reviews of the published scientific literature, curations and summaries using established best practice for systematic searches (e.g. as provided by Norecopa and available from EURL ECVAM), using systematic review tools such as SyRF and encompassing review of established resources for altenative methods (e.g. DB-ALM from EURL ECVAM and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) from the US government), and discussion with the research community. Computer-based models, studies of proteins in a test-tube and in lab-grown cells are the established non-animal approaches. We use these extensively, and indeed, have ourselves developed some of the most widely used. As a result, our project pipeline starts by using existing knowledge of how the composition of these proteins determines how they behave and use this knowledge in computer-based models to predict which proteins might have promising characteristics.

We then first study these candidate proteins in the laboratory by introducing them to lab-grown cells and, where possible, in miniature lab-grown tissues. Only once we have used these non-animal approaches to gain a good understanding of a light sensitive protein will we pick the most informative and promising to study in the intact animal. We have considered applying non-protected species for this work, but the aspects of cell function that the light sensitive proteins target vary quite substantially across the animal kingdom meaning that they can be poor predictors of activity in mammals. Previous studies from other groups and data from our own laboratory have been assessed before defining these approaches to confirm that there is no duplication of existing data, this process will be repeated before designing new experiments.

Why were they not suitable?

We will make extensive use of the non-animal alternatives in our experimental strategy. We first use computer-based models to predict how proteins will function and then do extensive testing and refinement of light sensitive protein design in purely laboratory experiments. This means that we only trial the most promising and/or informative potential light sensitive proteins in animals. However, in the end we need to know how such proteins function in native cell environments and how effective they are at modulating circuit and tissue level physiology. To address these questions we have to introduce the light sensitive proteins in living animals.

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?

We have used our long experience of undertaking experiments of this type to decide the best approaches to address our objectives and the minimum number of animals required to achieve those goals. Wherever possible we also used established mathematical approaches to determine the correct number of animals required to reliably answer our questions. We have considered our ability to employ methods that maximise the amount of information collected from each animal and to reduce the impact of variation between animals by comparing the physiology of the same animal with vs without presentation of light. We have included the benefit of pilot studies and the need in some cases to use 'negative control' animals that will undergo the same procedure but will not express light sensitive protein, this is very important to rule out the possibility that any effect we observe is due to some aspect of the experiment that we have not accounted for.

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

We applied good experimental design principles as encompassed by the NC3Rs Experimental Design Assistant in devising the project. Wherever possible we will compare a single animal's response to different conditions (typically with vs without light stimulation) rather than use two different animals. This reduces the number of animals by more than half because it minimises the impact of animal-to-animal variation. In our brain recording experiments we use the latest equipment that allows us to record the activity of large numbers of neurones simultaneously from a single individual, greatly reducing the number of animals required. We will minimise the number of animals bred by using efficient breeding strategies ourselves and obtaining animals from commercial breeders wherever possible.

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 regularly review progress towards our goals of developing validated light sensitive proteins, identifying where additional work may be required and, conversely, where we have already achieved our objectives. Reviewing the data we have already collected will help us to determine what magnitude of response to light we need to detect and how variable our data are, we can then apply mathematical approaches to this information to determine how many animals we need for future work. We will also feed new data into our computer simulations that we hope will increaseingly enable us to predict the behaviour of light sensitive proteins without trialing them in animals. We will use pilot studies the first time we are studying a new light sensitive protein, comprising a small number of animals used primarily to confirm that the protein is effectively produced in the cells of interest (we will though also collect information about light sensitivity from these animals to maximise their utility). At the end of all experiments, we will harvest as many tissues as possible at post-mortem. If we don’t need to analyse the tissues immediately, we will archive them and make them available to other researchers working on similar questions.

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.

We will be using laboratory mice. We use laboratory mice because we are able to build upon a wealth of existing information about the physiology of this species, and because we have access to animals carrying naturally occurring mutations or engineered genetic modifications that are very useful for our objectives. Gene expression will primarily employ viruses that we can genetically engineer to produce the proteins we are interested in the target cells. The viruses themselves are safe and do not produce any disease. We will inject a small quantity of purified virus to the appropriate part of the mouse. Light sensitive proteins will be restricted to the cells we are interested in by controlling where we inject and/or by using genetic engineering of the virus to make it selective for those cells. Light responses will be assessed by recording activity of cells using electrodes, light meters or microscopes. Wherever possible we will minimise pain, suffering and distress by undertaking assessments of light response either in tissue collected post mortem or under terminal anaesthesia.

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

There are substantial differences in cell physiology across branches of the animal family which make less sentient species imperfect predictors of the behaviour of light sensitive proteins in mammalian cells and tissues.

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

We will apply analgesics to ameliorate post-operative pain. We will provide moistened food pellets and open water in the cage during the recovery period. We will monitor all animals closely in the hours and days post surgery with particular attention to the potential of weight loss, piloerection, changes in mobility, abnormal respiration, eye defects, and wound inflammation. If these are observed animals will be treated accordingly, and animals that develop severe effects will be humanely killed. We will employ appropriate environmental enrichment (nesting material, nest boxes, running wheels) wherever suitable to mitigate harms.

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

We will consider the PREPARE guidelines and, where appropriate, follow those of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement (Morton et al (2000) *Laboratory Animals* 35:1-41).

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

We will regularly check information on NC3Rs website, we’ve signed up to the NC3Rs newsletter, we will meet our establishment's 3Rs Manager, and attend Regional 3Rs symposia.