Home Office

NON-TECHNICAL SUMMARY

The regulation of whole-body metabolism across the life course

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Obesity, Diabetes, Brain, Appetite, Body weight

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

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 understand how the brain controls appetite and body weight. This includes normal regulation and what can go wrong with the development of diseases like obesity and diabetes.

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 are experiencing an epidemic in obesity and diabetes, including an alarming increase in the incidence of these diseases in children. By better understanding how metabolism is normally controlled (that is how we control the flow of nutrients through our bodies), we can help to develop new treatments or improve those that are already available, but which may have unwanted side effects.

If an animal is metabolically challenged it will respond and adapt, bringing into play most body systems. We need to understand why and how metabolic adaptation occurs by studying it in different guises, including responses to voluntary interventions (such as dieting and exercise), pharmacological interventions (which may produce unwanted side effects), pathology (sickness and disease) or life events (such as pregnancy, torpor and ageing).

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

We will increase our basic knowledge of how the brain controls appetite and body weight. We will provide mechanisms of action of drugs currently undergoing development and which will assist in bringing them to market. We will provide novel targets for the development of new drugs. Our findings will be disseminated in scientific publications, at professional conferences and at meetings for patient groups.

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

The immediate beneficiaries will be the academic community who wish to understand body-weight regulation. Immediate benefits will also be to our industrial partners who will be able to use our discoveries to support their development programmes or, indeed, to bring programmes to an end. The latter is particularly important to reduce further experimentation and avoid the unnecessary use of animals. By separating different aspects of behaviour, the pathways involved and the factors that regulate them, we will also propose new interventions to bring benefit to those suffering with metabolic disease or who are struggling to control their body weight. We expect our findings to be adopted by

partners in the pharmaceutical industry within the lifetime of the project. However, any potential commercialisation of target would require several years beyond the project.

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

In addition to the dissemination of our findings outlined above, we will collaborate with pharmaceutical companies to understand how their drugs work and how we can overcome any unwanted side effects. On occasions, our findings may indicate that a drug is ineffective or unsafe. By maintaining our collaborations as academic, rather than commercial adventures, our findings will become accessible to the whole pharmaceutical industry and academia.

Species and numbers of animals expected to be used

- Mice: 25000 mice bred; 9000 of which will be used in regulated procedures. The remainder will be used in non-regulated procedures and for tissue collection. In addition, some of the mice we breed will not have the necessary genotype for us to use in our experiments.

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.

Although the brain’s wiring is complex, it is very similar between humans and mice. This gives us the opportunity to use mice to understand normal and abnormal brain function. In fact, the breeding of genetically modified mice, in which we can introduce transgenes that control body weight, has massively accelerated our understanding and our ability to target metabolic pathways with drugs. A transgene is simply a modified piece of DNA that is introduced into specific cells and which then allows us to control their function, or perhaps look at the consequences of its introduction when the transgene reproduces a natural mutation found in humans. By using transgenic mice, we can employ minimally invasive techniques and we need far fewer experimental animals than in the past in order to progress knowledge. Our project intends to look at metabolism across the course of life and will include challenges which are normal (for example, pregnancy) or abnormal (for example, in response to disease or infection).

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

The transgenic mice we breed tend to grow and behave in the same ways as normal mice, though occasionally they may be a little fatter or thinner. To minimise any adverse effects, such as stress, we like to handle our mice (often daily) to get them used to being picked up. We may wish to alter their metabolic status, for example by restricting their food intake or, instead, by giving them a high-fat diet to eat. If we need to implant a catheter or a fibre into the skull or into a vein, this is done under general anaesthetic, after which the mice are allowed to fully recover. This means that, when the time comes for an experiment, we can give them an injection (either under the skin, into a vein or directly into the

brain) without them hardly noticing. Usually, we will use a specific transgenic mouse which will allow us to change the activity of a pre-selected cell type in the brain. Thus, we might activate or silence those cells and look to see how the behaviour of the mouse changes. We can do a range of physiological tests on the mice, sometimes in their home cages, but often after acclimatising them to other cages. Thus, we might put them in a scanner to see how much fat they have, measure their blood pressure by putting a tail-cuff around their tail (rather than their arm!), or measure their metabolic rate. Occasionally, we even train our mice to poke their noses into holes to break an infrared beam or to press a little lever, which provides them with a sugar reward. This can tell us about their motivation to eat. Invariably, the parameters we measure are much simpler: for example, how much food do they eat or how much sugar is circulating in their bloodstream. For the latter, we can take pin-prick samples of blood from their tail and measure these in a sugar monitor, rather similar to how a diabetic patient would. We are very interested in why we lose our appetite when we are ill. This could be because we have an infection or because a drug has an undesirable side effect. Thus, on rare occasions, we will induce an illness, such as the flu or inflammation of the bowel. By understanding why these situations cause nausea and sickness, we hope to suggest treatments which will help improve symptoms.

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

As stated above we strive to minimise any stress to our mice by getting them used to handling. We need to do surgery on about a fifth of our experimental mice in order to manipulate how the brain responds to different signals or if we want to control the activity of pre-selected cells. In this case, we carry out the surgery with the mice under general anaesthetic, plus we give the mice pain killers and sometimes local anaesthetics, to make sure that they do not feel any pain. The mice recover very rapidly, so they can be returned to their home cages to carry on living as normal.

However, to study a disease it is often necessary to induce that disease artificially. This could be relatively benign, such as feeding mice a high-fat diet to make them put on weight. Mice adapt to being overweight and most of our animals will never develop any of the extreme complications associated with obesity, such as high blood pressure or diabetes. Conversely, many common diseases or the drugs used to treat them cause nausea and weight loss. Sometimes we will need to make manipulations to cause these adverse effects, so we can compare how the brain responds and how it is different to normal responses to eating a meal. Usually, the anorexia or nausea experienced by the mouse only lasts for a few hours.

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)?

Our project requires the breeding and maintenance of several mouse lines. Every time a litter is born, about half of the pups will be “wild type” and the other half will be “transgenic” (i.e. they have small piece of their DNA that is different to the normal, wild-type mice). Usually, the transgenic mice will be perfectly normal and so are classed as not being in a severity bracket (sub-threshold). However, sometimes they may have a mild trait (e.g. slightly fatter than wild-type mice) or we may make a mild intervention to them (such as giving them an injection or putting them on a high-fat diet). We estimate that about 80% of the mice we breed for our project will remain within or below the mild bracket of severity. Very rarely, we will generate a transgenic mouse which may develop an unforeseen trait.

Assuming that this trait remains within the mild or moderate brackets, we may wish to justify maintaining this line in order to study it further. We estimate that about 20% of the mice we use in this project will undergo some form of recovery surgery, most commonly so that we can later manipulate cells in the brain. The mice will experience some weight loss and mild discomfort as they recover from the surgery, but this soon passes. The mice are normally kept for several weeks before we make any other manipulation, during which time we can acclimate them to handling or sometimes different cages. Occasionally, we will need to make a manipulation that we know will cause the mouse some discomfort. For example, when we are examining brain pathways causing anorexia, we may induce nausea or sickness-like behaviour. In these instances, and each occasion involving recovery surgery, the severity bracket will be classed as moderate.

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?

It is impossible to study appetite, body-weight regulation, circulating factors or responses to drugs in anything other than a normally behaving animal. When an animal is metabolically challenged it will respond and adapt. Normally, this will involve the brain interacting with other organs or systems.

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

We would if we could, but there are no non-animal alternatives that would allow this project.

We can still find out a lot about brain cells by studying them isolated from the rest of the body. We have to kill the mice humanely, but this allows us to take slices of brain and put them in a dish. We can then record the minute electrical or chemical activity of individual brain cells. To enable us to identify the right cells in the complex brain, we have bred transgenic mice in which specific cell types glow fluorescently under our microscopes.

There have been major elements of discovery in our research ranging, for example, from the use of deep RNA Sequencing of transgenic neurons *ex vivo* to describe novel signalling molecules through to collaborating with clinicians to screen obese populations for previously unknown gene associations.

Why were they not suitable?

There are no alternatives, such as cell or organ cultures which will allow the study of physiology and behaviour. Unless a model shows normal physiology and behaviour, we cannot study regulation,

pathology or treatment. Furthermore, an ultimate aim is that we can make a difference to the human population and, therefore, we need to utilise the animal model which provides the best, overall translation to humans. Basic mouse physiology and behaviour is very similar to those of humans, plus we are able to make use of our vast range of transgenic mouse models.

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?

The numbers are based on our usage during five previous years. This is likely to be more accurate that trying to estimate numbers from as yet unplanned experiments in the following five years. The major determinant is the number of mice bred, not the number used in individual experiments. The level of breeding depends on maintaining over 60 different lines/crosses of mice and providing enough individuals to carry out all of our experiments. Several of these lines are used across multiple experiments by multiple users. However, many of the lines are crosses and are bespoke to a particular experimenter. By using these bespoke lines, we are able to reduce the overall number of mice used in experiments.

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

For an individual experiment, data provided from similar studies in the past or from pilot studies, allows us to make precise calculations of the minimum number of animals we will need to provide robust experimental results. When a line is not used regularly, we reduce the colony to a minimum or we end their breeding, having first cryopreserved sperm, eggs or embryos for future regeneration. By publishing our results in open access forums, we are obliged to share these resources with other laboratories, preventing the need to replicate line generation.

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

Whenever possible we use within subject comparisons (crossover designs) where an individual mouse receives both control and experimental treatments. This increases both the quality and statistical power of an experiment. Often, multiple parameters are measured in the same mice concurrently, which is possible due to our investment in complex behavioural and metabolic apparatus. This avoids the need for repeat experiments and also improves the value of collected data. To validate our mice *post mortem* (e.g. for the accuracy of surgical implants or injections), we administer a relevant stimulation before culling. Often this provides us with a further opportunity to collect additional data. We normally collect a

number of tissues which provide data for the ongoing or additional experiments. On occasions, we can provide tissues from our experimental mice to other research groups.

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 majority of experiments will use transgenic mice which will allow us to manipulate pre-selected cell types in the brain very accurately. We will be able to stimulate or inhibit cells selectively, or record from them. We will measure a number of physiological and behavioural outputs. In the last five years, we have used over sixty different mouse lines/crosses. However, by using these approaches we have massively refined experiments, so that mice do not experience the same adverse effects as older models/approaches.

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

It is impossible to study appetite, body-weight regulation, circulating factors or responses to drugs in anything other than a normally behaving animal. Using immature or anaesthetised animals would invalidate translation.

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

One major advance has been the use of remote radiotelemetry. This is where, during surgery, we implant a small radiotransmitter under the skin or in the abdomen of the mouse. Later, these devices allow us to monitor things like body temperature, blood pressure and brain activity without having to disturb the mice. Another example is to use the smallest needles possible when giving injections. In a more recent set of experiments, we have worked with our colleagues from a pharmaceutical company to refine further the use of “EpiPens” instead of normal needles – similar to children who suffer with allergic reactions. A notable recent improvement/Refinement is adoption of the Phenomaster system which maintains the mice in their normal, home cage, but allows the measurement of several behavioural and metabolic parameters at the same time (e.g. food and fluid intake, body weight, metabolic gases by indirect calorimetry and locomotor activity). This also means that fewer mice are required to gather the same information, leading to substantial Reduction in the number of mice required.

We now use transgenic mice to identify, control or record the activity of individual cell types in the brain. This allows us to determine how different cells respond to stimuli and how they communicate with each other without using the very invasive old techniques. Since we can manipulate the mice

while they are still in their home cage, we can record their behaviour, whether they are secreting hormones, or if their metabolism changes, with minimal disturbance. To do this we breed mice that have so-called “designer” proteins expressed in just a single cell type. The designer proteins lay dormant and the mice behave as usual. But, by then giving the mice a “designer” drug or by shining a light of precise wavelength through an optic fibre, we can activate or inhibit specific brain cells selectively, while studying changes in the mouse’s behaviour or physiology. All the time, our techniques are improving and our equipment is miniaturising, so it is now even possible to see and record the activity of individual brain cells in freely moving mice.

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

We are informed by publications from NC3Rs, LASA and both the ARRIVE (https://arriveguidelines.org/) and PREPARE (Smith et al., 2018, Laboratory Animals 52: 135) guidelines for planning animal experiments.

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

Members of the laboratory are fully integrated into the community of *in vivo* scientists both within our establishment, but also at other institutes around the world. Members of the group attend and present at 3Rs focussed seminars and workshops. In addition, local interaction with husbandry staff and named officers occurs on a daily basis. Staff within the animal facility have in the past and are always welcome to attend our lab meetings, where ideas are exchanged.