



Home Office

## NON-TECHNICAL SUMMARY

# Understanding visual and circadian control of physiology and behaviour

### Project duration

5 years 0 months

### Project purpose

- (a) Basic research

### Key words

vision, circadian rhythms, neuroscience, retinal degeneration, light

### Animal types

### Life stages

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Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult
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Rhabdomys pumillio	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult
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## 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?**

In addition to supporting visual perception, our eyes also influence a range of sub-conscious responses to light in the environment (collectively known as 'non-image forming' responses) such as controlling our body clock and keeping us awake. We aim to understand how the eye and brain work together to define our daily patterns of physiology and behaviour and how this relationship might be altered according to longer term patterns of light exposure.

**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?**

By supporting visually guided behaviours and driving non-image-forming responses, light can regulate virtually 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. Likewise, many disease states and disorders are associated with disrupted sleep and circadian rhythms which contribute to reduced quality of life and, potentially also core symptoms. Understanding the biological mechanisms underlying these responses can allow us to devise new approaches, such as better control of the lighting 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 light sensitive cells of the eye (the retina) and brain allow us to see and how light influences mammalian circadian rhythms, and behavioural and physiological state, both acutely and over longer timescales. 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 may benefit a large fraction of the human population, most immediately by providing a greater understanding of how light influences our biology, including by regulating circadian rhythms and sleep. Indeed, between 10 and 20% of adults globally experiencing severe sleep disturbances and more than 30% report issues at a subclinical level. Sleep and circadian rhythm disturbances are also common features of many disease states including blindness/retinal degeneration, neurodegenerative (eg Alzheimer's, Parkinson's, Huntington's) and neuropsychiatric disorders (eg Bipolar Disorder). Findings during the project timeframe may include new drug targets and/or potential approaches for adjusting lighting design that could, in medium to longer time frames (5-20 years), be used in humans to mitigate these issues and improve rhythmic health across the population.

## 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 and share data, wherever possible, in open access repositories. Where relevant, we will submit patents to support further clinical development of our approaches. We also have active collaborations with the lighting and consumer electronics industries to see our insights translated into new processes and devices. Likewise, we will continue to provide guidance to lighting standards and regulatory bodies around the world to ensure that built environments provide the best support for health and wellbeing.

## Species and numbers of animals expected to be used

- Mice: 6500
- Other rodents:
  - *Rhabdomys pumillio*: 1600

## 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 both about the mouse circadian system and how vision works in this species, providing the strongest possible foundation upon which to interpret the outcome of our experiments. Key elements of circadian system function are retained across mammals, from mice to humans providing good translational potential and mice have a visual system that is designed to work well under dim light allowing us to understand that aspect of human vision. Two limitations of mice are that i) they do not have such good vision in daytime conditions and ii) they naturally avoid light and are therefore less well suited to understanding vision under bright light conditions and the impacts of daytime light on non-image-forming responses. 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, primarily, on adult animals. Since our goals including understanding how light exposure influences circadian and visual system development and function, in some cases this will involve animals raised from birth under controlled lighting.

**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 levels of circulating hormones. It may also be a change in the electrical activity of the retina or brain. In some cases these methods of measuring visual

and circadian responses 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) or pharmacology (application of drugs by injection or in the diet/water) to change the function of circadian or visual system neurones and/or as a method of looking for new treatments for humans. Animals may be anaesthetised to record retina/brain activity or undergo surgery for injection of gene vectors into eyes and/or brain or, less commonly, implantation of probes to allow us to define how neurophysiological activity correlates with behaviour in awake, freely-moving, animals.

### **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 analgesics and is not expected to last for more than a few days. There will also be transient stress associated with handling for injections or blood sampling and when animals are placed in unfamiliar environments for some of the ways we record behaviour and mild discomfort/reduced mobility associated with wireless transmitter or tethers used for recording in awake animals. Some animals will be housed under non-24h cycles of light and dark for several weeks, which do not produce overt reductions in general health or welfare. Some animals will have inherited blindness or impaired vision, 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 used in this project?**

- Killed
- Used in other projects
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

## **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 circadian and visual systems function to control physiology and behaviour in health and disease. These functions are emergent properties involving multiple interconnected cell types across retina and the brain. As such, our goals can only be achieved by studies using humans or animals.

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

Drawing on the RAR Replacement checklist methodology (Dukes et al 2025), we searched PubMed and bioRxiv for the following potential non-animal methods {computational model, in vitro, cell culture model, tissue culture model, organoid, human} in combination with search terms reflecting major CNS regions of interest {suprachiasmatic, retina, lateral geniculate, superior colliculus}.

Computational models that capture phenomenological or cellular/network aspects of circadian (e.g. PMIDs: 24039566, 2230697, 12775757) and visual function (e.g. PMIDs: 30215284, 28848380) exist and we have contributed to developing these ourselves (e.g. PMIDs: 34845984, 29117548).

Cell lines exist that can be used to model some features of cell types in the retina (e.g. PMIDs: 40392392, 40402521, 39848559) and circadian system (e.g. PMIDs: 39092782, 36293078) in isolation. Retinal organoids can further be used to model aspects of the developing retina (e.g. PMIDs: 40943594, 40571178).

Studies in humans can allow investigations of certain overt aspects of visual (e.g. PMIDs: 40839165, 37450287) and circadian system function (e.g. PMIDs: 40587501, 38135734, 37343084). As above, our own work has contributed to the development of relevant methods for studying these systems in humans (e.g. PMIDs: 39672888, 38765485, 37812713, 36278538, 22633808).

## Why were they not suitable?

We do undertake experiments on human volunteers wherever possible and use computer simulations of the circadian or visual system to better understand our findings and to generate testable hypotheses. Where aspects of objectives can be addressed using in vitro cell culture models or using tissue collected from animals we do so. 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 achieve 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 modelling 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 minimise 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 laboratory bred 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 and key features of how the circadian system functions are conserved with humans. A limitation of laboratory mice is that their visual system is adapted for dim light vision and that they avoid bright light. 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, a model much closer to how humans operate. Working with four-striped mice allows us to understand how vision works and how the circadian system is regulated under daytime lighting 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 analgesics 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, non-invasive measures of behavioural state (based on 3D motion capture) 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. This includes general guidelines and best practice for planning and reporting animal experiments (PREPARE, Smith et al 2018; ARRIVE, Percie du Sert et al 2020), handling and restraint, euthanasia, humane endpoints, welfare assessment, anaesthesia, and analgesia (Morton et al 2001).

### **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 Establishment 3Rs manager.