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NON-TECHNICAL SUMMARY

Neuroimmunometabolism: Understanding Brain-Body Communication in Health and Disease

Project duration

5 years 0 months

Project purpose

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

Key words

brain, metabolism, diet, immunity, homeostasis

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 goal of this project is to understand how the brain helps control how our bodies use and store energy, both when we're healthy and when we're sick. We'll focus on how the brain talks to the rest of the body through chemical signals, like hormones and immune responses. By studying how this system works, we hope to learn what keeps our bodies balanced and healthy. We also want to find out what happens when this communication goes wrong, which can lead to problems like inflammation, weight gain, or diseases related to poor eating habits.

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?

A very large portion of the computational capacity of an animal brain - including humans - is dedicated to monitoring internal energy levels and ensuring balance. These fundamental brain functions are crucial for maintaining health by regulating food intake, metabolism, and related behaviors. When this system works well, it helps keep our bodies healthy and functioning efficiently. However, disruptions to this balance can lead to serious health issues, including obesity and diabetes.

These conditions are often accompanied by serious comorbidities like cardiovascular diseases, intestinal inflammation, allergies, and a predisposition to infections. By studying how the brain, immune system, and other body systems interact, we aim to understand how these issues arise and how they can be prevented or treated.

Our research seeks to uncover the mechanisms behind these diseases, which can lead to the identification of new therapeutic targets. This knowledge will contribute to better treatments for affected individuals, improving health outcomes and quality of life, while also reducing the societal and economic burden of metabolic and inflammatory diseases.

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

At the end of this project, we expect multiple types of outputs:

Increase in Knowledge: This research will provide new information on how the brain, immune system, and other parts of the body work together to keep us healthy. We will also learn more about what happens when this interplay goes wrong, leading to diseases like obesity, diabetes, heart problems, allergies, and chronic inflammation.

New Ideas for Treatments: By understanding how these diseases develop, we hope to find new targets for treatments. For example, this type of research has led to new medications like Wegovy and Ozempic, illustrating how the identification of specific brain-body communication pathways can effectively turn into tangible therapeutic opportunities. Interestingly, these or similar medications are also showing potential for treating other conditions, including inflammatory diseases and addiction.

New Methods: We always aim to continually improve or create new experimental techniques, models, and analytical tools to further advance both science and animal welfare.

Publications: We plan to share our findings in scientific journals and present them at conferences, making sure that other researchers can learn from our work and build on it.

Collaboration and Shared Data: Our work will encourage collaboration with other research groups, and we will contribute to shared databases that other researchers can use.

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

The outputs from this project will benefit multiple groups and areas of research, both in the short term and over the long term:

Scientific Community (Short and Long-Term): New knowledge and research tools will immediately contribute to the understanding of how the brain, immune system, and metabolism interact. Researchers studying related fields, such as neurobiology, immunology, endocrinology, and metabolism will be able to access to and build on our findings. Improved experimental techniques and refined model could potentially set new standards that other labs can adopt.

Medical and Health Research (Medium to Long-Term): By identifying new therapeutic targets, this research could pave the way for new drugs or therapies that manage conditions like obesity, diabetes, and chronic inflammation, ultimately improving both human and animal health. While it may take time to translate findings into clinical applications, fundamental research like this is an essential first step in developing novel therapies.

Public Health and Healthcare Systems (Long-Term): In the long run, as new treatments or preventive strategies emerge from this research, public health could benefit from better management of metabolic and inflammatory diseases. This could reduce the overall societal and economic burden of these conditions, leading to improved quality of life and lower healthcare costs.

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

We aim to maximize the outputs of this work through multiple channels:

Dissemination of Knowledge: We will publish our findings in peer-reviewed, open-access journals to ensure they are widely available, including to the general public. When possible, we will use open-access repositories and pre-print servers to expedite sharing, including negative or inconclusive results to help avoiding unnecessary duplication.

Communication: Our research will be presented at national and international conferences and shared through public engagement platforms and events,. We usually collaborate with our Institution’s press

office to further promote our findings to the wider public. Outreach activities will raise awareness about the importance of metabolic and inflammatory diseases and the science behind them. This may spark more interest in health and correct lifestyles, but also encourage future generations to pursue scientific careers.

Collaboration: We will continue collaborating extensively with national and international researchers, clinicians, and pharmaceutical partners to share findings, accelerate translation, and maximize the impact of the research.

Translation and Impact: Our team has access to institutional infrastructure that facilitate translation of basic research into clinical applications, supporting knowledge transfer and drug development.

Species and numbers of animals expected to be used

- Mice: 9000

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.

We are using mice because their biological systems, including metabolism, immune function, and brain-body communication, are similar to those of humans. This makes them an excellent model for understanding the fundamental processes that regulate health and disease. Mice also provide access to advanced genetic models, allowing us to target specific cells or pathways with high precision. The project mostly makes use of adult animals, but in some studies, we will use juvenile mice as tissue donors because their tissues are of optimal quality, remaining vital and functional for longer when maintained in laboratory conditions, such as in a petri dish. This approach ensures reliable and reproducible results while minimizing the number of animals required.

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

Animals in this project will undergo carefully planned procedures to study how specific pathways in the brain, nerves, or peripheral organs work together to control metabolism, and by doing so how they impact on inflammatory and infection responses.

These procedures may include injections of specialized agents, such as genetic vectors like those used in the clinic for gene therapy, to target or visualize specific cells or pathways that connect the brain with other body parts. In some cases, this will involve minor surgical procedures performed under full anesthesia to ensure precise delivery and minimize distress.

Following recovery, animals may experience controlled dietary changes or pharmacological treatments to study their effects on cellular activity and immune responses. Some animals will be exposed to

models of inflammation or infection to investigate how these pathways impact the body's ability to respond to such challenges.

The duration of experiments will vary depending on the study, ranging from a few days to several weeks. Tissues will be collected at the end of the study for detailed laboratory analyses.

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

Animals may experience minor post-surgical discomfort, temporary weight loss, or mild signs of inflammation as part of the experimental procedures. These effects are typically short-lived, with recovery occurring within a few days to two weeks, depending on the specific model used. Some animals may show brief changes in behavior, such as reduced activity or appetite, particularly in studies involving inflammation or infection. All animals are closely monitored, and care is provided as needed to ensure discomfort is minimized with use of analgesics where appropriate. If any adverse effects become more important or prolonged, humane endpoints will be applied to prevent unnecessary suffering.

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)?

Approximately 50-60% of animals in this project are expected to experience moderate severity, primarily due to surgical procedures required for precise delivery of agents to specific cells or pathways. These procedures are carefully managed to minimize discomfort, with full recovery typically occurring within a few days. The remaining animals are expected to experience minimal or mild severity. This includes animals subjected to non-surgical interventions, such as dietary changes or pharmacological treatments, which may result in temporary, minor physiological changes.

What will happen to animals used in 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?

This research focuses on how the brain, immune system, and other organs work together to control energy use and respond to challenges like diet changes, inflammation, and infections. We need to study these processes in living animals because it's impossible to fully recreate the complex signals and interactions between these systems in a lab dish or with computer models.

Mice are the best model for this research because they allow us to use special genetic tools to study specific cells and processes. Switching to a different species would require using many more animals to develop new models, which would slow down progress and may not give us the same level of detail.

While animals are necessary for this research, we are always looking for ways to reduce their use. As technology improves, we plan to include alternatives like lab-grown tissues or advanced computer models when possible. But for now, using living animals is the only way to fully understand these complex systems and achieve our research goals.

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

i) cell culture with in vitro derived primary cells, or cell lines.

ii) in silico (e.g. computational) analyses or meta-analyses of publicly available.

iii) parallel studies utilizing human materials.

Why were they not suitable?

Due to the complex interactions between the brain, immune system, and metabolism, these alternatives cannot fully replace the use of animals.

While cell lines can provide useful insights into basic cellular processes, they are not suitable for studying the complex, whole-body interactions that are central to our research. For example, the signaling between the brain and peripheral organs, such as those that regulate metabolism and immune responses, cannot be replicated in isolated cell lines. That said, we do plan to use cell cultures in specific experiments to minimize animal use.

We will make use of computational tools and meta-analyses of publicly available data to predict certain biological outcomes and refine experimental designs. While these approaches help in hypothesis generation and model testing, they cannot fully replace live animal studies, particularly when studying dynamic systems like behaviour, neuro-endocrine, and neuro-immune signaling. However, we will continue to explore and incorporate these methods as technology advances.

While we will consider future human-based studies, it will still not be possible to perform detailed mechanistic analyses in humans with the level of precision that contemporary genetic technologies allow in transgenic mice. These approaches enable us to selectively manipulate specific neuronal and immune pathways with unprecedented accuracy, which is essential for understanding the fundamental mechanisms of brain-body communication. Human studies will be considered once key mechanisms are identified, but they cannot afford the level of selectivity required for these initial mechanistic studies.

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 majority of animals we'll use in this project will be bred specifically to have certain genetic traits, while some will be standard animals purchased from trusted suppliers. Estimating the exact number of animals needed over a five-year project can be challenging. This is because animal numbers can vary depending on each study's needs, the distribution of different traits in the offspring, and the fact that some animals won't have the exact genetic traits needed for experiments. Additionally, the number of animals required may change if new funding or team members join the project.

To estimate the number of genetically altered animals needed, we used data from past projects, including experience with previous project licenses. This includes average numbers of breeding groups, typical offspring produced, and the frequency of breeding cycles. We also included the expected numbers of animals without the needed genetic traits that are part of the breeding process. These estimates were then checked against the requirements of each experiment in this project.

For other experiments, we estimated the number of animals based on past experience with similar studies. This approach helps us be as accurate as possible while making sure we only use the minimum number of animals necessary.

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

To minimize the number of animals needed, we worked with a statistician during the project's design phase. They helped us assess experimental sensitivity and optimal group sizes based on typical readouts, and we will use this approach across our studies. For example, in cases where we can predict the effect's direction (i.e., whether it will increase or decrease), we can plan experiments with fewer animals while still achieving reliable results. Predictable, directional effects enable us to design studies using statistical tests, which generally require fewer animals to detect meaningful changes.

We will also use tools like the NC3Rs Experimental Design Assistant throughout the project to ensure each experiment is precisely scaled to its intended readouts. Existing data from our lab and public databases will inform these calculations wherever possible. For new or exploratory experiments with unfamiliar genetic models or novel combinations of procedures, we will start with small pilot studies to gauge effects and optimize design for future work.

Furthermore, by pooling data from related experiments and assessing results in sequence, we maximize the insights gained per study, ensuring scientific rigor while reducing the overall number of animals needed.

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

To ensure that the number of animals used in this project is kept to a minimum, we will take several measures:

- Statistical Analysis: Before each experiment, we will perform statistical analysis to determine the minimum number of animals required for valid, informative results. We will also work together with biostatisticians employed by the Establishment to assist with biostatistics.
- Within-Subject Design: Whenever possible, we will use a within-subject design, where each mouse serves as its own control, reducing the number of animals needed.
- Use of the latest technology: We always try to implement the latest genetic technologies available, which given their precision and selectivity significantly reduce the number of animals compared to other methods.
- Breeding Practices: Where wild-type littermates are not needed, mice will be bred so that they inherit the same genetic variant from both parents, provided this does not result in harmful phenotypes. This approach increases the number of usable animals from each breeding cycle.
- Pre-Screening Substances: We will pre-screen substances in vitro using established cell viability assays to determine effective doses, reducing the number of animals required for dose-finding studies.
- Sharing Samples and Data: Biological samples and data will be shared with other scientists to avoid unnecessary animal breeding for similar experiments.
- Avoiding Duplication: To prevent unnecessary duplication of studies, we will regularly review the literature and attend scientific conferences to stay informed about current research.

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.

For this project, we employ a range of carefully selected models to explore metabolic, immune, and neuroendocrine functions while minimizing pain, suffering, or distress to the animals.

Primarily, we use genetically altered mouse models that allow precise control over specific genes related to metabolism, immune function, and brain-body communication. This targeted approach focuses only on relevant cells or pathways, reducing the need for invasive techniques.

We utilize advanced genetic tools to visualize, activate, or inhibit specific cell populations in a highly controlled manner. These methods enable selective manipulation without broader pharmacological

impacts, minimizing systemic effects and additional stress. For instance, optogenetic tools allow us to manipulate neuronal activity precisely in targeted brain regions using very narrow beams of light, thereby enhancing accuracy, reducing discomfort and unwanted off target effects.

We focus on using non-invasive or minimally invasive techniques whenever possible. For example, we use telemetry, which allows us to monitor things like heart rate and body temperature without disturbing the animals. We also use special home cages that track food intake and activity in a way that feels natural to the animals. Mice are typically housed in these cages for no longer than two months. When behavioral observations aren't needed, we carry out experiments under deep anesthesia, so the animals don't feel anything.

When studying infections and inflammation, we create controlled conditions that help us understand how the body's metabolism and immune system work together. This includes mild, reversible infections like the flu (influenza) or certain bacteria (like Citrobacter rodentium), which are carefully managed to ensure animals can fully recover.

To study inflammation without causing actual infections, we use safe, well-known methods. For example, we use a substance called DSS to create temporary gut inflammation, similar to what happens in conditions like colitis. We also use small doses of a bacterial component called LPS, which triggers an immune response without causing an infection.

In our cardiovascular studies, we specifically focus on models critical for understanding links between metabolic and cardiovascular health. These models mimic human cardiovascular conditions associated with obesity and metabolic disease. For instance, we use a gene therapy-like approach to mimic the progression of plaque buildup, as it would be seen in human atherosclerosis, carefully monitoring plaque formation and stability. This allows us to observe cardiovascular risk factors as they relate to brain-body communication and metabolic dysfunction.

Importantly, all infections and inflammatory conditions we study cause no more than moderate, short-term effects on the animals' well-being. We closely monitor them throughout the experiments to ensure their comfort, and they recover fully after the studies are complete.

In some cases, we will use behavioral tests in mice that involve mazes or specially designed cages where mice can receive a food reward for performing simple actions, such as nose poking or pressing a button. These tests help us assess their cognitive abilities and motivation levels, as well as how these change in response to our experimental interventions.

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

Using less sentient animals or immature life stages does not allow us to fully capture the complex neuroendocrine and neuroimmune interactions our research requires. The metabolic and immune responses we study, such as appetite regulation, systemic inflammation, and energy balance, develop fully only in mature mammals, making adult mice the most suitable model.

Wherever possible, we do use terminally anesthetized animals for experimental procedures that don't require behavioral data, such as specific neural recordings or tissue sampling. However, because many of our experiments depend on observing real-time behavior and physiology in a whole, behaving organism, we must rely on conscious animals to gather accurate and translatable insights.

Additionally, the long-term nature of many experiments, the need to study responses over multiple time points, and the requirement for sequential experimental steps prevent the use of terminally anesthetized animals for all experiments.

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

To protect animal welfare in our studies, we are taking extra steps across five main areas:

Improved Monitoring and Care: Animals will be closely watched, especially after any procedures that could cause stress or discomfort. If we notice signs of discomfort, like weight loss or dehydration, we can provide fluids or place soft food nearby to help them recover quickly.

Pain Relief and Aftercare: Animals that undergo surgery or other procedures will receive pain relief as needed. We work with veterinarians to ensure each animal's pain is well managed.

Reducing Stress Through Acclimation and Training: Where possible, animals will be introduced to specific equipment (like metabolic monitoring cages) before starting the experiments. This familiarization helps them feel more comfortable, reducing stress and leading to better results.

Non-Invasive Techniques: In some cases, we use methods that don't require physical handling, like monitoring devices that work remotely. This reduces the need for handling and helps keep animals at ease.

Using Terminal Anesthesia: Whenever feasible, we prioritize conducting experiments under terminal anesthesia, especially for procedures like recording neuronal activity where anesthesia won't impact function, and behavior isn't part of the experiment. This approach ensures animals experience no discomfort during the procedure.

Continuous Learning and Facility Collaboration: We work closely with facility experts to keep updated on the latest research and techniques. For example, using tunnel handling instead of direct contact has shown to reduce animal stress, and we adopt such improvements whenever possible. We also keep informed about new technologies and methods that could improve both scientific outcomes and animal care, adding these whenever possible to refine and reduce procedures.

By integrating these practices, we aim to maintain the highest standards of welfare, ensuring animals experience minimal discomfort while producing reliable and meaningful scientific data.

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

To ensure experiments are conducted in the most refined way, our lab follows established best practice guidelines from reputable sources such as Morton et al. (*Lab Anim*, 2011) and regularly consults the NC3Rs resources page (https://www.nc3rs.org.uk/3rs-resources) for the latest in 3Rs advancements. We also watch instructional videos showcasing best practices in techniques to stay current with refinements.

For the specific experimental models in this project, we review published studies from laboratories across the world using similar methods and engage with colleagues at local, national, and international levels to discuss potential improvements and refinements. Furthermore, our NTCOs and NIOs regularly provide updates and suggest refinements, helping us incorporate the latest standards into our work.

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

We stay routinely informed about advances in the 3Rs through internal newsletters, updates on the NC3Rs website, publications, and by attending seminars and workshops from organizations such as the NC3Rs. We use refined handling methods to reduce stress in rodents and provide environmental enrichment as standard practice.

Additionally, we maintain regular communication with our Named Veterinary Surgeon (NVS), Named Animal Care and Welfare Officer (NACWO), Named Information Officer (NIO), NC3R champions and other named staff, who advise us on facility-specific refinements. We actively discuss potential refinements in experimental approaches based on our ongoing research to implement these advancements effectively throughout the project.