Home Office

NON-TECHNICAL SUMMARY

Central mechanisms of appetite regulation and neuroprotection

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
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

neurodegeneration, obesity, metabolism, neuroprotection, therapy

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant 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 overall aim of our work is to understand how appetite is regulated by the brain, and how genetic, chemical, and dietary factors acting in the brain can modify metabolic and neurodegenerative disease.

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?

Obesity and the loss of brain cells (neurodegeneration) are common and often deadly diseases.

Obesity and its related diseases (like diabetes) affect over 1 billion people around the world, and is predicted to affect over 2 billon in the next decade according to the World Health Organisation. It leads to millions of deaths each year, and according to the Tony Blair Institute it costs the UK over 100 billion pounds each year in lost earnings and health care costs that take up 10% of the NHS's budget. Telling people to eat better and exercise has not proven to be an effective way for the whole population to achieve a healthier weight. While there are some new treatments for obesity like the drug Ozempic/Wegovy that act on brain cells that regulate appetite, these treatments are expensive and have unpleasant side effects in some people like vomiting and diarrhoea, which limits the number of patients who could benefit. Surgeries to reduce body weight are relatively dangerous and cannot be applied to billions of people. Studies in mice therefore have the potential to identify new treatments that are more effective, have fewer unwanted side effects, and can address the global reach of this disease.

Neurodegenerative diseases like Alzheimer's Disease or Parkinson's disease can be fatal, and affect millions of people around the world and are a leading cause of death and disability among older people. There are often no effective treatments, so any treatment that could slow disease could have a huge impact. Work from our group and from others have shown that some treatments used for obesity or diabetes can also slow the loss of brain cells both in mice and in people. Our work will test which treatments are most effective in mice and therefore might also benefit people more than currently available treatments.

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

This project is likely to lead to new information, publications, and hopefully also new treatment strategies for common brain diseases. Specifically, we will study how brain cell types regulate appetite, and how factors that act on these cells can improve the health of mouse models of obesity. These studies are likely to lead to new datasets and publications. Factors that can treat obesity in mice could

also be useful in humans, and lead to studies in humans. We will also study how candidate factors could slow the loss of brain cells in mouse models of neurodegeneration. These studies will identify which factors are effective, and how they might work, which could lead to the development of factors that are even more effective at slowing the rate of brain cell loss. Again, these studies could lead to ideas for treatments that could be used in humans. To maximise our chances of having benefit to people, we will also offer to work together with companies who are developing treatments, so that our expertise and models can contribute to new therapies.

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

In the short term, we aim to identify new brain pathways regulating appetite that may reveal new treatment strategies for obesity, and new treatments for brain diseases that lead to the loss of brain cells (neurodegeneration). This information is likely to benefit other scientists in a similar field of work, and also companies trying to generate new treatments for obesity and diabetes.

In the longer term, these insights can benefit the millions to billions of people worldwide suffering from obesity, diabetes and related diseases, as well as the millions of people suffering from the loss of brain cells each year. The diseases we are studying also cost over a hundred billion pounds to the UK each year, so our work could help save these costs.

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

We will openly publish the outputs of our work regardless of whether the results are the ones we expected to find or not, and make those findings accessible to everyone free of charge. Wherever possible, we will publish these results on ‘pre-print’ servers like BioRxiv to share them before they are published by a journal. To help other groups repeat our studies, we also openly share the methods we used to generate our results, and not just the results themselves.

We will also share our research results on social media like Twitter/X, at scientific conferences, and via public engagement events. Strategically, we work with a dedicated Public and Participant Involvement and engagement programme including dissemination of scientific advances with Social Media, engagement in Science Festivals etc. Our funders also have dissemination strategies to complement our internal channels.

More broadly, we are focused on allowing the "translation" of pre-clinical science into real advances in healthcare that can impact people's lives. There is also wide support for this with a specific office for translation keen to guide early translation pathways from pre-clinical models.

Species and numbers of animals expected to be used

- Mice: 6500 mice

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.

To model human disease, it's important to work with other mammals that have brains more similar to our own (rather than flies, for example). Mice are widely used to study brain disease, so our own studies can build on a vast amount of knowledge about their brains and behaviour. Since mice mature quickly, we can carry out our studies in a way that maximises the knowledge we will gain while minimising the number of animals we will need to study, and minimising the suffering of animals.

Specifically, since cell types involved in appetite and neurodegeneration are similar in mice and humans, results in mice are likely relevant to humans. Working with adult mice rather than newborn or juvenile mice allows us to ensure that any changes we see in their behaviour or body weight is not due to changes in their development, but is instead due to changes in the diseases we want to study. In some studies, we will use juvenile mice to induce the model of neurodegeneration, since injections into the brain can be done more easily and painlessly at this age than in adults.

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

Typically, we will use group-housed male and female adult mice to study obesity and neurodegenerative disease.

For studies of obesity, we will typically feed mice a high-fat diet for four weeks to make it obese, and then treated for two weeks with candidate factors (e.g. drugs) to test if they can promote healthy weight loss by monitoring body weight. We will include control groups that we feed a normal diet, and control groups that we treat with a non-active substance (called 'vehicle') to allow us to interpret the results of our candidate factor.

For studies of neuroprotection (slower or reduced loss of brain cells), we will typically induce neurodegeneration when mice are young by injecting their brains with a substance (for example misfolded proteins that induce neurodegeneration), and then treat mice with candidate therapeutic factors (like drugs used to safely treat obesity in humans) using the methods described above. We will then observe mice for several months to identify which factors keep them healthy for longer. We typically monitor them for signs of neurodegeneration such as loss of body weight and motor coordination (e.g. how well they can walk), and end studies before mice become very sick.

Less typical experiences that mice may undergo are listed below:

- In some cases, we will feed mice different diets to study how this affects their body weight and other features of their metabolism or brains. In some cases, we will inject chemicals to model different aspects of human disease associated with obesity such as diabetes (high blood sugar) or hypertension (high blood pressure).

- Typically, mice will have access to food and water whenever they want, but in some cases we will restrict access to food (fasting) for a short period of time (e.g. overnight).

- In some cases, we will first test whether candidate factors are toxic before carrying out larger studies.

- Wherever possible, we will deliver candidate factors via the food or drinking water. In some cases, we will inject mice via a syringe into the stomach through the mouth (oral gavage), under the skin (subcutaneous or SC),into the body cavity (intraperitoneal, or IP), into the muscle (intramuscular or IM), into the bloodstream (intravenous or IV), into the brain (intracerebroventricular, ICV). In some cases, we will implant mice with a pellet or small pump under the skin (SC) to deliver a drug at a constant level for a prolonged period of time, avoiding the need for multiple injections.

- In some cases, we will surgically implant a small device under the skin to allow us to more easily identify mice (RFID tag) or track their movements (telemetry tag).

- In some cases, we will also collect blood from mice to measure factors in their bloodstream, or test how mice respond to sugar (glucose) or the hormone insulin that causes sugar to be taken up from the blood into cells. These studies are called a glucose tolerance test (GTT) and insulin tolerance test (ITT), respectively.

- In some cases, we will house mice in special cages that allow us to more easily measure how much food they eat, how much they move, and how much oxygen they consume.

- In some cases, we will put mice into a special arena and take videos of their movement so that we can measure the way the move and the way they interact with their environment.

- In some cases, we will surgically inject mice (typically ICV) with a virus that does not make them sick but allows certain brain cells to be labelled or genetically modified to test how these brain cells are connected to other cells, and how changes in these cells affect the behaviour or disease state in the mouse.

- In some cases, neurodegeneration will be caused by breeding mice that have been genetically modified (e.g. transgenic), in which case we would not induce neurodegeneration by injection.

- In some cases, we will image mice in special devices to study how their bodies are composed of fat, muscle, and other organs (DEXA scan), or to study the activity of their brains or other organs (PET or fMRI imaging). This imaging is routinely performed in people and is non-invasive meaning that it does not require surgery or cause anything other than minor discomfort. Some mice will be injected as young animals with a substance that will cause neurodegeneration over the course of several months.

In the worst case scenario for studies of obesity and metabolic disease, a young adult mouse will be implanted SC with a telemetry tag, fed a high fat and high sugar diet, and be injected twice (several weeks apart) with the drug streptozotocin to induce a model of diabetes. It would then be moved to a metabolic cage for 72 hours to measure food intake and energy expenditure, followed by a DEXA scan, GTT, and ITT. It would then be injected every other day for 60 days with a candidate drug followed again by metabolic cages, DEXA scan, GTT and ITT before being killed.

In the worst case scenario for studying appetite-regulatory brain cell types, transgenic mice would be implanted SC with and RFID tag, be surgically injected with a candidate virus, and then fed a high-fat

diet and monitored for body weight, food intake, and energy expenditure as described above before being killed.

In the worst case scenario for studies of neurodegeneration, a mouse will be injected with scrapie as a juvenile, then implanted with an RFID tag, injected IP every other day with a candidate factor for 60 days, monitored behaviourally in specialised arenas for behaviours, and allowed to progress until clinical signs of neurodegeneration have been reached before being killed.

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

Mice undergoing breeding protocols without undergoing procedures will be classed as reaching a "sub-threshold severity" meaning no expected impact or harm. Most animals undergoing experiments will experience effects that are classed as mild or moderate severity. For example, a mild severity might be a mouse being fed a high-calorie diet to become obese, having a blood test or injection, and undergoing measures of memory and behaviour. Moderate severity will typically involve animals undergoing anaesthesia and surgery on brain. We expect their normal functioning to be disturbed after surgery for 2 or 3 days. They may lose weight during this time if eating and drinking less and may groom themselves less.

Some mice put on modified diets or injected substances to model obesity and metabolic disease will experience weight gain, high blood pressure, or elevated sugar concentrations in the blood for several weeks to months. Injections will only cause temporary discomfort. These conditions are also seen in millions of people and do not necessarily lead to suffering. Temporary fasting will increase hunger and/or thirst, but this procedure is routinely performed in people as well without causing adverse effects.

If candidate factors have never been used in mice before, we will first carry out ‘pilot’ experiments to ensure they are safe, staring with low doses in a small number of mice and then increasing doses and terminating experiments as soon as mice show signs of adverse effects.

Delivering candidate factors like drugs can cause temporary discomfort similar to that humans experience routinely. For example, vaccinations are given via intramuscular injections to people, and we will aim to use the least invasive method, such as subcutaneous injections or ideally delivering drugs via the food or drinking water wherever possible to reduce stress from handling and discomfort from repeated injections.

We will inject the brains of some juvenile mice with a substance that causes neurodegeneration. These injections are performed under anaesthesia, but do not require surgery. Some adult mice will be injected in their brains with drugs or viruses. These procedures require surgery that is performed under anaesthesia. Some mice have minor surgery to implant a device under the skin that can release a medicine slowly, or allow them to be identified or tracked. In all of these cases, mice are expected to recover quickly and will be given painkillers and post-operative care just like people recovering in hospital.

Similarly, blood collection is routinely performed in people and is not expected to have lasting adverse effects. Mice may also be briefly anesthetised to enable them to be imaged, as is sometimes performed for people undergoing similar treatment.

We will routinely house mice in social groups, but in some cases it will be necessary to separate them, for example if they are fighting (to prevent injury) or to carry out experiments in specialised cages for short periods of time. To reduce the stress of social isolation, and we will allow mice to acclimatise to their new surroundings and minimise handling. We also will sometimes move them temporarily to new surroundings to study their behaviour, which we do not expect to cause any harm beyond modest stress lasting approximately 30 minutes.

Imaging mice may require temporary anaesthesia lasting about 30 minutes, after which we will monitor them to ensure they make a full recovery. We do not anticipate these studies to have a lasting impact.

For studies of neuroprotection, mice will show no obvious signs of disease for many months, but will then experience loss of motor coordination and weight loss for approximately 1 month, but we will stop experiments before animals become too sick.

Rarely, mice may die unexpectedly after undergoing surgery or due to complications of neurodegeneration. In our previous licence this affected only about 1% of the total number of mice.

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)?

We will only use mice, and do not expect any mice to exceed moderate severity. Remaining proportions are estimated to be:

- Sub-threshold (e.g. breeding): 10%
- Mild (e.g. injection or diet): 20%
- Moderate (e.g. neurodegeneration), 70%

What will happen to animals used in this project?

- Killed
- Used in other projects
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

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 essential to use animals since they can show changes relevant to disease like putting on extra fat, eating more food, or becoming less coordinated over time. These changes help us to understand if the

treatments we are testing for metabolic and neurodegenerative disease in mice are likely to be useful to humans. There are some areas though where it is possible to avoid animal work, as described below.

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

Cells. We extensively use human cellular models, that allow us to reduce the total number of animals we use by ensuring that only the most promising approaches are selected. Specifically, we can work in a culture dish to turn human stem cells into some of the brain cell types that regulate appetite and are related to obesity, as well as some of the brain cell types that are affected in neurodegeneration. We can expose these cells to drugs and other candidate factors to test how they work in a culture dish. We can also generate more complex three-dimensional combinations of cells known as "organoids" that attempt to reproduce the complexity of groups of cells arranged into an artificial organ in a test tube.

Tissues: We can study human tissues (e.g. frozen brains) relevant for obesity and neurodegeneration by examining them under a microscope or examining the genes they express in single cells.

Humans: It is also possible to study the effects of candidate factors in people in ‘experimental medicine’ studies involving a few people, or in larger studies called clinical trials. We can gain insight into how these factors work by measuring food intake, hunger, energy expenditure, and by using non-invasive brain imaging methods like functional magnetic resonance imaging using high-powered magnets, much as we can also do in animal studies.

Why were they not suitable?

Cells. Cellular models allow us to understand how individual cells respond to candidate factors, but not how tissues or organisms respond. Organoids have potential, but still lack many of the cell types and interactions that are present in animals. Together, these studies will allow us to test out ideas in a culture dish before we have to use animals, reducing the number of animals we need to use, and refining the design of the studies we do.

Tissues: Human tissues are dead and do not allow experiments to be performed on them, but studying them can help us come up with better experiments to do in human cell cultures and in animals.

Humans: Before we can carry out these studies in humans, we typically need to know if the candidate factors are effective in animals. We also do not have access to human brain tissues, to understand how an experiment has affected cells in the same way that we can achieve using animals.

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?

To estimate animal numbers, we consulted with experts and considered previous studies by our group and others so that we could obtain the most meaningful results using the smallest number of animals. We considered both how large effects in our experiments were likely to be, and how consistent results would be between mice. We will carry out many studies in both male and female mice to identify effects that are different between males and females. Where effect sizes are unknown (e.g. novel factors), we will first carry out 'pilot' experiments where we treat a small number of mice to determine if a candidate factor has an effect and how large the effect is, in order to better plan larger future experiments.

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

We have applied an online tool (the NC3R's Experimental Design Assistant) to help design studies and plan to continue using this too, and will consult with experts whenever needed. We also ensure that mice of the same age, genetic background (breed), and sex are used in the same experiments, and are housed under similar conditions. To ensure that the data we collect are as useful as possible, we collect it at the same time of day, and use electronic software to record the identify and the procedures we do on each mouse. Wherever possible, we will randomly assign mice to different treatment groups to ensure that groups are balanced, and only after performing an experiment and collecting the data will we reveal what group each mouse was in. Sometimes, separate people will assign mice to groups, collect data, and analyse data to ensure that the results we get are not accidentally influenced by our initial ideas. We also describe experimental methods in detail to allow others to repeat our work and build on it to make new discoveries.

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

We follow Home Office guidance on breeding GA animals to maintain efficiency and help optimise numbers used and the NC3Rs breeding and colony management resource (https://nc3rs.org.uk/3rs-resources/breeding-and-colony-management).

Wherever possible, we will utilise refined and widely-accepted methods, such as feeding mice a high-fat diet to generate models of obesity. We write up methods into standard operating procedures (SOPs) that we share with all members of the group to ensure that methods are carried out consistently and to a high standard to help ensure the success of our experiments using a minimal number of animals, and reducing the likelihood that studies will need to be repeated.

We will carry out most studies in a widely-used and well-characterised and genetically consistent breed of mouse (C57Bl/6J) rather than using transgenic mice. This approach allows us to build on the knowledge of many other groups when designing experiments to minimise animal numbers. It also reducesanimal wastage from transgenic breeding, and facilitate the generation of sufficiently powered experimental cohorts matched by age and sex.

Wherever possible, we adopt approaches that will maximise the data collected from individual mice, such as non-invasive behavioural analysis (e.g. food intake) followed by physiological analysis (e.g.

DEXA scan and GTT/ITT) and terminal biochemistry and histology (e.g. brain clearing and light sheet microscopy).

Where appropriate, we will perform initial ‘pilot’ studies to check feasibility and review the likely effect size and chances of a successful outcome before committing larger numbers of animals to a full study. Typically a pilot up to 8 animals will allow us to assess the technical feasibility of performing the study and to estimate the likely effect size and variability in outcome measures (hence an estimate of numbers needed for scientific outcomes). Finally, we collect and share tissues, and also share surplus animals where appropriate through the University's initiative to limit animal use.

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.

Choice of species and strain: We will work with male and female mice, typically housed together in cages to provide social enrichment. We largely work with mice that are not genetically modified and wherever possible work with the C57Bl/6J strain, a breed of mice that all have similar genes (like a pure-bred pet animal) since this strain is routinely used in studies of neuroscience and metabolic disease. Since many other groups have already worked with this strain, we can build on existing knowledge to design better studies and reduce the number of mice we need to use. Since many studies do not require genetically modified animals, we can minimise wastage of animals due to breeding.

Metabolic disease modelling: We will typically induce obesity by feeding mice a high fat diet since this approach is not harmful and is widely used, allowing us to directly compare our results to other studies in the field. This approach is preferable to genetic models of obesity (e.g. ob/ob mice) that can have features (e.g. diabetes) that reduce animal quality of life.

Neurodegenerative disease modelling: We typically inject the brains of young mice with a substance to induce neurodegeneration. After the injection, mice gradually start to lose brain cells within a predictable time frame. For the first approximately three months they show no signs of disease or discomfort, then after approximately one month of modest weight loss and loss of motor coordination (such as walking on uneven surfaces), and approximately one month later they show greater changes in their behaviours such as their memory and motor coordination . We have developed refined methods to measure these behaviours, allowing us to determine how far neurodegeneration has progressed quickly and accurately. We can therefore end experiments before mice experience any unnecessary suffering.

Experimental agents: When treating mouse models of metabolic and neurodegenerative disease with experimental agents, we will preferentially select agents known to be safe in humans. Wherever

practical, we will deliver these agents in a manner that minimises pain and distress, such as via food or drinking water, or via a time-release pellet rather than by daily injections with a syringe. This approach both improves the experience of the animal, and generates more reliable data since the bioavailability of the experimental agent is more constant.

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

The aim of our research is to understand human neurodegenerative and metabolic disease (e.g. obesity), so we require a model system that has cell types and behaviours similar to humans that are relevant to these diseases. Other animals that are commonly used in research, such as flies, worms, frogs/toads, and fish have different brain cell types and different behaviours, and would therefore be less informative about human disease. We also cannot use very young mice, since the diseases we wish to study typically affect adult humans.

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

We have made extensive refinements over the past years to minimise harms. For example, when we inject mice with substances like streptozoticin that can induce a diabetes-like state in mice, we typically use low doses that provide a more accurate model of that most common form of diabetes in humans, and reduce harm to animals. If mice become more sick toward the end of our studies, we supplement their normal diet with tasty and calorie-dense food and gel packs to allow them to eat and drink more easily.

Furthermore, we have established ways to monitor the behaviour of mice by video in our studies of neurodegeneration, allowing us to collect data at earlier time points, improve well-being of mice during the study, and terminate experiments earlier to avoid suffering. For example we gently take mice out of their home cage and put them into an arena that they can freely explore. After they have gotten used to the arena, we take movies of them to how much they move, how good their motor coordination is on a tricky surface, and how well they remember where an object in the arena was to test their memory. These studies are less stressful than regular observation and handling, the data we collect are not influenced by the opinion of a particular individual and are therefore more consistent, allowing us to use fewer mice and to end studies sooner. We use AI-based analysis of the movies to learn as much as possible from each animal, and as technology improves we can return to data gathered in earlier experiments to learn even more. To further refine these studies in the future, we will explore the use of continuous video monitoring of mice in home cages to eliminate any stress associated with handling or new environments, and providing richer data. Our aim is to carry out these studies in group-housed mice in home cages to allow for social and environmental enrichment and more natural behaviour. To achieve this goal, we would use ear tags or a similar method to clearly distinguish between individual mice.

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

We will use PREPARE (doi:10.1177/0023677217724823) guidelines when designing experiments and ARRIVE guidelines (doi:10.1371/journal.pbio.3000411) when preparing work for publication. We will

regularly check for updates to the Essential 10 and Recommended Set of guidelines (https://arriveguidelines.org/arrive-guidelines). For survival surgeries, we will apply the LASA aseptic technique guidance “Guiding principles for preparing and undertaking aseptic surgery” (https://www.lasa.co.uk/wp-content/uploads/2018/05/Aseptic-Surgery.pdf).

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

To stay informed about 3Rs advances, we are subscribed to the monthly NC3Rs newsletter and will regularly review the NC3Rs resource library (https://nc3rs.org.uk/3rs-resources) and practical guidance from the Laboratory Animal Science Association (LASA). Furthermore, we will attend workshops, events, and webinars where these are relevant to our project plans, including those organised by our University. We will also look for advice from Named Person(s) about advances in 3Rs, and utilise our university’s tissue sharing resource.

To effectively implement advances, we update the SOPs used by the group to ensure that refined methods are incorporated into existing protocols. Where more substantial changes are required, we have a track record of regularly revising our current animal license to ensure that we can take approaches that reduce animal numbers and improve animal welfare, and we anticipate continuing this practice with the new licence.