Home Office

NON-TECHNICAL SUMMARY

# Understanding vision and developing therapies for blindness

**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**

vision, retinal degeneration, neuroscience, circadian rhythms, blindness

| Animal types | Life stages |
|---|---|
| Mice | adult, pregnant, juvenile, neonate, embryo |
| Rhabdomys pumillio | 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?**

The eye allows us to see. It also drives a number of sub-conscious responses to changes in our light environment (collectively known as 'non-image forming responses) such as resetting the phase of our biological clocks and keeping us awake. We aim to understand how the eye and brain work together to allow us to see and to support 'non-image forming' responses to light. We also determine new ways to manipulate cell activity using light in the search for ways of restoring vision in the blind.

**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?**

Vision is one of our most valued senses and impairments in vision reduce quality of life. Many of the most common types of visual impairment are currently untreatable. A more detailed understanding of how vision works, holds the promise of better appreciating disease conditions and developing new therapies. We will also use our knowledge of mechanisms of light detection to explore ways of making cells light responsive and apply this to developing new approaches to treating currently incurable forms of blindness.

A particular interest of our work is such 'non-image forming' responses to visual stimuli. Light can regulate practically all body systems either directly or by its impact on our internal (circadian) body clock. We know that disrupting this control can lead to widespread and intractable public health problems such as obesity and mood disorders. Understanding how vision drives these responses has led us to better control our light environment to support human and animal health.

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

We will achieve a deeper understanding of how the retina and brain allow us to see, what goes wrong during blindness, and how vision may be restored. We will also improve our knowledge of how light influences mammalian circadian rhythms, and behavioural and physiological state. We will publish our work in scientific papers and make our data available for others to use in their studies. We hope also to use our findings as a starting point for clinical trials of new therapies in patients.

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

Our work will benefit people suffering from retinal degeneration (by some estimates 1 in 3500 people in industrialised societies). It may also benefit a much larger fraction of the human population by providing

a greater understanding of how light influences our biology, which can be used to improve design of architectural lighting to better support human health.

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

We have active collaborations with the lighting and consumer electronics industries to see our insights translated into new processes and devices. We also provide guidance to lighting standards and regulatory bodies around the world to ensure that built environments provide the best support for health and wellbeing.

We have ongoing collaborations with the biopharmaceutical industry to see our discoveries in the field of retinal degeneration translated to patient benefit (including a clinical trial in preparation).

**Species and numbers of animals expected to be used**

- Mice: 7800
- Other rodents: No answer provided

# 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.**

Most experiments will be on mice. We have chosen these animals because we already know a great deal about how mouse vision works and this will make it easy to interpret the outcome of our experiments. Mice have a visual system that is designed to work well under dim light allowing us to understand that aspect of human vision. Finally, mice naturally have many of the genetic mutations that cause blindness in humans, allowing us to use mice to study human disease. One limitation of mice is that they do not have such good vision in daytime conditions. In order to get a full answer to these questions we will therefore also include a day active species with good bright light vision. We have chosen to use the African striped mouse (Rhabdomys) that has good daytime vision. The rodent visual system develops post-natally. We will therefore work on adult animals.

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

The most common experiment will be to present an animal with one or more visual stimuli (anything from a single brief light flash to a change in the colour/intensity of the light in its home environment lasting several days or weeks), and measure the animal's response. The response could be a change in the animal's pattern of activity, pupil constriction, or performance of a task to receive a food reward. It may also be a change in the electical activity of the retina or brain. In some cases these methods of measuring vision will be applied to animals which have inherited problems with vision to study human

disease, and we also may use techniques of gene therapy (injection of gene vectors) to change the function of visual neurones and/or as a method of looking for new treatments for humans. Animals may be anaesthetised to record retina/brain activity and for injection of gene vectors.

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

Surgery will cause pain during the recovery period, this will be treated with pain killers and we do not expect it to last for more than a few hours. There will also be transient stress associated with handling for injections and when animals are placed in unfamiliar environments for some of the ways we record behaviour. Some animals will be put on restricted food for several weeks in order to motivate them to seek a food reward during training based visual discrimination tasks, the level of feeding is set such that it does not impact general health or wellbeing. Some animals will be housed under non-24h cycles of light and dark for several weeks, which may theoreticlly result in discomfort but does not in practice produce signs of reductions in general health or welfare. Some animals will have inherited blindness, this does not produce signs of suffering in mice.

**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)?**

20% animals will experience sub-threshold severity. A further 50% will experience mild suffering, and 30% moderate severity.

**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 are interested in how the visual system functions in health and disease, and in the utility of gene-based therapies for retinal dysfunction. Vision is an emergent property of the retina and the brain and, as such, it can only be studied using humans or animals.

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

We considered using computer-based simulations of the visual system, human volunteers, stem cell derived retinal 'organoids' and neurones, and immortalised cell lines. We also considered working with tissue collected from animals.

**Why were they not suitable?**

We do undertake experiments on human volunteers wherever possible. We also have developed methods of recreating light sensitivity in cell lines in the laboratory and use these extensively as an alternative to animal experiments. We use computer simulations of the visual system to better understand our findings and to generate testable hypotheses. Wherever possible we use tissue collected from animals. However, there are important reasons why none of these approaches can replace animal experiments. Vision and circadian light responses are produced by multiple regions of the retina and brain working together. We are a long way from being able to recreate such a complex system in the laboratory (either with engineered cells or with a computer simulation), and so if we wish to understand the capacity and characteristics of this system we ultimately have to work with animals. We can undertake some experiments in humans, but the level of control over experimental conditions (e.g. long-term patterns of light exposure) and the range of techniques suitable for measuring and manipulating brain activity is far smaller in humans than laboratory 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 acheive those goals.

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

Wherever possible we will compare a single animal's response to different conditions rather than use two different animals. This more than halves the number of animals used because it reduces the impact of inter-individual variation. In our electrophysiological 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.

**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 routinely make use of tissue collected from animals at the end of experiments for analysis in the laboratory. We also undertake advanced statistical analysis and modeling of these data so that we can refine our questions and employ the most informative experiments. We also provide our data to other

groups so that they can analyse it to answer their own questions. We will minimse the number of animals bred by using efficient breeding strategies ourselves and obtaining animals from commercial breeders wherever possible.

# 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 and four-striped mice (*Rhabdomys pumillio*). Both of these species are accustomed to laboratory conditions. We use laboratory mice because we are able to build upon a wealth of existing information about the visual and circadian systems in this species, and because we have access to animals carrying naturally occurring mutations or engineered genetic modifications that are very useful for our objectives. Mice also allow us to study how vision works under dim light. A limitation of laboratory mice is that their visual system is adapted for dim light vision. Traditionally, this has led researchers to employ primate or companion animal species (especially cats) in vision research. We have established the four striped mouse as a rodent alternative which has good daytime vision. Working with four-striped mice allows us to understand how vision works under daytime conditions.

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

Although non-mammalian vertebrates have retinas that are rather similar to those of humans, their visual systems differ from our own in other key respects. Most importantly, unlike mammals, they have a wide variety of light sensitive cells outside of the retina. For this reason we have no alternative but to work with mammals.

Rodent visual systems develop after birth, meaning that we cannot address our questions at a more immature life stage. We include work on tissue harvested from terminally anaesthetised animals whenever possible, but this cannot replace studies of the intact visual system or when we wish to understand how behaviour is controlled.

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

We will apply pain killers to reduce welfare costs following surgery. We carefully monitor animals during the recovery period after any procedure capable of causing pain or suffering. Wherever possible we will undertake brain recordings from animals under terminal anaesthesia. We use advanced 3D pose analysis methods developed in the group to provide automated and objective analysis of mouse

behaviour, maximising the amount of information available from each experiment and avoiding the potential for observer bias in scoring behaviour.

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

We consult protocols, training resources and guidelines on best practices in animal experiments available through the NC3Rs website (https://www.nc3rs.org.uk/3rs-resources) and will adhere to them whenever relevant. Topics covered include handling and restraint, euthanasia, humane endpoints, welfare assessment, anaesthesia, and analgesia.

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

We receive regular 3Rs updates through the animal unit and our 3Rs Regional Program Manager.