Home Office

NON-TECHNICAL SUMMARY

Understanding the regulation of behaviour and physiology by genes and nutrients.

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

1-carbon, Circadian rhythms, Nutritional deficiencies, Diet, Metabolism

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult
Rhabdomys pumilio	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 define how what we eat interact with our genes to regulate our behaviour and physiology.

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?

Our genes are made of DNA, but to produce the encoded proteins, the DNA must for be copied, or transcribed, into a short-lived copy called RNA that is then translated into protein. How quickly the RNAs and proteins are produced, and how long they can remain functional shapes our body and actions.

Just like bookmarks can flag pages of interest in a book, we know RNAs and proteins can be flagged by a molecular tag called a methyl group. Our bodies can attach or remove these methyl groups to or from other molecules to change how these target molecules behave.

Previously, we have shown that methyl groups are important for daily rest/activity cycles and other biological rhythms in mammals as well as in many other species across the tree of life. Moreover, the circadian clock (a ~24 hours clock ticking in our body) appears to directly control the metabolism of these methyl groups, such that a bidirectional crosstalk between biological rhythms and methyl metabolism appears to operate in our body.

This proposal aims at defining how our diet and our gene regulate physiology and behaviour, focusing on methyl metabolism.

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

Open-access academic publications, datasets in public repositories to facilitate new discoveries, increased public awareness through media and public events. Importantly, our results will uncover previously unknown mechanisms, of genetic or dietary origins, that can contribute to the development of motor disorders or metabolic diseases; these new mechanisms and associated genes may in time become the basis for new ways to detect and treat such disorders.

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

- **Academic beneficiaries**

In the short term (1-2 years), students and scientists will present data from our work at conferences and public open days, which will prompt potential collaborations.

In the mid term (3-5 years), via the publication of open access articles and published datasets submitted to repositories, the scientific community will benefit from these results in a way that can help them progress with their own discoveries and increase our collective knowledge.

In the longer term (>5 years), this knowledge may find clinical or health applications and lead to new ways to detect, prevent or treat diseases in human and animals.

- **Public**

In the short term (1-2 years), we will share our data with the general public during opens days and university events.

In the longer term (3-5 years), open access academic publications and newspaper articles will increase public awareness on the importance of a balanced diet and the questionable use of dietary supplements.

In the longer term (>5 years), new diagnostic techniques and treatments may be available to improve human and animal health and wellbeing.

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

Positive and negative research results will be published in Open Access journals, and data produced by this research will be deposited in repositories (e.g. NCBI's Gene Expression Omnibus, EMBL's Proteomics Identifications database) when appropriate. Research results will be presented at (inter)national conferences and host laboratories. Scientific papers will be accompanied by media releases to reach the general public. Collaborations with (inter)national laboratories have already been set up and will develop further in the course of this research. When appropriate, tissues and data will be shared with direct collaborators.

Species and numbers of animals expected to be used

- Mice: 14000
- Other rodents:
    - Rhabdomys pumilio: 50

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.

Our body moves tiny carbon building blocks around to help build important things like DNA, produce energy, and keep cells healthy. Known as 1-carbon metabolism, it works in a similar way across all living things with complex cells. However, there are important differences between species.

In human, methyl and folate metabolism requires the essential nutrients methionine and choline, as it does in mouse. However, requirements for these nutrients are different in "less complex" organisms that are often able to synthesise their own folate or methionine. Similarly, while most eukaryotes studied so far have circadian rhythms, there are species-specific differences that are more pronounced the further away from human the species in question are. These differences not only occur at the level of genes but also at the behavioural level. Therefore, to study the physiological function of key genes and nutrients in methyl metabolism in adult mammals, for the sake of understanding animal physiology but also intending to gain insights on human physiology for clinical applications, the simplest possible species is the mouse. Moreover, mice are excellent models at the genetic levels, with many state-of-the-art tools available for genetic alterations to define the specific function of genes.

Since humans are diurnal and most mice are nocturnal, we have selected Rhabdomys - the African four-striped mouse. This species of mouse is awake during the day and so is closer to humans in terms of biological rhythms. We will, however, mainly use nocturnal mice as we have a more precise understanding of the genetic makeup of this species.

Animals may be used at all life stages, depending on the aim of the experiment.

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

70% of animals may be genetically modified, 30% will be wildtype.

70% of animals will have their ear notched for identification and husbandry.

5% of animals may be provided with a diet containing chemicals that trigger genetic alteration or may be given such substance via subcutaneous injections.

10% of animals will have the composition of their food or water changed for a duration of up to 2 months.

20% of animals will be single-housed and monitored for biological rhythms under normal light/dark cycles or under specific illumination schedules including constant darkness, constant light and phase shifts, for up to 3 months.

5% of animals will undergo non-invasive physiological monitoring and/or imaging using specialist equipment requiring brief restraint.

1% of animals will undergo recovery surgery for the implantation of compound delivery or telemetric devices.

Less than 1% of animals will be anaesthetized in a non-recovery manner.

1% of animals will be fasted for up to 2 days.

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

This work examines normal behaviours and physiological processes in the animals. Therefore, most animals experience only transient and mild adverse impact such as temporary stress, discomfort and/or weight loss caused by alterations in their housing environment (e.g. changes in lighting schedule) and/or cage type. Mice only show some mild stress temporarily after a sudden change of their environment, which resolves after a few days when the mouse become familiar with its new environment, even if it is alone in a cage and as long as it has environmental enrichment such as a running wheel or tunnel. 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. Pain will be managed with the use of analgesics.

Some genetically altered animals may present chronic health issues including tremors and motion disorders, or developmental delay. For such mice, they may be smaller and weight less then their age-matched wild-type siblings, and may have altered gait or shaky movements, akin to a patient with essential tremors or Parkinson's disease.

Alterations in diet/water composition may lead to weight loss that may be attributable to a change in palatability or a nutritional deficiency, which may cause changes in behaviour, including temporary lethargy and reduced activity.

Mice fasted for up to 48 hours can lose up to 20% weight, and may show lower levels of activity during the last 12 hours, but this is completely reversible upon adlib refeeding, with mice regaining over 10% of their body weight and normal activity levels during the first 24 hours of refeeding. Unlike dogs with nutritional deficiencies, fasting mice in a controlled environment will NOT try to eat inedible items (phenomenon called pica in dogs), and in any case the bedding and cage enrichments mice are provided with are made of wood or cardboard, which are often given to rodents as safe chewable materials to control teeth growth. During fasting, mice will have free access to water.

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)?

Sub-threshold: 30%

Mild: 50%

Moderate: 20%

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?

This project focuses on mammalian physiology. Our scientific objectives require us to examine whole animal physiology in adult animals in the short and long-term. These studies are aimed at understanding how the metabolism of essential nutrients and the genes that are involved in the metabolism of these nutrients can influence the physiology and behaviour of human and animals, and to define what pathologies arise when metabolic deficiencies occur. Not using animals would defeat this aim, and less complex animals including insects do not have the same metabolism or nutritional requirement, and do not express the same range of behaviour and do not develop the same pathologies as mammals.

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

Where possible, human and mouse cell cultures will precede all investigations involving animals.

We have considered the use of flies or fish.

Why were they not suitable?

While cell cultures are key to study molecular mechanisms, investigating physiology and behaviour, or how pathologies that affect behaviour arise, can only be done with a complete organism expressing such behaviour.

Less complex organisms do not exhibit the same array of behaviour that mammals do, and do not have the same dietary requirements. Moreover, less complex vertebrates including fish do not have the same range of genetic tools available, and have dramatically different genetic make-up compared to mammals.

While less complex animals including flies may not be appropriate as a complete replacement for mammals, they may still be part of the research to investigate what is conserved between animals, and may provide potential avenues for future replacement opportunities.

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 animal models to be used; ii) the scope and objectives of the current project; and iii) careful consideration of experimental design taking into accounts the variability between animals and the expected effect size.

For example, in the past 5 years working on a similar programme of work, we have performed around 1,000 procedures per year (based on our own returns), often with genetically modified mice whose genetic design meant that only 25-50% of the mice were of the appropriate genotype for procedures, which means that an estimated total of 2,000-4,000 mice were born per year, i.e. 10,000-20,000 for a period of 5 years.

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

We are working with the NC3Rs experimental design assistant to help us ensure the experimental designs are appropriate.

For each experiment, animals studied will have the same genetic background and age to reduce variability, but we will use both sexes for all experiments.

To avoid experimental bias, whenever possible random allocation of mice to treatment groups or to cage number and position within the animal house will be carried out, and the investigators assessing the outcomes of experiments will be blinded to the nature of the groups to be compared.

Typically, randomisation will be carried out using a computer's randomize function to avoid human bias.

We have strong experience of using statistical tests in circadian research and have data scientists among our collaborators. An independent professional statistician approved all study designs.

At each stage of the project and within each study conducted, we will ensure that animal use is minimized.

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

When applicable, *in vitro* experiments using cell cultures will be carried out to first test the validity of our hypotheses before deciding whether *in vivo* experiments should go forward.

Pilot studies will be used where the impact of procedure is unknown; they will enable us to determine the most efficient and least stressful methods, as well as to obtain a first idea of the effects triggered by the procedures. Another benefit of running pilot studies is that it will allow a more accurate estimation of the required number of animals.

We will genetically modify our mice to express the luciferase gene which produces bioluminescence—light emission through a biochemical reaction famously found in fireflies. That will allow us to easily measure at multiple time points with minimum stress to the animals.

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.

The methods we use are mostly non-invasive, with the animal provided with environmental enrichment including a running wheel. Animals may bear genetic alterations that affect their physiology and behaviour but do not as such cause pain. Animals may also be provided with diets with excess or deficiency of key nutrients that may cause weight loss when given over a long time but that is reversible. 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 and physiology; this reduces stress to the animals under study, and ensures that animals are well monitored and cared for. Our past experience has allowed us to refine the diet composition to avoid inflammation of the liver, a common symptom of nutritional imbalance, and to prevent weight loss associated with a sudden change in food palatability.

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

Immature life stages are not appropriate because inactivation of the genes studied here cause early developmental arrest, and less sentient animals do not show complex human-like metabolism and behaviour. As most of our studies involve long-term assessment, they cannot be conducted in terminally anaesthetised animals.

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

In relation to how the welfare of the animals is affected by the procedures, monitoring may increase or decrease in frequency and details. Should the animals develop pathologies to a level that is more serious or earlier than anticipated, increased care, with consultation with NVS and/or NACWO, will be provided and protocols will be updated accordingly.

Animals that are single-housed are ether provided with a running wheel or environmental enrichments including tunnel and wooden toys.

For procedures with unknown outcomes, such as new genetic alterations or novel dietary composition that are potentially detrimental for health, pilot studies will be set up to be able to determine the most

refined methods.

As much as possible animals will be acclimated when transferred to a new environment.

Aseptic procedures and analgesics will be used for all surgical procedures by trained and licensed persons, conducted after consultation with NVS and/or NACWO.

Fasting of a shorter duration than 48 hours does not allow the animal to exhibit a wide range of metabolic switches to mitigate the lack of food. After the first 24 hours, the loss of weight is mainly due to the loss of food in the stomach and guts, and the metabolism of the animal shifts to first use sugar stored as glycogen in the liver. Fasting of up to 48 hours is necessary to trigger the use of fat and amino acid for energy. During fasting, water is freely available and mice have access to safe bedding and nesting materials, as well as environmental enrichments including tunnel and wooden toys.

The licenced personnel will be trained to use the most refined methods of mouse handling and husbandry, providing environmental enrichment in the cages so that the animals can display an appropriate range of behaviour as in the wild.

We have consulted and external statistician to refine our experimental designs.

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

The Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes published by the Home Office will be followed.

LASA guidelines will be considered regarding surgical procedures, behavioural analyses, and general good practices for animal welfare. Specifically, single-housed mice will be provided with environmental enrichment including a running wheel, cardboard tunnel or wooden blocks, as appropriate for the experiment being conducted.

Administration of substances and removal of blood will follow the guidelines published by Morton *et a*l. (2001, https://journals.sagepub.com/doi/10.1258/0023677011911345) and Diehl et al. (2001, https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/jat.727).

The ARRIVE and PREPARE guidelines will be followed when planning the research and reporting research results using animals.

These best practices will evolve whenever these guidelines are updated.

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, developments in the literature and scientific community, as well as through our own developing work and discussions with our colleagues, NVS and NATCO.