Home Office

NON-TECHNICAL SUMMARY

Gut hormone modulation of metabolism

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

enteroendocrine hormones, gut-brain-pancreas axis, diabetes, obesity

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 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 characterize how gut hormone secretion is controlled and how gut hormones modulate metabolism and body weight.

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 Diabetes are major diseases putting considerable strain on health services worldwide. Patients suffering from obesity, definable as excess storage of fat, and/or diabetes, definable as failure to control blood glucose adequately, often develop complications affecting their hearts, blood vessels, liver and kidneys, leading to organ failures, amputations and early death. Modulating eating behaviour, energy expenditure and blood glucose handling are obvious therapeutic interventions for obesity and diabetes and there is good evidence that gut hormones modulate nutrient handling and eating behaviour. An example is glucagon-like peptide-1 (GLP-1), which is secreted from special cells in the intestine during a meal. Analogs of GLP-1 are relatively frequently in the News, as they have been approved for the treatment of obesity by the NHS in March 2023, adding to their well established role in the treatment of diabetes. We are investigating how GLP-1 secretion is initiated during a meal and which cells this hormone targets to reduce appetite. There are about twenty different gut hormones, many of which also affect eating behaviour. New drugs are under development which combine the action of GLP-1 with some of these other hormones, promoting even better weight loss in pilot studies. Our research aims to understand what mechanisms underlie these better outcomes and if and how we might be able recruit the bodies own spare capacity of hormones to treat obesity and diabetes.

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

We expect to gain new insights on how gut hormone secretion is controlled and how in turn these hormones modulate metabolism and eating behaviour. We expect that we will continue to publish our findings in open access scientific journals and at scientific conferences. We also have established collaborations with the pharmaceutical industry, which is actively developing gut hormone based therapies for the treatment of diabetes and obesity. Any new mouse models developed during this project will be shared with the scientific community, as will be data arising from the project, by uploading the data to appropriate data depositaries.

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

The scientific community and pharmaceutical industry is actively developing gut hormone based therapies for obesity and diabetes. Our findings should guide these attempts and clarify the mechanisms of action these new therapies employ. This might open new avenues of development or highlight potential risks, such as unexpected and unwanted side effects. On the longer timescale we hope that new and effective treatments will be available for patients - glucagon-like peptide-1 (GLP-1) based therapies are already licensed and newer therapies combining GLP-1 action with that of other gut hormones have shown promising improved therapeutic outcomes in pre-clinical and pilot studies. This project will explore how these better outcomes come about and hopefully identifies further

improved combinations. Our group is aware of at least one company developing agents that target GLP-1 secreting cells in the intestine - our research should help to inform and improve these attempts.

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

We will continue to publish in open access peer reviewed scientific journals, including, if appropriate, open access publishing platforms which do not employ editor based publication decisions, but allow ongoing peer review of deposited data (such as F1000Research), and participate in scientific meetings. We will continue to collaborate widely, sharing mouse models and ideas internationally, as evidenced by our collaborative publication record. The group is involved in outreach programs to the wider public, such as school visits - although only loosely connected to the specific questions asked in this project, this raises a wider awareness of how the gut affects body weight and health; we hope this to have further impact by promoting healthier eating choices.

Species and numbers of animals expected to be used

- Mice: 18050

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.

Mice as a mammalian species are close in their regulation of metabolism to humans. The suitability of mice to explore the role and ability of gut hormones to modulate obesity and glucose handling is exemplified by the successful development and subsequent translation of GLP-1 based therapies. We will predominantly use adult mice (>6-8 weeks old) for our experiments. Given that one focus of our research is on the regulation of feeding behaviour by the central nervous system this is the stage at which the underlying neuronal circuitry is established and cross talk with gut hormones (either arising from the gut or being released locally within the brain) can be studied. In addition, mice genetics are very advanced and it is possible to perform bespoke and restrictive manipulations of rare cell types, like the cells releasing gut hormones, which are found scattered in the gut lining and only constitute about 1% of the gut lining, or their neuronal targets.

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

To be able to identify and/or manipulate the rare cell types underlying the gut brain axis, we need to produce transgenic animals that tag the cells of interest, gut hormone producing cells in the gut and neurons in the central nervous system, either expressing the same hormones as neuromodulator or the receptors for these hormones. Many animals will be bred for this purpose and not undergo any further regulated procedures - some will be used as tissue donors for experiments performed on tissues harvested after they have been killed, as for example for the preparation of brain slices, in which we will monitor neuronal activity through electrical recordings or live cell imaging.

Animals used in regulated procedures might need additional modifications before they are assessed. This might involve feeding of altered diets to make them fat or lean and application of drugs, which induce or silence a genetic alteration or are considered to target the cells along the gut brain axis; these will typically be given through the drinking water, food or injected, typically directly into the blood stream through a vein, or into the body cavity or under the skin. Some animals will also be surgically modified. This is needed for example to manipulate specific areas in the brain, either through targeted injection of viruses into this area, thereby avoiding the delivery of a specific genetic alteration to other areas in and outside the central nervous system or through similarly targeted delivery of cell manipulating agents. Brain surgery is performed under general aneasthesia on specialist frames aiding the correct positioning of the agents and takes typically less than 30 minutes, with mice usually recovering to normal behaviour within 2 hours. Cell manipulating agents include for example gut hormones and viruses but also light in mice which express light sensitive ion channels to modulate neural activity or light sensitive sensors that allow us to monitor intracellular components such as calcium, as good measure for neural activity. In cases where a administration of agents (for example light) will have to be repeated frequently, a permanent access to the brain is implanted and mice will be single housed after surgery to prevent them from damaging each others access. Most mice will only undergo a single brain surgery, but some viruses used to identify the neural circuits a specific neuron sits in require two surgeries typically with a 3 week interval between surgeries. A minority of mice will undergo surgery aimed to interfere with known neuronal pathways connecting the intestine with the central nervous system performed under general anaethesia and typically lasting 10-20 minutes; most of these will not also undergo surgery to the central nervous system, but a few will, which then would typically be performed at the same time. Some mice will be surgically altered to enable direct infusion of nutrients and other enteroendocrine cell modifying agents directly into the intestinal lumen, bypassing the mouth and/or the stomach. These surgeries will be performed under general anaesthesia with adequate pain relieve and animals will be closely monitored for the rest of their lives with frequent flushing of the artificial access tubes to prevent complications due to food entering from the intestine getting stuck and blocking the tubes. We expect animals to recover from this surgery within a few hours and behave normally - a minority of animals might undergo this surgery in conjunction with brain surgery in which case we will aim to combine both surgeries.

Once prepared, animals will be tested for metabolic and behavioural parameters. In the most simple experiments we will measure their food intake and body weight whilst being fed special diet, for example containing a high fat content, which mice usually prefer to their standard diet. For this animals might be fasted for a few hours before the food intake quantification and single housed. Animals might undergo metabolic fitness assessment - for example their glucose tolerance will be assessed either by administering a glucose bolus intraperitoneally or given through a tube directly into their stomach at a defined time. Typically such tests will be performed whilst agents that modify gut hormone release or act on gut hormone receptors are also administered, typically directly into the blood stream or into the main body cavity. Occasionally longer term administration will be achieved by placing slow release devices under the skin under anaesthesia before the metabolic assessment. Metabolic fitness test like these will typically involve taking small amount of blood from superficial veins to be able to monitor blood glucose and/or hormone levels.

Some animals will be placed into specialist equipment to measure food intake and energy expenditure in which they are typically single housed for 3 days, including 1 day adaptation to the new environment. These "calorimetric chambers" resemble the home cage environment the mice normally life in and mice appear to be happy in these for days without obvious signs of stress. Sometimes we

will assess the body composition of animals for which they will be placed in specially designed tubes made of material identical to that found in the home cage to enable them to remain still and secure and quickly have a scan without the need for sedating drugs. Animals will also be trained to perform task in "operant chambers" - these are modified cages in which mice can work for treats (for example poking a computer touch screen), which will be provided in small quantities through special dispensers, whilst the mouse is monitored through video cameras. Training for these typically will take several daily sessions over a couple of weeks and typically individual training sessions will not last more than 2-4 hours; however, on occasions animals will be kept in these operant chambers for longer, in which case we will provide extra housing and enrichment material to simulate a home cage environment as much as possible and food and water provision, whilst intake is monitored, will not be limited to task performance. Some mice (typically whilst in these operant chambers) will be linked to light guides enabling light driven activation or inhibition of specific neurons in the central nervous system or neuronal activity monitoring whilst performing feeding tasks. Other mice, which express designer receptors exclusively activated by designer drugs (DREADDs), will typically be injected with vehicle or designer drug on different experimental days (cross-over design) whilst performing these tasks. Other test mice will undergo involve conditioned taste aversion or preference in which mice are given the choice between different flavoured liquids or meals which are paired with gut hormone releasing or gut hormone receptor modifying drug administration or control agents known to induce aversion or preference.

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

Most animals will experience no more discomfort than that experienced by any rodent bred in captivity and residing in a modern animal facility.

Some animals will experience transient (seconds) discomfort when given injections or when having blood samples taken. The injections will often be of naturally occurring hormones, or compounds closely related to them. On occasion, animals may be given compounds that are recognised to produce circulating levels of hormones that are seen in acute illness. These may reduce the animals' drive to seek out and eat food in the hours after they have been given.

When given a different diet or treatment, some animals will gain or lose weight. This will be within closely monitored parameters that take into account other important aspects of their appearance and behaviour. This weight change will typically occur slowly over weeks.

A minority of animal will also undergo surgery that will require a general anaesthetic. Inevitably, as with any operation, animals will have some discomfort in the immediate hours after the operation at the site of the incision. However, this will be minimised by administration of painkillers under instruction from a veterinarian. The general anaesthetic needed for this surgery may also make the animals less active and less hungry in the first day after the operation but we expect them to recover their appetite and vitality within 48 hours. Animals undergoing brain surgery that leaves access tubes for subsequent substance administration of light monitoring will need to be single housed to prevent them from dislodging each others access tubes - they will be closely monitored and in our experience they show normal behaviour without obvious signs of distress.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Sub-threshold - 40%

Mild- 30%

Moderate- 30 %

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?

We are researching the gut-brain axis. We are particularly interested in hormones released by the intestine after a meal which not only affect how the gut handles digestion of food but also affects future food intake by modulating hunger and fullness perception. There are already new therapies for diabetes and obesity based on the actions of one of the gut hormone glucagon-like peptide-1 (GLP-1), with other experimental medicines currently being developed that combine GLP-1-action with other gut hormone activities; these show promising better weight loss in experimental animals and human volunteers. To develop these further we will need to understand better i) how release of the different hormones is regulated in the intestine and the central nervous system, where some of the hormones are also made and ii) where and how they act to change feeding behaviour and/or energy expenditure, neither of which can be assessed in vitro.

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

To understand how hormone release is regulated in the gut we have now mostly moved to intestinal organoids - these are cell cultures of just the intestinal epithelium that can in principal be kept indefinitely and appear to give rise to all gut hormone secreting cells. We have established mouse and human derived intestinal organoids is which we have genetically labelled different intestinal hormone secreting cells with fluorescent proteins. These allow identification, characterisation and manipulation of stimulus-secretion coupling pathways within the hormone secreting cells themselves and also to investigate cross-talk between different cells in the gut epithelium. However, they lack the other tissues interacting with the epithelium, such as the enteric nervous system and currently there are no in vitro models that recapitulates these complex interactions. We also for example find that glucose-triggered GLP-1 secretion is totally dependent on a particular transporter in intestinal organoids preparations, but that mice lacking this transporter nevertheless elevate GLP-1 levels in their blood in response to

glucose in the diet, a mechanism that might be exploitable to stimulate GLP-1 secretion therapeutically, but can currently only be explored in live animals.

To understand how gut hormones affect feeding behaviour we need to measure behavioural outcomes, which is not possible with isolated cells in a dish. We are using acute brain slices made from mice killed for this purpose to monitor which neuronal circuits are modulated by gut hormones, however, to link these to behavioural outcomes we also need to perform experiments in freely behaving animals. Both, acute brain slices and cultures of differentiated neurons can also be used to characterize what a gut hormone might affect within a single neuron. To some extent the connectivity of neurons underlying behavioural pattern generation can also be investigated in brain slices, but many longer distance connection are lost, whereas connections in primary neuron cultures do not adequately replicate connectivity found in the brain.

Why were they not suitable?

See above - intestinal organoids are very powerful and we use these, but outcomes need to be further verified in animal experiments and more complex mechanisms like the unexpected GLP-1 response to dietary glucose when glucose uptake in the intestine is inhibited, are not well modeled.

Neuronal cultures can be used to identify molecular signals within a neuron, but lack the connectivity needed to produce specific behavioural outcomes. Connectivity and cross-talk between neurons can to some extent be explored in brain slices isolated from animals, but behaviour has to be explored in freely behaving 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?

We are interested in physiological outcomes, so are interested in robust effect sizes. In most cases we are able to observe effects in group sizes of 8 and whenever possible we use a cross-over design in which each mouse is its own control.

Often we need to bring together different genetic alterations (usually at least 2). Each animal has two copies of each gene and for some experiments both copies need to be altered. To bring these together with genetic alterations in a different locus is often challenging and produces many animals which only partly have the desired genetic change - such animals are killed early in life, when they cannot be used as controls for experiments or for future breeding, but contribute to the number given above.

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

I consulted the NC3Rs experimental design assistant and we discuss all animals experiment within our group. Our field is rapidly evolving and experimental designs are refined through discussions with both our collaborators and competitors directly or through the literature. For example until fairly recently glucose tolerance was frequently assessed after prolonged fasting of mice, but a newer consensus takes into account that mice can go into torpor after prolonged fasting. Torpor is a state of usually reduced body temperature and metabolic rate, enabling mice to survive times of food scarcity or famine. As this is not likely a good model for the physiological states we want to treat in obesity and diabetes, most groups are now fasting the mice for no longer than 16 hours and ideally only for 4-6 hours - whilst this has no direct impact on the group size used in a particular experiment, the better data quality achieved indirectly reduces the number of animals used.

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

Our research is shaped by guidelines for animal research, such as PREPARE and ARRIVE.

Whenever possible we share tissue within our group and with other groups in our institution. However, many of our animal models bring together several genetic alterations enabling specific manipulation or observation of a specific subset of cells within the intestine or the brain and therefore do not qualify as adequate wild-type controls.

We are using the Cre-lox system; Cre is a recombinase that changes the expression of "f-lox-ed" genes when expressed; by putting the expression of Cre under the control of specific promoters we can therefore modulate "floxed" genes just in cells that would normally express from this promoter, for example a promoter that drives the expression of a gut hormone. By sharing floxed reporter genes between different Cre-lines we are able to keep breeding pairs of these different Cre-lines low whilst still minimizing the risk of genetic drift due to excessive inbreeding. Cre-negative offspring can be used to cross-breed or as controls for Cre-positive animals.

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.

We use genetically altered animals in which rare cells either in the gut lining or in the brain are specifically labeled and manipulatable. These genetic alterations have no impact on the animals wellbeing.

Some animals are surgically modified to allow live monitoring and/or manipulation of these cells during behaviour. We will continue to work with our colleagues and collaborators to improve these so that they inflict as little harm to the animals as possible. All animals will receive pain relieve medication after surgery. Surgery will be performed aseptic and under general anaesthesia, if this is beneficial for the procedure (minor surgery, like the placement of a slow release pharmaceutical agent, will be performed with local anaesthesia).

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

To understand the gut brain axis and develop new modifying drugs useful to treat diabetes and obesity in humans we need to work in a species that has a similar physiology to humans. Mice have a proven track record, with research on the GLP-1/GLP1R axis having translated well into treatments now widely used in type2 diabetes and obesity therapy.

As we are interested in behavioural outcomes we cannot use terminally anaesthetised mice, as these are not showing any feeding behaviour driven by nutrient availability or by pleasurable experiences in response to eating, which are strongly influenced by the gut brain axis we wish to understand better, so that on the long run we will be able to exploit this therapeutically.

We need to work in mature mice, as only in adult mice the underlying neuronal circuits and feedback mechanisms are fully functional.

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

All our animals receive close postoperative monitoring and analgesia as required and are checked frequently (initially hourly and later daily after surgery). For example, post central nervous system surgery postoperative analgesia is provided as standard. For other procedures with potential to induce pain (for example stimulation of serotonin producing EC-cells, which have been implicated in gastrointestinal nociception) we will use pain scoring schemes like for example the McGill Grimace scheme and postural/behavioural clues and provide analgesia as required. We will also use scoring sheets rating animals body condition (for example BCS1-5 scores defined in the application) rather than relying solely on body weight changes, as substantial loss of body weight is a desired outcome in diet induced obese mice. These will also be used for example when new diets, potentially resulting in neophobia (the fear of all new things often seen in mice refusing to eat unfamiliar diets), are introduced. When animals are exposed to new diets or environments they receive training sessions. New diets are usually introduced as an add on before a total switch, which we find reduces neophobia and increases the acceptance of diet switches.

Behavioural arenas ("operant chambers") are introduced in short training sessions, with the animals returning to group housing when this is possible or at least to their home environment before data is collected in subsequent experimental sessions once the animals are familiar with the setting. Whilst the behavioural arenas have a flooring that allows excrements and wasted food to fall through and out of reach, which is necessary to adequately monitor feeding behaviour and motivation, the floors are not wired grids, but fully weight bearing (small holes punched into a flat surface). When animals are housed for more than 4 hours in these arenas we will provide housing with nesting material to which the mice can retreat, and other environment enrichment components provided as standard in our home

cages (e.g. wooden gnawing sticks), attempting to replicate a home cage environment as much as possible. To minimize disturbances during the experiment individual behavioural cages are in noise cancelling cabinets and mice are monitored through video cameras.

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

We are part of a very active scientific community that tries to evolve and refine methods constantly. New methods and method modifications are shared through peer-reviewed publications and at meetings, either during collaborations or at conferences. We expect that methods will be refined further during the duration of this project license. We will also aim to implement guidance provided by the Laboratory Animal Science Association, (LASA; https://www.lasa.co.uk/current_publications/) and other sources of 3Rs advice as they arise during this project.

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

Our institution runs a 3R enquiry email group, which informs about updates and developments and has in recent years implemented an animal and method sharing platform. In addition group members are encouraged to consult open resources, such as https://nc3rs.org.uk/3rs-resources or https://norecopa.no/databases-guidelines.