Home Office

NON-TECHNICAL SUMMARY

Understanding the neuronal network involved in the regulation of glucose metabolism, feeding behaviour and energy expenditure

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

Obesity, Diabetes, Blood glucose levels, Energy Expenditure, Brain regulation of metabolism

Animal types	Life stages
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand how the central nervous system senses hormones like Insulin to regulate blood sugar levels, food intake and energy expenditure (how we burn calories).

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 study of the brain regulation of energy balance has the potential to improve current treatments of diabetes and obesity. Insulin is released by the pancreas and goes to the liver to decrease glucose production (sugar released by the liver) and to promote glucose storage as glycogen. However, when insulin reaches the brain, it can act at multiple levels and have important functions like regulating the nervous system that controls how much we eat, blood sugar levels, cognition and memory and can affect mood. Deficits in brain insulin signalling have been linked to several neurodegenerative diseases including Alzheimer’s and Parkinson’s disease. Indeed, with Type 2 Diabetes (T2D) older adults suffer from decreased verbal learning, abstract reasoning, and complex psychomotor tasks. Restoring insulin sensitivity in the brain could be a good strategy to counteract the negative outcomes of obesity and diabetes. In addition, it could be beneficial for people suffering from neurodegenerative disease. The aim of this study is to investigate how the brain regulates glucose metabolism, feeding behaviour and energy expenditure. We will identify which brain cells are sending signals to the liver in order to control glucose levels and to the brown fat in order to control energy expenditure. We will also study how alteration of these brain cells can have deleterious consequences for the metabolism in particular in obese and diabetic models.

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

The involvement of the brain in controlling metabolic functions is well known, however, the impact that obesity and diabetes have on brain functionalities is understudied. The long-term effect of brain insulin resistance (decreased ability to respond to insulin) can increase the chances of developing neurodegenerative diseases. Memory loss is a big problem in diabetic individuals and this is due to the brain not functioning properly. Understanding the molecular mechanism that triggers insulin resistance in the brain is the first step for developing a specific treatment that targets the brain to ameliorate metabolic functions and also to prevent long term deleterious effects. In addition, we plan to determine the specific brain cells that are involved in insulin sensing and are affected by insulin resistance, diabetes and obesity.

Our work looks at mechanisms, our major output will be in the form of peer-reviewed publications. We are also involved in public engagement activities where we make aware the public of the effects that obesity and diabetes can have on brain functions.

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

The study of the brain regulation of energy homeostasis has the potential to improve current treatments of diabetes and obesity. Insulin is released by the pancreas and goes to the liver to decrease glucose production and promote glucose storage as glycogen. However, when insulin reaches the brain, it can act at multiple levels and have important functions, for example, regulating neural circuits that control appetite, blood glucose levels, cognition and memory and can also affect mood. Deficits in brain insulin signalling have been linked to several neurodegenerative diseases including Alzheimer's and Parkinson's disease. Indeed, with T2D older adults suffer from decreased verbal learning, abstract reasoning, and complex psychomotor tasks. Restoring insulin sensitivity in the brain could be a good strategy to counteract the negative outcomes of obesity and diabetes, in addition, it could be beneficial for people suffering from neurodegenerative disease.

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

The major output for this research will be of interest to the scientific community, the outcomes will be published in peer-reviewed journals. We will also present our data at conferences. The focus of this research is of significant interest to the general public, most people know someone who has T2D. In order to bring this research to the attention of academics in other disciplines and to the general public, we will work with the University Communications Office to ensure timely Press Releases. In addition, I will also present the work at the local Café Scientifique or during the Pint of Science events.

As obesity is increasing amongst the young population, this project could bring awareness regarding the dangerous effect that overnutrition has on the brain and the bad consequences that this could have during development. An activity focused on this topic and targeted to young generations will be extremely important. To engage with young people, I will develop an exhibition based on our work to be used at the Festivals of Science organized locally. These events are attended by many 9-19-year-old schoolchildren. Both events are run annually and draw in around 1200 people per day from the local community.

Species and numbers of animals expected to be used

- Rats: 1200

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.

The experimental procedures proposed require the use of 8 to 12 weeks-old Male Sprague Dawley (SD) rats. These are fully developed rats that are still growing. We measure changes in body weight, and we aim to look at body weight increase over time. This is the best system to perform metabolic studies since their glucose levels are very stable. All our previous data are based on 8 to 12 weeks old

male SD rats and in addition, they have a higher than 95% rate of survival after brain surgery. The stereotactic surgery is very easy to perform in rats and, due to their anatomy, is very easy to get the right coordinates to insert the Intracranial (ICV) cannula in the dorsal vagal complex (DVC) of the brain. In addition, the rats have fast and complete recovery after surgery. We measure changes in blood glucose levels using a pancreatic euglycemic clamp, SD rats have a stable blood glucose that make our analysis reliable over time. We have vast experience on using this model and we can make the best use of these rats with the confidence to achieve the highest level of care and rate of survival until the end of the experiment.

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

First, rats will be subjected to brain surgery in order to insert a cannula in a specific brain area. The surgery lasts 10 minutes and is done under balanced general anaesthesia. The cannula is held with a small implant made of dental cement. Once in place, the implant does not cause any distress or pain to the rats. Rats are expected to make a full recovery within 6-12 hours after surgery. We will use the brain cannula to inject inactivated viruses that will be used to express specific proteins in the brain area we are interested in. In addition, the cannula will be used to deliver specific treatments in the brain.

On the same day of brain surgery, a subgroup of rats will also receive a small incision either in the abdominal cavity or the scapular area in order to expose the liver and the brown fat respectively. This procedure is done in order to inject an inactivated virus that can be taken up by neuronal terminals in these peripheral organs and can be visualised in the brain. After the viral injection, the small incision will be sutured. The viral injection will not cause any harm, disease or pain to the rats and the cut will be small enough to not cause post-surgical discomfort. Pain-relieving drugs are also given routinely to all animals that are subjected to surgical procedures and pain score is recorded post-surgically.

Insertion of catheters in the jugular vein and artery will be done in a subgroup of rats a week after brain surgery. This is also a moderate procedure. We will use the catheters in order to infuse substances and withdraw blood for analysis at the end stage of our experiments, which involve the injection of substances and serial withdrawal of blood for glucose tolerance test (GTT). These catheters also allow us to withdrawn blood from fully conscious rats and without the need for manual handling and repeated punctures of the vein. After vascular surgery, the rats are expected to have a full recovery and the catheters do not cause discomfort.

In order to monitor the effect that our brain treatment has on vital parameters like heart rate and blood pressure, we will implant subcutaneously telemetric devices. The implant has no effect on the day-to-day life of the rats and allows continuous monitoring of important physiological parameters.

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

The majority of the procedures will require recovery from anaesthesia following surgical procedures. The surgical procedures will involve the insertion of cannulas in specific areas of the brain for the purpose of infusing substances and the insertion of catheters in the blood vessels in order to infuse substances and withdrawal of blood. Brain cannulas are inserted using specialized equipment. We have extensive experience in performing such surgical procedures and do not envisage adverse effects during the post-surgical period. Post-operative pain relief is routinely given to all animals and pain response is monitored on a score sheet. After surgery, the rats will be housed individually to avoid the

dislodging of the catheters. We will use transparent cages near each other so the rats do not feel isolated. At the end of the experiments, animals will be humanely killed to allow for tissue collection. The genetically modified rats will not have an adverse phenotype. The use of the Cre recombinase expression system under a specific promoter will allow us to target only specific cell populations. This will be very important to identify the neuronal network involved in insulin sensing and resistance in the DVC. We will use PET, MRI or CT scan to monitor fat deposition ad brown fat activity in high fat diet-fed obese rats. This will help us understand how changes in brain activity can affect whole-body metabolism. These are terminal procedures performed under full anaesthesia. Finally, during imaging sessions, we will use telemetric devices implanted subcutaneously to monitor the heart rate of the animals.

Rats will be also placed in a cold environment (cold challenge) to stimulate the release of heat by the brown fat (thermogenesis). This experiment will be performed in fully anaesthetized rats at the end stage of our experimental procedure.

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)?

All the procedures we will perform are expected to be of moderate severity. We expect a full recovery after brain and vascular cannulation. We expect that both the brain implant and the vascular tubing will not cause discomfort to the rats.

Our procedures include glucose clamp and intravenous tolerance tests to measure how the rats control their blood glucose. In both cases, the rats will be fully awake and free to move in the cage. The use of vein cannulas and long tubing will allow us to infuse substances and withdraw blood without causing any distress or harm to the rats.

The brain infusion is also painless and done through a long tubing so that the rats can be kept unrestrained in their cages.

Cold challenge and bioimaging (PET, MRI and CT scans) will be done in fully anaesthetized rats so no discomfort will be caused.

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?

Since this study focuses on understanding the neuronal network involved in the brain control of metabolic functions and the physiological impact that insulin resistance, obesity and diabetes have on brain metabolic regulation, it is not possible to substitute the use of animals with alternative systems. We will study how the brain controls sugar metabolism, food intake, body weight and energy expenditure. For these studies, there is no possibility to use an alternative system for animals.

The experiments will be performed in rats; a vast amount of background data in relation to metabolic parameters is available which make these the most appropriate species in which to perform our studies. In particular, rat glucose levels are very stable, in addition, stereotactic brain surgery is very easy to perform in rats and, due to their anatomy, it is very easy to get the right coordinates to insert the brain cannula. Our research team has extensive experience in this kind of surgery. In addition, the rodents have a fast and complete recovery after ICV surgery and also recover well after vascular surgery.

However, where in vitro work can be undertaken we will do so e.g. all the validation of the adenoviruses and lentiviruses will be performed in cells, and only viruses that show a clear effect in vitro will be then used in vivo. Whenever an analysis can be performed using cells, we will do so instead of using rodents. We have available a plethora of brain cell lines that we can use when is needed.

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

No animal alternative can be used.

Why were they not suitable?

Since we need to do neuronal tracing to look at the connection between different organs and the brain. The changes in whole-body metabolism can be studied only in the whole organism.

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 number of animals that will be used in this project is based on power calculations that are based on prior experiments. To determine the required number of animals we used the variance estimated from prior work as well as expected effect sizes. We will conduct our experiments to be able to publish to the Arrive guidelines: https://www.nc3rs.org.uk/arrive- guidelines.

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

The number of animals will be minimized in several ways:

Tissue and plasma from the same animal will be used for different objectives where possible. This reduces the total number of animals that would otherwise be required if a single animal was used for each experiment.

Tissue for neuroanatomical procedures such as immunohistochemistry will be stored long-term for future use where possible.

The NC3RS experimental design assistant will be considered for planning:
https://www.nc3rs.org.uk/experimental-design-assistant-eda

Rodents are subjected to seasonal changes; this could affect the results in terms of high variability. In order to minimise this event, we will make sure to conduct all the experiments with the appropriate controls and to minimise the environmental changes that could affect the analysis. We will randomise the animals starting from the brain surgery (the first procedure that we perform) and when possible, we will match the body weights of control and treated subjects

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

At the end of each experiment, we will harvest as many tissues as we can even if they are not useful for the moment because they might be needed in future. In this way, we will not have to repeat the experiment again.

For example, in our recent study published in Molecular Metabolism, we performed feeding studies and harvested at the end of the experiment different organs including the brown fat (BAT). We then realized that there were changes in BAT and we performed a series of analyses that led us to a completely new project for which most of the data has come from animal tissues that we already have.

We also collaborate with other groups in the university. The hearts, muscles and spinal cord of our obese and diabetic rats will be taken by different groups in the university that are interested in the effect of obesity and diabetes on heart functions, muscle activity and the spinal cord’s ependymal cells functions.

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 aim of this study is to look at the brain regulation of glucose metabolism and feeding behaviour. We will look at the effect that the insulin resistance in the CNS has on the whole-body metabolism. The experimental procedures we propose require the use of 8 to 12 weeks old male Sprague Dawley rats.

These animals provide the best system to perform metabolic studies. In particular, their glucose levels are very stable and this makes them the perfect model to perform pancreatic clamp studies. All our preliminary data and previous studies have been performed with rats.

We have been performing brain and vascular surgeries for years now and we have been able to optimise the procedure in order to minimise the suffering of the rats and to obtain close to 100% success and recovery after surgery. Here are some examples of how we proceeded to refine our procedure:

1) At first, we were using injectable anaesthetics that remain in the system longer, delay the recovery after surgery and sometimes can cause an overdose of the rats. We now mainly use isoflurane that is well accepted by the rats and allows the rats to wake up soon after surgery, in this way we have a faster recovery and no issues with overdosing.

2) After surgery, the rodents are housed in single transparent cages in order to avoid any potential fights that could affect the surgical areas. Initially, we tried to pair housed surgically prepared animals but noticed that animals were chewing at each other's surgical area and losing their cranial implants, rendering the animal unusable. For this reason, single housing after surgery is imperative in this study. Animals are kept in visual contact with each other and are only singly housed for a maximum of 18 days

3) After vascular surgery we prepare food mushed with water and leave it in a small dish on the cage floor. This makes it easier for the rat to eat and keep hydrated right after surgery. In addition, extra bedding and nesting materials are also provided to all surgically prepared animals for cage comfort. Heated pads are also available to aid thermoregulation during recovery after anaesthesia

4) We experienced that Adenoviral injection in the dorsal vagal complex (the area of the brain we are mainly interested in) if injected on the day of surgery, is sometimes not well tolerated by the rats. This area controls the breathing and if the rats are not fully awake after surgery, they might stop breathing. We now inject the virus via intra-cranial cannula the day after surgery, this allows the virus to be well tolerated and no side effects have been seen.

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

Since we are studying how the brain controls blood glucose levels, thermogenesis and feeding behaviour, we need a fully developed animal. We are looking at how the brain regulates peripheral organs so using organoids won't be suitable for us.

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

All surgical procedures are performed under balanced general anaesthesia. We use inhalation anaesthesia using isoflurane which causes rapid induction and uneventful rapid recovery. In addition, full aseptic conditions are maintained for all surgical procedures and we follow LASA Guidelines for Aseptic Surgery.

Brain surgery requires the insertion of an ICV cannula in a specific brain area. The surgery lasts less than 10 minutes and is done under full anaesthesia. After surgery, the rodents are expected to have a

full recovery within 1 day. The cannula implanted has a very mild impact on the day-to-day life of the rodents and will be kept for up to 18 days.

Vascular surgery requires the insertion of catheters into the jugular vein and artery of the rodent. The surgery lasts ~30 min and is done under full anaesthesia After surgery, the rodents are expected to have a full recovery within 2 days. The implanted catheter has a very mild impact on the day-to-day life of the rodents and will be kept for up to 4 days.

All the surgical procedures will be carried out under aseptic conditions. After surgery, we will constantly monitor until animals regain full consciousness and we will give analgesic to the rodents for 3 days until we can see a full recovery. To limit suffering, animal responses will be monitored.

Viral injection in the liver will require a laparotomy of the abdominal area to expose the liver and perform the viral injection. The injection of the virus will not cause any harm and we will do the smallest incision we can in order to avoid discomfort after surgery.

Similar attention will be observed when we will do the brown fat viral injection that occurs at the level of the scapulae in the back of the rat.

The feeding protocol is very mild, we just remove the food in the morning (the rodents eat less in the daytime). The brain infusion is carried out with a long catheter that allows the rodents to move freely in the cage to cause them minimum stress. The infusions through the vein catheter and blood withdrawal through the arterial catheter are also carried out with long tubing that allows the rodents to freely move to minimize stress.

For MRI/PET/CT imaging, motion artefacts from breathing and heartbeats can degrade the quality of imaging – meaning that the imaging may take longer to get quality data. By implanting telemetric ECG monitors, we can get the imaging on the heartbeats and make sure that images are only taken at the correct time, resulting in better quality images and shorter overall scans.

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

We follow the NC3R best practice guidance. We attend webinars from Nc3R like "the best practice in experimental design": https://www.nc3rs.org.uk/events/webinar-best-practice-experimental-design.

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

We receive newsletters from the NC3R, we rely on the website to find important information regarding ways to reduce, refine and replace (https://www.nc3rs.org.uk/). We also have regular talks with NACWO and veterinarian in order to refine our procedures.

Over the years we have changed the way in which we anaesthetize the rats, the way in which we perform post-surgical care and the way in which we keep the rats after surgery in order to improve the success rate of our procedures and the wellbeing of our animals. This has also reduced the number of animals needed for our experiments.