Home Office

NON-TECHNICAL SUMMARY

Circadian regulation of behaviour, physiology and metabolism

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

metabolism, circadian biology, cardiovascular health, adipose, heart

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult
Rhabdomys	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 aim of this work is to define how circadian timing systems (our bodies' internal clocks) regulate rhythmic physiology, energy metabolism, and our overall cardiovascular and metabolic health.

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 circadian timing system regulates daily rhythms in virtually all aspects of our biology, from sleep/wake timing and eating habits, through to how individual cells of the body produce and use energy. Uncovering how this internal clock system works is fundamental to understanding not only our basic biology, but also how our health is impacted by aging and the pressures of modern life (which can undermine the normal function of our body clock). It is becoming increasingly clear that disruption of our circadian clock is detrimental to health and well-being, and has been linked to numerous disease states ranging from cancer to metabolic diseases, including type 2 diabetes and cardiovascular disease. Our work examines this link between the clock and disease states at both a cellular and whole organism level. These studies continue to reveal important events which drive pathology and identify novel approaches to improving human health and well-being.

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

Major Benefits. The primary outcome and benefit of this project is the advancement in understanding of how the biological timing mechanisms regulate physiology and behaviour. A specific context of our work is in the development of metabolic diseases, such as type 2 diabetes and cardiovascular disease, and how disruption of our bodies' clock system (e.g. through aspects of modern life such as shift work) contributes to these conditions. Thus, a primary output from this work is knowledge creation, which will be delivered through publication in academic journals, conference seminars, and communication with regulatory and advisory committees, the clinical community and wider public.

The potential for this research to benefit human and animal health is high, given the scale of the unmet need in clinical medicine for new ways to deal with metabolic diseases and pathologies associated with circadian dysfunction. Our work will identify key cell types and molecular pathways that initiate and propagate such disease processes. These basic science advances are essential in the long-term for medical breakthrough and therapeutic advancements. Development of therapeutic interventions (both pharmacological and non-pharmacological) which target the circadian rhythm to improve well-being is achievable. For example, outputs from our work have, and will continue to inform on the use of timed feeding, specific light interventions and improvement of behavioural routine to achieve health benefits in humans and animals. Benefits will include the identification of molecular targets at which new pharmaceutical products could be aimed.

The research also benefits the health and welfare of animal in the laboratory, farm and at home by providing better understanding of how environmental conditions impact on the circadian clock, and in turn downstream clock-related physiology.

Additional outputs are likely to include the development of new research methods and protocols. This may be in the form of refining and/or enhancing current methods, or through the development of new tools and approaches (such as longitudinal non-invasive ECG recording). The scientific community will benefit from our work through collaboration and data sharing. Our data will be made available to other researchers in the scientific community at the earliest appropriate time, and therefore enable further/secondary use of the outputs for scientific discovery.

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

Our internal body clock is fundamental to our biology, and how we live our lives (e.g. when we eat, sleep, work, stare at computer or phone screens…) can profoundly affect our health. Our work will define new aspects of cardiovascular and metabolic physiology, reveal mechanisms of circadian clock control over physiology, and determine how clock function (and dysfunction) contributes to metabolic disease. The outcomes will benefit and inform the following communities:

Scientific community (short and long-term) through knowledge advancement, data sharing, and technical, analytical and tool development.

Health care sector, occupational health regulators, government policy advisors (medium to long-term) through advancing understanding about our circadian biology, the potential harms of circadian disruption, and advancement in strategies to combat circadian disruption.

commercial sector (medium to long-term) through knowledge advancement and the identification and understanding of novel therapeutic targets in circadian disruption and cardiometabolic disease.

The general public (short to long-term) in terms of both understanding the potential positive/negative impacts of daily routine on health, but also how light and/or food may be used to alleviate such consequences.

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

We aim to maximise the outputs of this work through multiple channels:

Dissemination of Knowledge: We will publish our findings in peer-reviewed, open-access journals to ensure they are widely available, including to the general public. As we have done routinely, we will use open-access repositories and pre-print servers to expedite sharing, including negative or inconclusive results to help avoiding unnecessary duplication.

Communication: Our research will be presented at national and international conferences and shared through public engagement platforms and events. We usually collaborate with our institute’s press office to further promote our findings to the wider public. Engagement activities will raise awareness about the importance of circadian disruption and cardiometabolic disease and the science behind

them. This may spark more interest in health and correct lifestyles, but also encourage future generations to pursue scientific careers.

Collaboration: We will continue collaborating extensively with national and international researchers, clinicians, and pharmaceutical partners to share findings, accelerate translation, and maximize the impact of the research.

Translation and Impact: Our team will use Institutional infrastructure that facilitate translation of basic research into clinical applications, supporting knowledge transfer and drug development.

Species and numbers of animals expected to be used

- Mice: 13500
- Other rodents:
    - Rhabdomys: 250

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 use adult mice for the vast majority of studies undertaken within this project. This includes normal control and genetically modified mice. Importantly, the systems that we are studying (the body clock, regulation of energy metabolism, and development of obesity) are well reproduced between mice and humans, and mice are therefore appropriate for this work. The inclusion of day-active mice, Rhabdomys Pumilo, further increases the translational relevance of our work.

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

Common procedures (~65% of the mice that we study will undergo one or more of these procedures). i) Animals may be monitored for natural behaviours under normal or altered environmental conditions (e.g. alterations in light/dark cycle, modulation of ambient temperature across a natural range, altered patterns of food availability). ii) Animal may undergo physiological monitoring and/or imaging using non-invasive specialist equipment. iii) Animals may also receive injections or have small blood samples collected. iv) Animals may have the amount, composition (e.g. increased fat content) or timing of their food changed.

Rare procedures (~12% of the mice that we study will undergo one or more of these procedures). i) Surgery in order to insert a device for physiological monitoring, slow release drug delivery, or to induce a change in gene expression (minor surgery with rapid recover of typically 1-3 days). ii) experience changes in ambient temperature (within a naturally occurring range) to assess adaptive metabolism.

In addition to these procedures, mice will be used in this project for breeding, and for provision of cells and tissues for laboratory-based studies.

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

Much of this work examines normal behaviours and physiological processes in the animals. Therefore, most animals experience only transient and mild adverse impact (e.g. temporary stress, discomfort, and/or weight loss) caused by alterations in their housing environment (e.g. changes in lighting cycle, ambient temperature, timing of food availability) and/or cage type. Removing food access for 1-2 days may lead to more pronounced weight loss, but this is rapidly recovered upon re-feeding.

Some animals used here will also experience transient discomfort and mild pain due to surgical implantation of monitoring devices and/or injections.

Alterations in diet composition may lead to weight gain and obesity. Over time (~4 months in mice), obesity may lead to insulin resistance and low level inflammation, akin to that observed in individuals with type 2 diabetes. This may cause subtle changes in behaviour (e.g. reduced activity), but is not associated with any suffering or distress.

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)?

The studies to be undertaken in this project will result in a cumulative impact to the animals that are of a sub-threshold to mild (~60% of animals used) or moderate (~40% of animals used) severity rating.

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?

The diseases we are studying involve the complex interaction between multiple organ systems, and often progress over long periods of time.

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

We routinely use in vitro (e.g. using cells) and information from human data resources (e.g. UK BioBank). In vitro cell/tissue assays can offer powerful models to test how genetic or pharmacological alteration of cell function may impact on clock or metabolic activity and vice versa, and can inform on the best design of subsequent in vivo animal experiments. The complex neural pathways controlling sleep and metabolism however make it unlikely that such approaches can replace the need to use animals. Human studies are important for informing our work, but cannot replace the need to do mechanistic studies in animals.

Why were they not suitable?

We always explore alternatives to using animals in research, as well as developing methods which allow us to use fewer animals to achieve the same scientific objective. However, since circadian biology and metabolic diseases involve multiple tissue systems acting across the body, it is impossible to wholly mimic this biology in a culture dish or by computer.

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 extensive experience with these approaches and in running projects of similar scope. Thus, estimates of animal use is based on i) previous work and experience with the methodologies used, the physiological parameters to be studied, and the specific mouse models to be used; ii) the scope and objectives of the current project; and iii) careful consideration of experimental design.

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

The projected number of animals used over the 5-year duration of the licence is an estimate. At each stage of the project and within each study conducted, we will ensure that animal use is minimized. We employ careful planning of experimental design including the use of supporting purpose written software (including the National Centre for the Replacement, Refinement and Reduction of animals in research, Experimental Design Assistant). We have consulted with statisticians in preparation of this licence, and will continue to do so throughout the project.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In addition to good experimental design, we work to optimise animal use wherever possible. This starts with efficient breeding of transgenic mouse lines. For example, well planned breeding minimise age

variation and breeding numbers, while achieving adequately powered group sizes. Where new approaches are being employed, we optimise experimental conditions through pilot studies. Wherever possible, we share tissues, data, and results with other research groups to maximise the outcome and use of each animal study.

Moreover, through continuous development of our approaches we seek to reduce the use of animals. For example, our development of novel mouse lines which allow longitudinal measures of gene or protein expression (via bioluminescence, where genes or proteins are coupled to a protein that emits light) to be recorded from individual animals or isolated tissue samples. This can replace the need to study individual animals (or collect numerous samples) at each of a series of discrete time points.

In addition, we will take several measures:

Statistical Analysis: Before each experiment, we will perform statistical analysis to determine the minimum number of animals required for valid, informative results. We will also benefit from expert biostatistical collaborators, who input into study design and analyses.

Within-Subject Design: When possible, we use a within-subject design, where each mouse serves as its own control, reducing the number of animals needed.

Pilot studies: For new or exploratory experiments with unfamiliar genetic models or novel interventions, we will start with small pilot studies to gauge effects and optimise design for future work.

Sharing Samples and Data: Biological samples and experimental data will be shared with other scientists to avoid unnecessary animal breeding for similar experiments.

Avoiding Duplication: To prevent unnecessary duplication of studies, we will regularly review the literature and attend scientific conferences to stay informed about current research.

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.

This project focuses on mammalian physiology with all our studies being conducted in mice. Our scientific objectives require us to examine whole animal physiology in adult animals over both short and long-term time frames. The methods we use are, for the most part, non-invasive or involve only temporary discomfort due to surgical implantation of a monitoring device (e.g. to monitor heart rate). Animals may also be provided with alternative diets (e.g. to drive obesity), but these conditions do not themselves lead to suffering and/or distress. To minimise any impact to the animals, we always end the studies as soon as the study objectives have been met. Our researchers are well trained and

experienced in animal behaviour, physiology, and welfare; this reduces stress to the animals under study, and ensures that animals are well monitored and cared for.

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

Using less sentient animals or immature life stages does not allow us to fully capture the complex neuroendocrine, circadian and cardiometabolic interactions our research requires. The behavioural and physiological responses we study develop fully only in mature mammals, making adult mice the most suitable model. As most of our studies involve long-term assessment, they cannot be conducted in terminally anaethetised animals.

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

As we study natural physiological responses and behaviours in the animals, minimising stress, discomfort and other general impacts is critical to our work.

We take many steps to minimise adverse impact, including acclimatisation of the animals to our researchers and any test conditions (e.g. new cages) during study. Examples of relatively simple, yet highly effective refinements include using appropriate handling techniques (e.g. tube handling for cage extraction) and use of appropriate home cage enrichment. We provide active monitoring and care of our animals. Animals are closely monitored, especially after any procedures that could cause stress or discomfort. If we notice signs of discomfort, for example unexpected weight loss or dehydration, we provide fluids or place soft food nearby to help them recover quickly. Where animals do undergo invasive treatments (e.g. surgery to implant monitoring devise), we ensure full recovery of the animals under close monitoring and post-operative care to minimise any suffering (e.g. pain management with analgesics, provision of extra fluids). Where appropriate we will assess whether acute studies can be undertaken under general anaesthetic rather than in conscious mice (e.g. acute ECG assessments).

Examples of our past and ongoing refinement of approach include increase efficiency in breeding through the use of conditional and inducible transgenics (which allow for the activation or deletion of genes in specific cells and tissues at specific times), development of genetic reporters to reduce animal use, transition to advanced technical approaches which provide higher quality of information with less impact to the animals, and development of sophisticated analyses to maximise the data obtained from in vivo studies.

As major refinement to current studies, we aim to develop and implement a non-invasive approach for longitudinal recording of heart rate and ECG.

We work closely with facility experts to keep updated on the latest research and techniques, and adopt improvements whenever possible. We also keep informed about new technologies and methods that could improve both scientific outcomes and animal care, adding these whenever possible to refine and reduce procedures.

By integrating these practices, we aim to maintain the highest standards of welfare, ensuring animals experience minimal discomfort while producing reliable and meaningful scientific data.

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

We will ensure best-practice through continual assessment of experimental design and study outcomes, relative to the cumulative impact to the animals. We will stay informed about regulatory standards (e.g. through local animal facility communications and NC3Rs publications) and developments within the scientific community (e.g. publications on standardisation and best practice for metabolic phenotyping of mice).

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

We stay informed about advances in 3R approaches, and will continue to implement these into our work where appropriate. This includes, but is not limited to, staying up to date with NC3Rs recommendations and developments in the literature and scientific community. Additionally, we maintain regular communication with our Named Veterinary Surgeon (NVS), Named Animal Care and Welfare Officer (NACWO), Named Information Officer (NIO), NC3R champions and other named staff, who advise us on facility-specific refinements. We actively discuss potential refinements in experimental approaches based on our ongoing research to implement these advancements effectively throughout the project.