NON-TECHNICAL SUMMARY

Dissecting central mechanisms of obesity and neurodegeneration

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

Key words

No answer provided

Animal types | Life stages
---|---
Mice | adult, embryo, neonate, juvenile, 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 project’s objectives, for example the scientific unknowns or clinical or scientific needs it’s addressing.

What is the aim of this project?

We aim to identify shared mechanisms and treatments that both reduce obesity and slow the progression of neurodegeneration.

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?

The proposed project addresses metabolic disease (e.g. obesity) and neurodegenerative disease (e.g. brain cell damage and loss), which are both major human health issues lacking broadly effective solutions. Our studies may reveal the processes that produce these diseases, which could lead to new treatment strategies. We will also test whether certain drugs can reduce obesity or slow the progression of neurodegenerative disease in mice. If these studies are successful, they would pave the way for potential treatments in humans.

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

The intended outcome of these studies are 1) increased biological understanding of the molecular and cellular pathways involved in metabolic and neurodegenerative disease, and 2) drugs that act on these pathways to reduce obesity or slow the progression of neurodegeneration, which could be advanced to pre-clinical studies.

We expect the data from the experiments covered in this license will lead to several publications in peer-reviewed journals. In addition, we will publish protocols and results, sometimes also including data on experiments that did not work, in databases that are open to the general public (e.g. protocols.io and BioRxiv).

Outputs based on our broader goals are more difficult to predict, but we intend them to have a wide and positive impact. In particular, neurodegenerative diseases and obesity contribute to more than a third of the total burden of disability-adjusted life years (DALYs) in the elderly population. By shedding light on the processes in the brain that may lead to metabolic disease (e.g. obesity) or damage to brain cells (e.g. neurodegeneration), we may contribute to the future development of more effective treatments for these major diseases.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

Diet and neurodegeneration: In the short-term, our studies would prompt us to isolate the relevant dietary or metabolic factors that affect the neurodegenerative progress. In the longer term, these
findings could affect how individuals at risk for neurodegenerative disease might alter their lifestyle to reduce their risk.

**Drug treatments for neurodegeneration:** In the short-term, we aim to understand how some drugs given to treat diabetes and obesity (e.g. Liraglutide) also improve brain function in Alzheimer’s disease and Parkinson’s disease. In the longer term, we would test additional drugs and additional mouse models of neurodegeneration, or even test promising drugs in humans suffering from neurodegeneration.

**Drug treatments for metabolic disease:** Our plans to test whether drugs that act on cultured human brain cells also affect food intake and body weight will test how well cultured cells can predict effects on body weight, and may reveal new ways in which food intake is regulated. In the longer term, these drugs or drug combinations could be tested in humans, especially since we will preferentially work with drugs already approved for use in humans.

**Central mechanisms of metabolic disease:** In the short-term, these studies will explore and identify genes that act in the brain to regulate food intake in order to link genes associated with obesity and behavior that contributes to obesity. In the longer term, drugs targeting these genes might lead to new treatments for obesity and diabetes.

**How will you maximise the outputs of your work?**

Our research work will be carried out in collaboration with research teams who have years of experience in working with mice that develop neurodegenerative disease. All our research findings will be made available to other scientists through presentations at scientific conferences and publication in peer-reviewed journals. In addition, protocols developed for this work will be freely distributed to the community (e.g. Protocols.io). Negative results may be published on open-access servers (e.g. BioRxiv). Data will stored in electronic format wherever possible and stored for at least 10 years to enable our group and collaborators to easily retrieve and build on past results, or reanalyse data using new methods.

**Species and numbers of animals expected to be used**

- Mice: 19,000

**Predicted harms**

**Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.**

**Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.**

Mice will undergo a brief surgical procedure which involves injecting infectious proteins (Prions) into the brain to cause brain damage (neurodegeneration). About a week later, mice will be given food that
is high in fat and/or carbohydrates, which they like to eat so much it makes them fat. Following this, mice will be given experimental drugs to reduce obesity, or reduce the signs of neurodegeneration. The effect of drugs on obesity and/or diabetes will be analysed by measuring their food intake and activity along with body composition imaging to find changes in body fat. Blood samples may be taken to measure hormonal changes in the blood and mice may also be exposed to a brief test similar to one used in humans to see if their diabetic state has been reversed. To analyse the effects of the drug treatment on neurodegeneration, mice will be tested for changes in behavior (e.g. burrowing pellets of food) and the amount of certain proteins in the post-mortem brain tissue. Experiments will typically take 4-24 weeks.

**Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.**

- Prion-injected mice will develop defects in their movements and memory at a predictable time after injection, unless they are killed sooner. In case of obese mice, these defects may appear sooner. All prion-injected animals will be regularly monitored for onset of early signs of clinical disease. Once these signs are observed, mice will be inspected daily to determine if additional signs of the disease emerge, and twice daily if prion signs are progressing quickly. We will monitor the general condition of the animal and pay specific attention to breathing, coordination, posture and gait. All signs will be documented on clinical observation sheets. Mice will be killed as soon as clinical prion disease can be diagnosed.

- Most animals will experience transient pain or discomfort due to drug administration, which will be treated with appropriate anaesthesia/analgesic.

- Mice fed with modified diets will exhibit long-lasting increase in food intake (hyperphagia) and obesity which may cause type 2 diabetes. These conditions may be reversed in the presence of therapeutic interventions as a part of the experimental design.

- Food restriction and insulin tolerance tests may cause a drop in blood sugar (glucose) levels (hypoglycaemia), which will be treated by either giving mice food, or directly giving them glucose. Mice will be killed if hypoglycaemia is not reversed within two hours of these interventions.

- Mice that undergo surgery (prion injection, osmotic mini-pump implantation and guided injection into the brain) may develop complications such as failure to eat and/or to move normally, or failure of the wound to heal normally. In these cases, the NVS will be consulted. In rare cases (expected < 2%), animals may experience wound breakdown or infection, which will treated and wounds re-closed within 48 hours post-surgery.

- Treatment with substances (e.g. drugs or viruses) targeting genes and cell types important for regulating metabolism are expected cause a change in food intake, body weight and body condition score (observed as a readout of the experimental design as well as a measure of animal health). Mice will be killed if their body weight drops more than 15% below the weight of age-matched and sex-matched control mice or if they show other clinical signs or deterioration in body condition score.
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 species)?

The expected severities are mild (approximately 30% of mice) and moderate (approximately 70% of mice).

What will happen to the animals at the end of the study?

- Kept alive
- 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?

Metabolic and neurodegenerative disorders affect millions of people worldwide. These diseases are mostly untreatable, and represent a large, unmet clinical need that cause a substantial economic impact. These diseases may have shared causes, which are still poorly understood, but likely interact at the level of the whole animal rather than a single cell type. For example obesity leads to increased blood pressure and activation of the immune system, which are thought to independently contribute to neurodegeneration. As such, it is essential to use animal models to understand which of these factors are most relevant, and to test possible treatment options.

We work extensively with cell culture systems to understand the behavior of individual cell types that contribute to metabolic and neurodegenerative disease. However, due to the complexity of the brain and the lack of culture systems that can fully mimic this complexity, animals remain irreplaceable. Furthermore, animals provide insight into the clinical, behavioral, physiological and neuropathological outcomes that can lead to development of better therapies.

What was your strategy for searching for non-animal alternatives?

The proposed work cannot be readily replaced with non-animal alternatives, however:

- wherever practical, we will use our cell culture systems to prioritize drugs that act on specific brain cells before carrying out targeted animal studies
- wherever practical, we will use our cell culture systems to prioritize genes that act on specific brain cells before carrying out targeted animal studies
- where possible, we will use human tissue samples to confirm the significance of key target genes in human neurodegenerative disease and obesity
Why were they not suitable?

Although brain cell culture systems provide useful insights into obesity and neurodegeneration, they cannot completely replace the need for animal studies. Specifically, cell culture models do not mimic complex interactions between cell types and body systems that likely contribute to human disease. They also do not allow measurement of behaviors or physiological outputs relevant to disease, making it difficult to predict how results obtained from cell culture systems will be relevant to human disease progression or treatment. Therefore, we will conduct both cell culture and animal experiments to identify the most promising strategies for treating obesity and neurodegenerative disease.

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 will use the minimum number of mice needed in all experiments for reproducible results and statistical validity in line with the ARRIVE guidelines www.nc3rs.org.uk/ARRIVE. We will maximize the use of each experimental animal by maximizing the readouts obtained from each animal thereby reducing the number of animals needed. For most of the quantitative experiments, we will determine the sample size required based on relevant literature. Otherwise, statistical power analysis will used to set sample sizes (significance level of 5%, power of 80%).

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

We have referred to the PREPARE guidelines (https://morecopia.no/prepare) and the NC3RS Experimental design assistant (https://www.nc3rs.org.uk/experimental-design-assistant-eda) to help plan and design our experiments. We have considered the number of independent experiments that will be performed when calculating total animal numbers and have consulted the “Guiding Principles for Behavioral Laboratory Animal Science” paper to ensure that our experimental design incorporates the most appropriate tests.

What other measures apart from good experimental design will you use to minimise numbers?

The following steps will be taken to ensure best breeding practice to efficiently supply mice for research while minimizing animal wastage:

- We will ensure high standards of animal care and welfare and the breeding of animals will be conducted according to the best practice.
Dosage and safety of all drugs and drug combinations will be validated by a pilot study.

Where possible, we will use published data to inform safe dosing limits and adverse effects of drugs, thereby reducing the number of animals needed for screening and testing.

We will use cell culture systems to prioritize and select drugs and genes that act on specific brain cell populations of interest before carrying out targeted animal studies.

Mice will be grouped into experimental cohorts large enough to allow sufficient numbers for sampling at regular intervals.

We will randomly assign mice to experimental groups and where possible, we will conduct blinded studies to minimize bias.

Suitable experimental controls will be used for each study, based on the study design and specific aims.

We will maintain minimum colony sizes that are required to meet the demands of our research experiments and avoid overproduction.

Detailed breeding records will be kept, enabling the selection of the most appropriate breeding stock.

Where appropriate, genetically altered animals will be used to reduce the number of animals required to achieve specific aims.

Cryopreservation of genetically altered mouse lines will reduce animal wastage and costs associated with mouse colony maintenance.

We will ensure that researchers and technicians working on this project are appropriately trained and competent to maximize the success rate of experiments and thus minimize the number of animals used.

Detailed study plans describing the aims and objective of each experiment along with the experimental steps, treatment groups and sizes and methods for data analysis will be written down to guide our work.

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.
Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Models:

- Mouse models of prion disease are superior to those available for other human neurodegenerative diseases since they show many of the same features of human prion disease, including the loss of brain cells that are not seen in other mouse models. Prions are essentially infectious proteins. They have a particular three-dimensional structure and can cause normal proteins in the brain to fold into that same structure, causing a “chain reaction” of misfolded protein. Therefore, injecting normal mice (e.g. C57Bl/6J) with prions from mouse causes misfolded proteins to spread throughout the brain leading to brain cell damage and loss. Injected mice develop clinical signs of prion disease (ruffled fur, rigid tail, hunched posture, loss of coordination and balance) after about 4-5-months in a manner that has been described in detail and that is very consistent between individual animals. This model aids better understanding of the how neurodegenerative disease progresses, and how treatments might delay or slow disease progression.

- Genetically altered mouse lines will enable us to more easily count connections between brain cells to determine how they change with metabolic or neurodegenerative disease. Similarly, genetically altered mouse lines will be used to facilitate change in gene expression in specific brain cell populations and evaluate the gene function with respect to metabolic and/or neurodegenerative disease.

- We may use genetically altered mouse models of obesity (and/or other metabolic diseases associated conditions such as type 2 diabetes) or models that are resistant to obesity (and/or other metabolic diseases associated conditions such as type 2 diabetes), typically on a C57BL/6J genetic background. For e.g. we may use Melanocortin-4 receptor (MC4R) knockout mouse model that exhibits obesity, hyperphagia, hyperglycaemia (excessive levels of blood glucose) and hyperinsulinemia (excessive levels of blood insulin). These models will enable us to identify the effects of metabolic associated genes on neurodegenerative disease progressions and/or therapeutic interventions.

- Diet-induced models of obesity and metabolic disease are well-established in the field, and complement existing and emerging genetic models. Specifically, the time-course of diet-induced obesity in C57BL/6J mice and the accompanying changes we intend to measure (e.g. body weight, body composition, food intake, energy expenditure, hormone levels, and glucose homeostasis) are all well-described in the literature. These mice serve as useful tools in evaluating the effects of anti-obesity compounds and therapies. We will also use them in combination with prion infection to study the links between metabolic disease (and substances used to treat metabolic disease) on neurodegenerative disease progression.

In drug-treated diet-induced obese mice with or without prion infection, we hope to see reduction in body weight and related symptoms, but it is possible that there may be unintended side effects such as anxiety or difficulty moving. We will therefore monitor mice regularly for potential adverse effects. We will use body-condition scoring charts following published standard criteria rather than solely relying on weight changes, since weight loss in an obese mouse may actually indicate improved health rather
than deteriorating health. Implementation of body condition scoring, regular body weight measurements, and observation charts for neurological signs and general welfare will allow us to accurately monitor the neurodegenerative process and define humane endpoints to reduce suffering.

Humane endpoints have been chosen to ensure that animal suffering is minimised whilst obtaining the most valid scientific output. Most prion-infected mice will be killed at early time points during disease progression, well before they develop prion disease. Tissue collected at these time points will be analysed for histological and biochemical readouts of the disease state; such as neuronal loss, detection of PrPSc (disease-associated form of the prion protein) and markers of inflammation. However, a cohort of mice will be allowed to develop clinical signs of prion disease to assess how metabolic disease and therapeutic interventions affect the later stages of neurodegenerative disease progression. In rare cases (estimated < 5%), mice left to develop clinical signs of prion disease may exhibit rapid disease progression. However, this is very unlikely since prion disease can be confidently diagnosed at relatively early stages by the appearance of early indicators such as decreased motility and tail rigidity, and confirmatory signs such as ataxia and loss of righting reflex. Therefore, suffering is limited by the daily observation and monitoring of these signs.

Methods:

All procedures (breeding transgenic mice, prion inoculation, and administration of drugs, behavioral and physiological analyses, and guided viral injections) will be conducted based on previous publications.

Animal suffering will be minimised by:

- performing all surgical procedures (prion inoculation, osmotic pump implantation and guided brain injections) under general anaesthesia and providing analgesia (painkillers) before and/or after surgery.

- minimizing the number of surgical interventions: most animals will receive prion inoculation followed by one or two osmotic fusion pump implantations (with at least 4 weeks in between subsequent surgeries). Surgical implantation of telemetry device will be carried out at the same time as osmotic fusion pump implantation. In other experiments, mice will receive one set of brain injections on either side of the head (at most two injections, one on each side of the head) on a double transgenic background. For embryo transfer, preference will be given to non-surgical method whenever possible.

- giving mice pain relievers where appropriate and administering substances (e.g. drugs) via the least invasive route that is practical. All administration protocols adhere to published guidelines, LASA Good Practice Guidelines, and as advised by the NC3Rs. For most drugs, daily dosing will be required for a period of 50-60 days, corresponding to the onset of clinical signs or defined endpoints. Wherever practical, mice will be given these drugs via implanted osmotic fusion mini-pumps rather than daily injections. In case of daily injections, mice will be monitored for adverse reactions and body condition (as standard for all dosing studies). Pain relief may be given as recommended by a Named Veterinary Surgeon (NVS)/ Named Animal Care and Welfare Officer (NACWO) if mild signs of discomfort are noted. Dosing will be stopped and the NACWO/ NVS/ Home Office Inspector (HOI) contacted if moderate signs develop.
• using the most refined compounds identified from the literature, our cell culture work, or from drug libraries. After identifying the dosage routes, frequency and concentration, the minimal likely effective dose will be used. In case of new compounds or combinations, small pilot studies will be conducted in mice to provide this information.

• using ultra-fine needle syringes, such as Hamilton NeurosTM syringes for stereotaxic injections, therefore reducing injection site damage.

• group housing mice with enriched environments as much as is practical.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

We are unable to use animals that are at a more immature life stage/less sentient/ terminally anaesthetised due to the following reasons:

• To study the complex interrelation and course of disease progression (obesity and/or neurodegeneration), a multi-organ living organism with a developed brain that can closely mimic human disease needs to be utilized as an appropriate model.

• In order to understand the mechanism of disease progression and identify therapeutic strategies, we need to analyse brain structure and animal behavior that cannot be obtained from immature or terminally anaesthetised mice.

• Less sentient species cannot provide accurate insight into the human disease progression as they lack sufficient similarity to humans.

What are you going to do to refine the procedures (for example increased monitoring, post-operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

• Where permissible by the experimental strategy, mice will be group housed in small numbers (3-4 per cage) and will be provided with environmental enrichment wherever practical. Male mice may exhibit aggression and therefore will be regularly monitored for the presence of fight wounds. Any mice with evidence of fighting will be closely monitored and animals may be separated if fighting continues. The NACWO/NVS will be informed and analgesia may be given as directed by the NVS. If an animal sustains injuries from fighting that are noted to cause distress and suffering and that cannot be treated with topical or systemic therapy (as directed by the NVS), it will be promptly killed by Schedule 1.

• Post-surgery (Prion inoculation, osmotic mini-pump implantation, guided brain injection), mice will be monitored daily for a minimum of 72 hours and maybe given appropriate care (analgesic for pain, re-closing of uninfected and minimally inflamed wounds).
- For experiments requiring short-term single housing or transfer to a different type of cage (e.g. metabolic cages), mice will be allowed to acclimatize to the individual housing and/or the new cage for minimum period of 24 hours.

- Body weight, body condition scoring, glucose and ketone levels will be measured regularly (daily to weekly) to assess animal health. Mice exhibiting poor tolerance to fasting may be re-fed or given an intra-peritoneal dose of glucose.

- Mice will be regularly (daily/weekly) assessed for any changes in their motor function (e.g., disturbances of gait and abnormal posture or muscle tone), level of arousal (e.g., hyperactivity and lethargy), and psychological status (aggression, biting, licking).

- All prion-inoculated animals will be regularly monitored for onset of early indicators of clinical disease. Once noted, mice will be inspected daily thereafter for clinical confirmatory signs of the disease and twice daily if prion signs are progressing quickly. The general condition of the animal will be assessed with specific attention given to breathing, coordination, posture and gait. All signs will be documented on clinical observation sheets.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

We will follow the LASA Good Practice Guidelines and PREPARE guidelines to ensure all our experiments are conducted in the most refined way. We will refer to the NC3Rs (https://www.nc3rs.org.uk/3rs-resources) and use our university biomedical services search tool (https://www.ubs.admin.cam.ac.uk/3rs/3rs-search-tool) to stay informed and continuously refine our experimental strategies. We will consider the number of independent experiments that will be performed when calculating total animal numbers and have consulted the “Guiding Principles for Behavioural Laboratory Animal Science” paper to ensure that our experimental design incorporates the most appropriate tests.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

During the project, we will regularly follow updates on the NC3Rs and RSPCA websites specially pertaining to the 3Rs and animal welfare. Experimental strategies will be modified to incorporate new technologies and approaches. We will ensure our participation in review meetings held in our establishment to discuss validity and usefulness of our mouse models and review animal wastage. We will stay updated on scientific literature in our field which would enable us to further refine our experimental approach.

Explain the choice of species and the related life stages

We intend to understand how genes, cell types, dietary factors, and drugs contribute to the development (or treatment) of metabolic disease (e.g. obesity) and neurodegeneration. To understand how these contributing factors are related, it is essential to use animals where behaviors related to obesity (e.g.
food intake) and neurodegeneration (e.g. defects in memory) can be studied. Mice serve as the most appropriate species to use for these studies since mice and humans share more genes, cell types, and behaviors than flies, worms, or fish that are commonly used in research. While in theory monkeys would be even more similar to humans, mice have been used more extensively so obesity and neurodegenerative models in mice are very well established. Furthermore, there are many genetically altered mice that will aid our studies to understand the details of how obesity and neurodegeneration is caused, and how these diseases might be treated.

Since obesity and neurodegenerative disease largely affect adult humans, we will perform our studies in adult mice. Specifically, most of our studies will be carried out using young or adult mice (males and/or females) to enable the study of metabolic and/or neurodegenerative disease progression and the effect of interventions (metabolic and environmental factors, agents (e.g. drugs), and genetic). Prion infection in young mice results in the development of clinical signs of prion disease after about 4-5 months. Their disease onset and progression is well-described in scientific articles and is consistent between animals, enabling us to compare the effect of diet or experimental agents (e.g. drugs) on disease progression.