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NON-TECHNICAL SUMMARY

Mapping and controlling neural circuits

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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Neuroengineering, Neuroscience

Animal types	Life stages
Mice	adult, embryo, pregnant, juvenile, neonate, aged

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 main aim is to develop new tools and techniques that help us understand how the brain is altered in disease and help to correct those alterations. Ultimately, these tools can eventually be applied to diagnose and treat brain disorders.

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 most important discoveries that have been made recently in neuroscience have relied on new tools for analyzing and controlling the nervous system. For example, most of the neuroscientists at our institution rely on optogenetics, a system for controlling the activity of brain cells, in order to understand the effects that individual brain cells have on animal behavior. Most neuroscientists also rely on the use of specialized proteins called calcium indicators, which can tell neuroscientists when individual brain cells are active. These tools (calcium indicators and optogenetics) were both developed by neuroengineers like myself. In contrast to most areas of neuroscience, neuroengineers do not typically seek to understand how the brain works, but instead seek to build tools that will help other neuroscientists understand how the brain works. The tools that we develop lay the groundwork for the next generation of neuroscience.

In all cases, we thoroughly test new tools in cultured cells or in computational systems before using them on animals. However, our goal is to develop tools that can be used by neuroscientists to better understand the mammalian brain, or that can be used in humans for therapeutic purposes. For that reason, it is critically important that new tools be tested on animals to determine whether or not they work for their intended purpose. The protocols described in this license application are intended to allow us to test new tools in animals, before deploying them widely for other neurosciencists to use.

In the long run, the experiments we are doing will have a major impact on neuroscience and the treatment of neuropsychiatric disease. Some of the tools we are developing will enable neuroscientists to study the brain at a higher level of detail, revealing new aspects of how diseases affect the brain. In addition, we are also developing tools for controlling brain activity, which may eventually be used directly for therapeutic purposes. For example, we are exploring new ways to diagnose neurodegenerative diseases such as Alzheimer's disease, and are building new gene therapies for debilitating psychiatric and neurological disorders such as epilepsy. The use of animal models allows us to evaluate the safety and efficacy of these tools before they would ever be used in humans.

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

This project will advance our understanding of how networks of neurons are organized within the brain, how cell types are distributed throughout the brain, and how neuronal activity is organized within the brain. Moreover, this project will produce new technologies aimed at controlling the organization and

activity of brains. This knowledge will be disseminated through presentations at scientific conferences and peer-reviewed publications. In addition, any new techniques, reagents, or software tools generated as a part of this project that have potential to impact clinical or broader scientific practice will be disseminated either freely, through partnership with an appropriate company, or through commercialization in a startup company.

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

Our goal is to produce new technologies that lead to major advances in the diagnosis and treatment of brain disorders, including psychological disorders such as schizophrenia and depression; neurological disorders such as epilepsy; and neurodegenerative disorders such as Parkinson's and Alzheimer's. We will achieve impact in these areas both directly, through the creation of technologies that can be applied for therapeutic purposes in humans; and indirectly, through the creation of technologies that allow researchers to better understand the effect of these diseases on the brain, thereby allowing them to develop newer therapies.

The methods we develop with direct therapeutic potential will include new techniques for delivering gene therapies to the brain as a way of producing gene-replacement therapies for debilitating diseases such as Fragile X. In addition, we will produce new methods for inhibiting gene expression in specific cell-types, for example to allow us to knockdown expression of genes that cause disease such as the Huntingtin gene in Huntington's disease.

On the other hand, some of the indirect methods we will generate will include methods for characterizing brain circuits and the distribution of cell types throughout the brain, thus allowing us to observe how tissues are affected by disease.

Our goal is ultimately to make our technologies available as widely as possible, so scientists, doctors, and patients can all benefit from them.

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

Whenever possible, we will seek to maximise the utility of data and tools generated as a part of this project through collaboration with other experimental or theoretical groups, and online distribution of raw data and software tools. We will also make use of preprint platforms, such as bioRxiv, to rapidly disseminate our findings to a broad audience.

Species and numbers of animals expected to be used

- Mice: 27150

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 developing many different kinds of tools, all aimed at recording or manipulating different properties of the nervous system. The overall organization of the brain and the function of cells in the brain is similar across mammals. Therefore, insights gained through studying the effect of molecular tools on the structure and function of the mouse brain will help understand what effects these tools would have on the human brain. In addition, the availability of genetically altered mice makes it possible to measure and manipulate neural circuits with unprecedented precision.

Most experiments will be conducted in adult mice. In the case of some tools that are aimed at recording properties of the nervous system during development, or that are aimed at manipulating neural development (e.g., gene therapies that could be used as interventions for neurodevelopmental disorders), we will use neonatal animals. In addition, mouse embryos or oocytes will be used when required for the development and maintenance of genetically altered mouse lines.

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

Typically, animals will be subjected to a single surgery under suitable anesthesia and analgesia with post-operative follow-up. During the surgery, a molecular tool we have developed (such as a gene therapy) will be injected into the brain, but these tools are not expected to have adverse effects. Animals will typically then be allowed to recover for an extended period of time, unless they exhibit adverse effects requiring early termination, or must be terminated early for scientific reasons. Rarely, animals will be subjected to a second procedure, under appropriate anesthetic and analgesia, in which a chemical will be injected to stimulate neural activity or changes in neural connectivity. Finally, animals will be terminated and tissue will be collected for further investigation.

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

Pain resulting from surgical procedures may reach moderate severity for short periods of time immediately following surgery. Animals will be closely monitored for signs of pain after surgery and appropriate analgesia will be provided.

Head fixation is expected to result in only mild stress during initial habituation to the experimental apparatus.

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)?

16350 mice at mild severity.

10800 mice at moderate severity.

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?

We are still far from understanding the effects of different neuroscientific research tools on the brain. For example, we have previously tried to develop research tools in cell culture, and have found that the levels of gene expression in cultured cells can be very different from the levels of gene expression in the mouse brain. Many other features of biology cannot be satisfactorily replicated in *in vitro* systems: for example, the blood-brain-barrier is a major barrier to the development of brain therapies, and despite several advances there are not yet satisfactory models for the blood-brain-barrier *in vitro*. Since our ultimate goal is to create tools that can be used to develop new therapies and diagnostics for use in humans, it is important that we use model systems that are as close as possible to human biology, hence the need for animals.

We will focus on mice, which are genetically tractable allowing highly precise measurements and manipulations of neural circuits and the development of disease models, and whose brains are organized according to similar principles to humans.

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

Computer simulations

Brain organoids

Stem-cell-derived model systems

Why were they not suitable?

Often, when tools fail, they fail because of some element of the underlying biology that is not yet understood. Elements of biology that are not understood cannot be modeled in computer simulations. This is why, for example, even simple organisms such as worms and flies still cannot be simulated in the computer faithfully.

Organoids can reproduce many of the aspects of the brain, but still differ in ways that are important and often unknown. Moreover, brain organoids lack normal sensory inputs or outputs, and as a result the connectivity and activity of neural networks present in organoids may differ substantially from the networks present in animal brains.

Finally, stem-cell-derived model systems are excellent for studying aspects of cellular biology, and we expect to use such systems widely. However, they are not effective for studying aspects of the nervous system such as connectivity or activity for the same reason as organoids.

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 numbers of animals are estimated based on our prior experience breeding genetically altered mouse lines and with experimental approaches used in the project.

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

We will always ensure that we use the minimum number of animals possible to achieve our research goals. We will conduct pilot studies and adopt new methods as they become available, to ensure that we are always extracting the most information possible from any particular experiment or animal. In addition, we will develop better analysis methods and experimental design to ensure that we achieve maximal power and minimal variability.

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

We will always conduct pilot studies to test the efficiency and applicability of new tools and techniques before employing them broadly to pursue the scientific objectives of the project. We will use computer modeling and first-principles modeling to refine engineering designs and hypotheses. Whenever possible, brain tissue and experimental data will be shared between researchers or will be used for multiple experiments or analyses.

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.

This project will exclusively use mice. Surgical procedures will be conducted under deep anaesthesia following aseptic technique and mice will be carefully monitored following surgery to ensure complete and uneventful recovery.

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

For projects that have direct clinical application, it is very important to use models that are as close to humans as possible: for example, the adeno-associated viruses that we are experimenting with only infect mammals, and previous studies involving adeno-associated viruses have been found to be invalid because they were used in animals that were not sufficiently similar to humans. Carrying out experiments in this project solely in terminally anaesthetized animals is not feasible since many of our experiments require gene expression, which takes place over the course of several weeks.

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

We will ensure always to use appropriate analgesia and anesthesia, with close monitoring following surgery. We will habituate mice gradually to any new apparatus in order to minimize stress, for example in the case of head fixation.

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

We will follow the guidelines set out in LASA Guiding Principles on Preparing for and Undertaking Aseptic Surgery.

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

We will utilize online resources such as the NC3RS and RSPCA, and will work with the staff of the biological research facility, such as the NIO, NACWO, and NVS, in order to improve and refine our procedures and adopt new techniques as they become available.