



Home Office

NON-TECHNICAL SUMMARY

Circadian regulation of chronic inflammation

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Circadian, Inflammation, Arthritis, Colitis

Animal types

Life stages

Mice

juvenile, adult, pregnant, neonate, embryo

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 project is to explore how the function of the immune system varies over the course of the 24 hour day, and how these daily changes impact on the development, progression and treatment of chronic inflammatory conditions.

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?

Chronic inflammatory diseases such as rheumatoid arthritis (RA), colitis and psoriasis are debilitating conditions. The estimated prevalence of these conditions within Western society is 5-7%. The symptoms of chronic inflammatory diseases often show daily variation. For example, RA patients report increased pain and joint stiffness in the early morning. Similarly, biological markers used in the clinics to assess disease severity fluctuate over the course of the 24 hour day.

The circadian (24 hour) clock is a timing mechanism which synchronises animal physiology to the 24 hour environment created by the earth rotating on its axis. This biological timer regulates numerous aspects of physiology, including sleep-wake cycles, feeding and metabolism, the gut microbiome, hormone secretion and the immune system. Disruption of the circadian clock, through environmental disruption (e.g. shift work) or through genetic disruption is associated with increased incidence of inflammatory diseases.

The circadian clock plays a critical role in regulating the normal working of the immune system and ensuring appropriate inflammatory responses are mounted when the system is challenged. This work investigates mechanistic links between the biological timing and immune systems to understand the involvement of the clock in regulating chronic inflammatory disease. An ultimate goal of these studies is to reveal novel therapeutic targets or improve existing therapeutic regimes to treat disease through the use of biological timing (chronotherapy).

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

A major output from this project will be an **advance in our knowledge** regarding clock control of immune responses. More specifically, information generated from these studies will contribute to our understanding of how the circadian (24 hour) clock affects the development and progression of human chronic inflammatory diseases. This information will further our understanding of how circadian disruption (a consequence of rotating shift-work) may impact on the function of the immune system in health and disease.

It is predicted that data generated by these studies will have a **positive impact on the diagnosis and treatment of human chronic inflammatory disease**. An example here is the implementation of chronotherapy - timing therapeutic interventions with the peak of disease symptoms. To maximise these positive benefits, it is essential that information obtained from studies outlined here is disseminated effectively. This will be achieved through: **publication in academic journals**; **presentation of data at conferences**; and through **public engagement** events.

A secondary benefit of this programme of work is the **advancement of research methods**. This may be in the form of refining or enhancing current methods or through the development of new transgenic mouse lines. The data generated from this project will be made available to other researchers in the scientific community at the earliest appropriate time therefore **informing further scientific discovery within our research community**.

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

Healthcare sector: There is a growing understanding of the importance of considering circadian time in the diagnosis and treatment of inflammatory disease. This work will further our knowledge of how the circadian timing system interacts with processes underlying human chronic inflammatory conditions. It is hoped that these studies will promote further incorporation of "clock logic" into clinical practice in the long-term. That may be through standardising the time of day at which a patient's blood is sampled for a disease biomarker, or through recommending the best time of day at which to take medication.

Scientific community: This programme of work will generate new research tools and advance research methods which will be shared with the scientific community. Data generated in this project will advance our basic understanding of clock control of immunity and will be of benefit to the wider research community.

General public: The importance of the circadian clock and good sleep hygiene for maintenance of health is becoming widely recognised by the general public. Information obtained through this project will be of interest to people engaging in shift-work and patients suffering with chronic inflammatory disorders. It is becoming more and more evident that disruption of the circadian clock has negative consequences on health. Whilst sometimes circadian disruption cannot be avoided (e.g. shift-workers), for some individuals small lifestyle changes may have a positive impact on health. In order to engage the general public, we will continue to reach out via public engagement events run by the University and charities.

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

Outputs from this work will be published in highly visible journals targeting a multi-disciplinary audience. Through the use of social media (Twitter) and the establishment press office, we will publicise these publications as widely as possible to engage with the general public. In the past, this has led to opportunities to present our research on the radio, television and news websites. We appreciate that it is important to disseminate negative findings to minimise replication of experiments across research institutes. On going work will be presented at international and national meetings aimed at circadian biologists, immunologists and specialised clinicians. To maximise the benefit of our research we will continue to engage with the general public and relevant patient groups through organised events. These activities are important for maintaining public interest in our research and also further developing trust in UK research.

Species and numbers of animals expected to be used

- Mice: 24 750

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.

These studies will utilise juvenile and adult mice, which may be genetically modified. Mice are the most appropriate species for these studies as the systems which we are studying here (the body clock and immune system) are well reproduced between mice and humans.

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

Animals may be monitored non-invasively for behaviour under normal or altered environmental conditions (typically for periods lasting 2-6 weeks). These environmental manipulations include changes in the light/dark cycle, changes to the composition of the diet or changes in timing of food availability. More rarely, animals may undergo physiological monitoring using implanted telemetry devices (requiring a brief surgical procedure) or imaging facilities (under recovery anaesthesia). Rarely repeated imaging may be utilised (up to 8 times in one day). Changes to rhythmic biological signals (such as hormones or microbial metabolites) may be instigated through implantation of hormone pellets under the skin (brief surgical procedure); through application of antibiotics; or through administration of a chemical reagent. On occasion when utilising specific strains of transgenic animals a gene inducing agent may be administered to the animal in order to "switch off" the gene of interest.

To instigate chronic inflammation different approaches will be utilised, each modelling a different human chronic inflammatory disorder. In each instance, mice will usually only be maintained in a chronic inflammatory state for 5-10 days. Each of these models will be used alone (never in combination).

(1) Joint inflammation: This may be induced via an injection of collagen which drives an autoimmune response and chronic inflammation of multiple joints (collagen induced arthritis, CIA) or via use of methylated bovine serum albumin which drives a resolving inflammatory arthritis affecting just one joint (antigen induced arthritis, AIA).

(2) Gastrointestinal inflammation: This may be induced via temporary administration of a chemical agent in the drinking water which causes damage to the gut barrier. This drives inflammation of the colon which resolves over time when the chemical agent is withdrawn.

(3) Skin inflammation: This may be induced via application of an agent to the skin of the ear or the back, which causes a resolving local inflammatory response.

Prior to, or during these procedures to induce an inflammatory response, the immune system may be manipulated. This may be via application of reagents to target specific pathways. On rare occasions the immune system may be manipulated further through irradiation to deplete host immune cells before replacement with donor cells. Additionally, reagents may be administered which target the clock or the immune system to establish their effects on inflammatory processes.

Animals utilised in these models of inflammation may be assessed using *in vivo* imaging, collection of small volume blood samples and/or assessment of metabolic function or gastrointestinal function. To assess metabolic function, animals may be administered an injection of insulin or glucose and blood samples taken, or may have harmless metabolic tracers added to the drinking water. To assess gut barrier permeability animals may be orally administered a fluorescent labelled sugar prior to collection of small volume blood samples.

In some instances, a line of mice will be utilised which express a green fluorescent protein which can be converted to a red fluorescent protein through application of violet light. Here, animals may be briefly anaesthetised and exposed to this light source in order to be able to track the movement of cells from one site in the body to another.

In addition to these procedures, mice will be used in this project for breeding, and for provision of cells and tissues for *ex vivo* studies.

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

Animals may experience mild adverse effect such as temporary stress or brief pain and discomfort. Temporary stress may be induced by a brief period of restraint (e.g. in order to administer an injection) or alterations in their housing environment (e.g. single housing). Mice may experience a brief period of pain and discomfort in response to dosing (e.g. injection or oral gavage) or blood sampling (e.g. tail bleed) or following surgical intervention to implant a telemetry device or hormone pellet or to apply a photoconverting light source. In these instances the stress and discomfort will be transient.

Weight loss may occur after manipulation of the diet, but will be transient and will either reverse or stabilise. Transient dehydration and weight loss may occur as a consequence of addition of an agent to the drinking water which may make it less palatable or as a consequence of irradiation protocols.

Models of chronic inflammation will induce localised inflammatory responses resulting in pain. Models of arthritis (collagen induced arthritis (CIA) and antigen induced arthritis (AIA)) will lead to swollen, inflamed joints and may result in reduced mobility. CIA is a polyarthritic model whereby multiple joints may be affected. Animals are usually maintained in an arthritis condition for 7-10 days. Affected animals exhibit reduced activity and may exhibit some weight loss. AIA is a monoarthritic model of resolving inflammatory arthritis whereby only one knee joint is affected. Here, animals are usually maintained in an arthritic condition for up to 10 days. These animals may exhibit a brief period of reduced activity (1-2 days) and rarely exhibit weight loss. On occasion (<20% AIA animals) mice may be manipulated to induce one or two subsequent "flares" after the initial insult in order to model periods of increased disease activity experienced in human disease.

Gastrointestinal (GI) inflammation is associated with pain, development of diarrhoea, occasionally the presence of blood in the stools and weight loss. The inflammation inducing agent is withdrawn after a period of time (usually 5 - 7 days) after which the inflammation resolves. On occasion (<20% colitic animals) mice may be manipulated to induce one or two subsequent "flares" after the initial insult in order to model periods of increased disease activity experienced in human disease.

Skin inflammation is associated with reddening, scaling and thickening of the skin. This is associated with pain and discomfort and weight loss. Here animals are usually maintained in this condition for 5-

10 days.

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 outlined in this project will result in a cumulative impact to the animals (mice) that are sub-threshold to mild (approximately 60 percent) or moderate (approximately 40 percent) severity rating.

What will happen to animals at the end of 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?

In the context of organ-level inflammation, it is currently not yet possible to replicate the complex multi-cell environment underpinning disease. Thus, *in vitro* cellular models and *in silico* models have limited application as a replacement for studies of chronic inflammatory disease. In order to study complex interactions between the circadian timing system and immune responses, mouse models are the most appropriate approach.

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

In vitro approaches include the use of single-type cell lines or co-culture systems (where two or more relevant cell types are studied together). We utilise these methods to inform the direction of animal studies. *In vitro* assays can be used to test how genetic or pharmacological interventions alter the function of the cell intrinsic clock and/or regulation of inflammatory processes. For example, *in vitro* assays using cell lines or primary cells allow us to first test a diverse range of potential therapeutic interventions and identify a small number of candidate molecules with the most potential for subsequent use *in vivo*. However, these approaches cannot replicate the complex environment of the joint, gut or skin and cannot replace the use of animals.

This programme of work is supported by parallel studies using tissue samples from humans. Synovial fluid and serum collected from healthy volunteers and rheumatoid arthritis patients will be analysed to assess time of day variation in inflammatory mediators and metabolites. Additionally the group routinely utilises online databases such as UKBiobank and NIHR IBD Bioresource to examine circadian features of patients with chronic inflammatory disease.

Why were they not suitable?

It is critical that any cell lines utilised possess a functional clock. Our experience is that a cell type may be rhythmic in a healthy animal, but once removed and cultured it becomes less rhythmic. This may be due to the absence of other signals (for example hormones) that these cells would normally be exposed to. Furthermore, *in vitro* assays cannot adequately model the complete array of inflammatory responses or address how systemic timing signals may modify these responses.

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?

Our grouping has extensive experience with the methods and approaches outlined here and of running projects of a similar scope. Consequently, estimates of animal numbers are based on previous experience and with careful consideration of the experimental design. Where protocols are new to the group, we will run small control experiments (taking advice from local colleagues with expertise in these models) to generate data on which to perform power calculations in order to plan future studies.

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

When considering the design of experiments underpinning this project we have consulted with statisticians to gain specialist advice on the types of experiments that will be undertaken and the nature of the datasets that will be collected. We will continue to do this as the work develops. We will be utilising purpose written software (such as the NC3Rs Experimental Design Assistant) to further support experimental planning, randomisation and blinding.

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

Throughout this project we will continue to optimise animal use wherever possible. This starts with efficient breeding of transgenic mouse lines, which we achieve through close monitoring (facilitated by specialist colony management software) and effective communication with technical staff managing the colonies. We aim to minimise numbers of animals bred whilst still achieving adequately powered, age and sex-matched groups of experimental animals. When individual projects have been completed breeding of relevant mouse strains will be minimised until a suitable time to preserve the colony by freezing down gametes.

Pilot studies are utilised to optimise experimental conditions when we are developing new approaches. Where it is appropriate we utilise technologies that permit longitudinal assessments in the same

animal. Furthermore, we always look to maximise the amount of data that we can gather from a single sample using the latest technologies to their full capacity.

At the end of each experiment we carefully consider which tissues to collect with future studies in mind. By building a well archived tissue bank we are able to utilise existing samples in the laboratory to test new protocols or reagents without the need to utilise further animals. We make our banked tissue available to our collaborators. Through providing the wider scientific community with access to data generated through our studies (through online data repositories) we maximise the scientific knowledge than can be obtained from our animal studies.

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.

A substantial proportion of the methods that we will use in the project are non-invasive and involve simple environmental manipulations to study effects on circadian physiology and/or immunity. This includes modification of the lighting, meal composition or meal timing. These manipulations are unlikely to cause suffering or distress, however occasionally may require an animal to be singly housed. To minimise potential distress in this situation, where possible we will supply mice with environmental enrichment (e.g. plastic tubes, wooden logs or nestlets). Some animals may be subject to a brief surgical procedure, for example to implant a device which permits remote monitoring of mouse activity.

Where we seek to administer reagents to experimental animals we utilise the most refined route of administration possible. For example, we routinely administer antibiotics in the drinking water, rather than through oral gavage. In order to make the solution of antibiotics more palatable to the animals we add a sweetener. We always seek to minimise the numbers of doses of a treatment in order to achieve our objective.

Pre-clinical models of human chronic inflammatory disease will be used in this project, including inflammatory arthritis and models of gastrointestinal inflammation and skin inflammation. We have chosen the most refined models available to address our experimental objectives. For example, we no longer use the K/bXN model of arthritis (in which mice spontaneously develop severe erosive arthritis from an early age). With each of these models, we restrict the period of inflammation as much as possible to minimise animal pain and suffering without compromising experimental aims.

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

We cannot replace these studies in mice with studies in another species (such as insects or fish) to achieve our objectives, as they lack the complex immune and circadian systems seen in higher order

species.

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

We routinely seek to minimise stress and discomfort to animals during our work and achieve this by ensuring all researchers utilise appropriate animal handling techniques (e.g. tube handling where possible to remove animals from their cage) and by using environmental enrichment where possible, especially in instances where it is necessary to singly house animals.

Occasionally animals may undergo a brief surgical procedure. After surgery, animals are closely monitored and post-operative care provided (pain management with analgesics and provision of extra fluids). Analgesics may be provided in the form of a palatable gel to encourage voluntary ingestion.

Animals which develop chronic inflammation are monitored regularly, including monitoring weight loss and general condition. Analgesics may be utilised in animals exhibiting chronic inflammation, however the use of pain relief is not always compatible with our experimental aims. Where transient weight loss is expected as a consequence of the experimental condition, soft food (wet mash) will be provided on the cage floor as well as environmental enrichment.

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

To ensure that our experiments are conducted in the most refined ways we continually assess our experimental design and re-assess approaches if the opportunity arises. We stay informed about best practice guidelines by referring to information provided by Laboratory Animal Science Association (LASA) and NC3Rs.

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

Our group stays informed about recent advances in 3Rs approaches by staying up to date with NC3Rs recommendations and developments. This information from the NC3Rs is obtained through interaction with their website, local seminars, contact with their staff and through social media (Twitter). We endeavour to adopt new tools as they evolve, such as the NC3Rs self assessment tools, in order that we focus our efforts here and keep up to date with successful initiatives utilised elsewhere. We also discuss further refinement opportunities with our NVS and NACWO and through interaction with colleagues at conferences, workshops and seminars.