Home Office

NON-TECHNICAL SUMMARY

Genes and essential nutrient influences on behaviour.

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

Methylation, Circadian rhythms, Methionine

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant

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 understand how essential nutrients and their metabolism can influence our physiology and behaviour, and in particular our biological rhythms.

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?

We eat food to stay alive; our body needs energy to keep functioning. Beyond simply providing energy, some nutrients dramatically influence how our body functions. This is especially true for vitamin B9 and the essential amino acid methionine, whose deficiencies can cause many pathologies including aging, diabetes, and neurological problems. These deficiencies can either arise from a poor diet or from genetic mutations that are often undiscovered until severe symptoms occur. Yet, the mechanisms underlying how these diseases occur are poorly understood.

In our body, vitamin B9 and methionine are involved in the metabolism of methyl groups. Methyl is a small chemical group, composed of only one carbon atom linked to three hydrogen atoms, which can be attached to other molecules in the cell, including DNA, RNA or proteins. The addition of a methyl group, or *methylation*, is a mechanism by which our body can regulate the function of virtually every genes, depending on what the methyl is attached to. Low amount of methionine or vitamin B9 in our diet, or mutations in genes involved in their metabolism, will inhibit methylation reactions and cause wide-ranging disruptions.

Our research has previously revealed a link between methyl metabolism and the body clock in many organisms from bacteria to humans. However, this research was based on *in vitro* experiments, using cell cultures. The nature of this link and how it is regulated is unknown. Using mice, whose methyl metabolism is virtually identical to ours, we seek to define which methylations are linked to our body clock, and whether and how they are regulated by our diet.

In addition to further our fundamental understanding on how nutrients can affect our body, this research will also provide potential new targets for the treatment of diseases related to methionine deficiencies.

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

Discoveries made during this research will contribute to our understanding on how our bodies work at the fundamental level but will also provide insights into two clinically relevant themes:
nutrients metabolism and the body clock.

The body clock is clinically relevant because our life-style and 24-h society have detrimental consequences on our biological rhythms, leading to poor general health and increased incidence of cancer, cardiovascular and metabolic diseases. Interestingly, deficiencies in essential nutrients when

unnoticed cause similar pathologies. These deficiencies can be caused by a poor diet but can also originate from genetic mutations.

The evidence
accumulated so far suggest that these two themes, clock and diet, are intimately linked in our body; understanding such a
link will therefore increase our understanding on how our body functions and responds to stress, and provide potential new avenues for the treatment of related pathologies.

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

- **Academic beneficiaries**

At the local and (inter)national levels, results from this project will find beneficiaries in fundamental as well as in more applied academic fields.

- **Society**

This research seeks to understand the link between our diet and our body clock, how they regulate each other, and what pathologies occur when this link is disturbed.

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: 11500

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.

In the first part of this research, the mice will be genetically modified but will develop and grow healthily just like normal mice. A drug called tamoxifen (used in the chemotherapy of breast cancer) can then be added to the food of these mice, usually at the adult stage, which will cause the inactivation of a

selected gene. This allows the physiological role of that gene to be investigated, without risking potential negative consequences on the development of the animal.

In the second part of this research, animals used will be wild-type and healthy adult mice that will be given food lacking essential nutrients such as methionine and choline, whose insufficient intake has been associated with hepatitis in humans. However, how these nutrients are used in our body, and what processes they regulate, is not well understood.

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

A typical experiment in our project will involve monitoring mice in a cage containing various items to interact and play with, notably a running-wheel that is connected to a computer to measure the activity of the mouse, or a system that measures when and how much the animal eats and drinks. Since these behaviours are controlled by the mouse body clock, when the mouse is in complete darkness these rhythms in wheel running and drinking can be quantified and used to measure the internal biological rhythms of the mouse. The effects of gene inactivation and nutrients deficiency on these behaviours will be studied. In addition, some animals may be killed by a Schedule 1 method in order to identify the molecular mechanisms triggered by these nutrients, or lack thereof.

The precise procedures that will be performed on the animals in this project, will be the following.

Part 1:

1) Animals will be genetically modified.

2) Animals from 1) will be provided with a diet containing the drug tamoxifen, or may be given tamoxifen via gavage (direct administration into the stomach through a tube) or subcutaneous injections (only if the diet method does not yield the expected results).

3) Some animals from 1) and 2) will be single-housed and monitored for biological rhythms under normal light/dark cycles and constant darkness for up to 2 months.

4) Some animal from 1) and 2) other than the one used in 3) will be single-housed and undergo physiological monitoring and/or imaging using non-invasive specialist equipment for up to 6 weeks.

5) Animals may also have small blood samples collected (microsampling), and their ear may be notched for identification.

Part 2:

6) Animals will have the composition (decreased levels of essential nutrients) of their food changed for a duration of up to 3 months.

7) Animals from 6) will be single-housed and monitored for biological rhythms under normal light/dark cycles and constant darkness for up to 2 months.

8) Animal from 6) other than the one used in 7) will be single-housed and undergo physiological monitoring and/or imaging using non-invasive specialist equipment for up to 6 weeks.

9) Animals other than those used in 6) will be fasted for a maximum of 38 hours.

10) Animals may also have small blood samples collected (microsampling), and their ear may be notched for identification.

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

Inactivation of candidate genes may cause chronic pathological consequences such as weight loss, anaemia and an inefficient immune system.

Single-housed mice can experience some level of stress and anxiety, that may be more pronounced when it is in constant darkness for 2 months.

The diets lacking methionine and choline, when given for a period of up to 3 months, are likely to cause health issues in mice as they do in humans, notably weight loss and hepatitis, although this will not be allowed in this project since healthy mice are needed.
Veterinary help will ensure mice showing these symptoms are treated appropriately.

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: 50%

Mild: 40%

Moderate: 10%

Severe: 0%

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?

These studies are aimed at understanding how the metabolism of essential nutrients can influence our physiology and behaviour. Therefore, an animal showing a complex array of human-like behaviours is

needed.

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

Human and mouse cell cultures will precede all investigations involving animals.

We have also considered simpler organisms, including fish, flies and even plants and bacteria.

Why were they not suitable?

While cell cultures are key to study molecular mechanisms, investigating physiology and behaviour can only be done with a complete organism.

Less complex organisms do not exhibit the same array of human-like behaviour that mice do, and do not have the same dietary requirements.

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?

11,500 is the maximum number of animals estimated, but the actual number of animals used is likely to be lower. This number was estimated on the basis of the number of different genes that will be investigated, each of which requires a separate genetically-modified mouse line and respective controls. An independent statistician has been consulted on the number of animals required to achieve the objectives of our research.

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

The experimental designs were approved by an independent consulting service.

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

Animals studied will have the same genetic make-up and age to reduce variability.

To avoid experimental bias, 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 blind to the nature of the groups to be compared.

Animals of both sexes will be used, which ultimately will decrease the total number of animals used.

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

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; 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.

Genetically modified animals expressing the luciferase gene as a reporter for molecular circadian rhythms *in vivo* will be measured longitudinally, at multiple time points, without the need for humane killing of different animals for every time point.

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.

Tamoxifen-inducible gene inactivation allows the physiological role of a given gene to be investigated without risking any negative consequences on the development of the animal. Tamoxifen administration via food intake is the preferred method because it is not invasive and based on the animal's own volition.

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.

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

Refinements throughout the project

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, under the veterinary surgeon's supervision, will be provided and protocols will be updated accordingly.

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

When animals are single-housed, environmental enrichment will be provided to alleviate potential anxiety due to social isolation.

Animals of both sexes will be used, which will increase the relevance of our research results.

Animals kept in constant darkness will be killed by a Schedule 1 method in the dark, using night-vision goggles, to prevent acute stress to the animal and avoid the effects of light exposure on the animals biological rhythms.

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.

Refinement specific to the first part of the project

Pilot studies have been set up to be able to determine the most refined methods in the administration of substance such as tamoxifen. A common problem of tamoxifen-containing diet is their low palatability, decreasing food intake, causing weight loss, and lacking efficiency. To refine this, the highly palatable sucrose will be added to the diet to promote food intake.

Refinement specific to the second part of the project

Pilot studies have been set up to be able to determine, with a view to stop or prevent, the effects of methionine/choline deficient diet on the animals' health.

Unfortunately, commercially available methionine/choline-deficient diets have been optimised to quickly induce fatty liver diseases, notably by adding sucrose and polyunsaturated fatty acids. However, we will not include these "improvements" in an attempt to prevent or delay the incidence of fatty liver diseases while still allowing the specific roles of methionine and choline to be detected.

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 (2014) will be followed.

The LASA guidelines for record keeping will be enforced to all personnel working on the project.

A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes (Diehl et al., 2001) will be followed whenever a procedure requires administration of substances and removal of blood.

The ARRIVE guidelines will be followed when 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?

Through discussions with colleagues and named persons at the institute, through keeping up to date with recent discoveries in the field, and through frequent visit to the NC3Rs website looking for resources. We have an account with the NC3Rs and receive frequent updates by email. The NC3Rs regional programme manager, available on site can be consulted about recent advances.