Home Office

NON-TECHNICAL SUMMARY

Circuit mechanisms of spatial learning and decision-making

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

brain, learning and memory, synaptic plasticity, neurodevelopmental disorder, neurodegenerative disorder

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult, Aged animal

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 understand how the brain represents and stores information about an environment and uses this information for navigation and decision making. A secondary aim is to understand how these mechanisms can go awry in human 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?

Understanding how we learn and remember has fascinated scientists for hundreds of years. Learning and memory are fundamental properties of the brain. They enable animals to adapt to their environments. Memory is also essential for our identities as humans. It is believed that learning and memory are mediated by changes in the connections between cells in the brain. However, how these changes affect behaviour is poorly understood. This work aims to identify how cell activity induces changes in the connections between cells. Conversely, we will identify how these changes in connections affect activity. Human brain disorders that affect memory have devastating effects on the individual as well as the society. As such, insight into memory processes is important not only for understanding animal behaviour but also for the treatment of learning disabilities and memory disorders in humans.

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

We expect to generate a better understanding of how we learn and remember. We will also understand better what goes wrong in human brain disorders that affect learning and memory, including neurodevelopmental disorders in children and dementia in the elderly. This new information will be published in peer-reviewed journal articles. We will also share the data with other researchers, so they can analyse our results in more detail.

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

The short-term benefit of this programme of work is knowledge generation. This will inform other researchers, clinicians and engineers, as well as students. A medium-term benefit could be to inspire new algorithms for computer scientists for use in artificial intelligence and robots. A longer-term benefit is better understanding of how defects in learning and memory mechanisms lead to disease. The insights gained could lead to new strategies for treating such disorders, including learning disabilities and Alzheimer's disease. However, this is not likely to be realised until after the project has been completed.

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

We will aim to maximise the outputs of the work by collaborating with other scientists, including investigators working on human brain disorders in the clinic. We will also work with computational neuroscientists, who could help interpret the experimental results and make predictions that could be tested experimentally. We will publish in open-access journals. We may also publish unsuccessful outcomes on platforms such as F1000 Research. In addition, our research group is regularly involved in outreach activity to share our results with the general public.

Species and numbers of animals expected to be used

- Mice: 10,000

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 have chosen the mouse as the experimental species for three main reasons. First, mice are evolutionarily quite close to humans. This makes our research findings relevant to mechanisms in the human brain. Second, mice are widely used to study brain mechanisms of learning and memory. Thus, we can build on previous results obtained by other investigators. Third, there are techniques available that can modify genes in mice. This genetic technology is powerful in addressing our scientific questions. We also want to find out what goes wrong with learning and memory in developmental disorders of the brain. In particular, we will use a mouse model of Rett syndrome, a rare genetic neurodevelopmental disorder primarily affecting girls, that impairs brain development causing loss of speech, motor skills, and purposeful hand use, in order to develop better treatments. In addition, we will study mouse models of Alzheimer's disease, the most common cause of dementia. Therefore, we need to do experiments at all life stages, from embryos through new-borns to adolescent, adult and ageing mice.

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

The majority of animals will be used for breeding of genetically altered mice. A relatively high number is necessary because several generations of mice are often required to generate the mouse lines which we will use experimentally. During breeding the animals would express their natural behaviour. Some of these animals may show mild signs of brain disease, such as problems with learning and memory. Very few animals will show other problems, such as abnormal gait, hindlimb clasping and occasional seizures in a mouse model of Rett syndrome.

Most of our experiments will be done on isolated brain tissue from mice that have been humanely killed.

A smaller number of experiments will involve brain surgery on animals that have been anaesthetised. This is to introduce genes locally in the brain; to make tiny brain incisions; to insert electrodes, light

fibres, or tubes to deliver drugs into the brain; or to mount a bracket, which would later be used to keep its head still. At most two such surgeries would be made in an animal. Prior to surgery, the animal would be given medication to remove pain, either as a local cream or via an injection; during surgery, the animal would be under general anaesthesia; and after surgery, the animal would be carefully monitored during recovery to assess and treat any pain.

After surgery, we would prepare brain tissue or record activity in the brain during awake behaviour or sleep.

Sometimes we would record while the animal performs simple memory tasks for food or water reward, after the animal has been made slightly hungry and/or thirsty. This is to be able to find out whether different motivations change the way an animal learns and remembers. The animal may be on food restriction for up to 28 days while the memory tasks take place, being given 80-90% of its normal food consumption each day, while its weight is monitored to ensure that it does not fall below 85% of its free-feeding weight. The animal may be without water for up to 4 hours each day. The memory tasks may take up to four weeks, with testing every day.

To investigate possible treatment of disease by targeting specific brain cells, we would use a technique known as chemogenetics. This technique introduces artificial drug receptors in some brain cells and we would test the effect of otherwise inert drugs designed to activate only these receptors.

We will always strive to use the least harmful methods to achieve the results required to answer our scientific questions.

For each of the protocols in the project, a typical experience of the animal would be:

Protocol 1: Superovulation

Female mice will typically experience two injections, approximately 48 hours apart, followed by mating.

Protocol 2: Embryo recipients

Female mice will be mated with a sterile male and embryos implanted under general anaesthesia.

Protocol 3: Vasectomy

After surgery, the vasectomised male will be mated with female mice until they are too large or too old, when they will be humanely killed.

Protocol 4: Breeding and maintenance of genetically altered mice

Animals will be used for mating. Offspring will be genetically altered but without clinical signs of disease.

Protocol 5: Maintenance and behaviour of genetically altered Mecp2 mutant mice

This is a mouse model of Rett syndrome. Female mice will have few clinical signs. Half of male mice will show movement problems by 5 weeks of age and all of them by 10 weeks of age. All male mice will be humanely killed before the age of 15 weeks.

Protocol 6: Tissue collection from wild-type mice

Animals will be put to sleep.

Protocol 7: Tissue collection preceded by neonatal intracranial injection

Animals will have a substance injected into the brain one or two days after birth, and several weeks later be put to sleep.

Protocol 8: Tissue collection preceded by recovery surgery

Animals will have a substance injected into the brain under general anaesthesia, and several weeks later be put to sleep.

Protocol 9: In vivo neural recording

Animals will be put to sleep while recorded from.

Protocol 10: In vivo neural recording preceded by neonatal intracranial injection

Animals will have a substance injected into the brain one or two days after birth, and several weeks later be put to sleep while recorded from.

Protocol 11: In vivo neural recording preceded by recovery surgery

Animals will have a substance injected into the brain under general anaesthesia, and several weeks later be put to sleep while recorded from.

Protocol 12: In vivo behaviour

Animals would have limited access to food and/or water so they are motivated to find food and/or water on a maze. They may experience weight loss during the testing period over a maximum of 28 days. They could experience water withdrawal for up to 4 hours each day.

Protocol 13: In vivo behaviour and neural recording

An animal would typically undergo one brain surgery to install optical fibres and a miniature camera. Following recovery, they would have limited access to food and/or water so they are motivated to find food and/or water on a maze while being recorded. They may experience weight loss during the testing period over a maximum of 28 days. They could experience water withdrawal for up to 4 hours each day.

Protocol 14: In vivo behaviour and neural recording during head-restraint

Animals would experience two surgeries under general anaesthesia. Following recovery from the second surgery animals would be water-restricted up to 4 hours prior to each training and recording session but have access to water during and following each session.

Protocol 15: Mouse ageing

Animals would typically undergo normal ageing in wild-type mice or accelerated ageing in dementia mouse models.

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

Most animals will not experience any adverse effects.

Some animals serve as models of neurodevelopmental and neurodegenerative disorders. They will show mild behavioural changes, such as learning deficits or mild motor impairment. Mice that show more than mild motor impairment will be humanely killed. In order to test a possible treatment effect, we would follow a few animals to a moderate level of motor impairment, such as abnormal gait and hindlimb clasping, or epileptic seizures. Any animal will be immediately humanely killed if it shows signs of suffering that exceeds those expected for the animal model.

Some animals will undergo a surgical procedure. Surgery will be done under anaesthesia and may inflict pain and distress, which we will aim to reduce with pain management. All these animals are expected to make a rapid and unremarkable recovery from the anaesthetic within two hours.

Some animals will be food restricted to achieve 85% of free-feeding weight for a period of up to 28 days and/or experience periods up to 4 hours without access to water before behavioural tests each day for up to 28 days. These animals will be hungry and/or thirsty and be motivated to seek food and/or water. We would only make the animals sufficiently hungry and/or thirsty to make the animals search for food an/or water, and this should have no long-lasting effect on the animals. The tests would take place no more than twice per day for up to 28 days and water restriction limited to a maximum of one time per 24 hours.

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)?

The severity limits of the protocols in this project are Non-recovery, Mild and Moderate, and the proportion of animals in each category is:

Mice:

Non-recovery ~10%

Mild ~80%

Moderate ~10%

What will happen to animals used in this project?

- Killed
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse
- 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?

The use of animals is necessary in this project, as there are no other experimental models that can adequately model the complexity of the nervous system, including intricate cellular interactions, neural circuit operations, and emergent behaviours.

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

We will complement our animal studies with models on computer, but the complexity of the brain cannot yet be fully replicated by computer simulations.

We will also complement our studies using cultured human neurons, which may better reflect mechanisms of human disorder, but lack the systemic complexity of living organisms.

Why were they not suitable?

They cannot yet replicate the full range of neural interactions, behavioural outputs, or physiological responses observed in intact animal models.

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 estimate is based on previous experience of breeding and animal experiments. The majority of the estimated number is related to the breeding programme of genetically altered animals (9,000). From these animals we will be collecting tissues for use in experiments in the laboratory, thus reducing the need for experiments in live animals. The remainder would be experiments under anaesthesia for collection of brain tissue. At most 1,250 surgeries would be made in which the animals would wake up again following surgery.

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

Our research is fundamental discovery science. As such the research proceeds in three stages. First, we notice an unexpected result in our data, which gives us new insight and ideas about learning and memory processes. Second, we articulate and develop those ideas to generate a research question. Third, we verify our insight by addressing the research question in experiments. It is very difficult to know in advance exactly how many animals would be needed to address our research questions, but statistics will help us to balance the number of animals used and the confidence we can have that the answers we obtain are correct.

Using statistics, it is possible to gauge in advance how many animals would be required to answer a specific research question. This is important in order to know how likely it is that other investigators would reach the same conclusion if they carried out the same or similar experiment. Typically, we would need at least 20 animals in each of two groups to be reasonably safe to detect a difference between the two groups.

The most important way in which we reduce the number of experiments conducted on live animals, however, is to do experiments in sections prepared from brains of both genetically altered and wildtype animals immediately after they have been humanely killed. These tissue sections are then used to investigate the mechanisms for the phenomena we observe in intact animals during behavioural learning and memory.

We will consider using NC3R's Experimental Design Assistant (https://www.nc3rs.org.uk/experimental-design-assistant-eda) when appropriate for our behavioural experiments.

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

To reduce the number of animals in the breeding programme:

a) Efficient breeding: We will use a breeding programme that maximises offspring with the required genes.

b) Use of spare animals: We will use animals without the required genes in other experiments, for example to prepare brain tissue.

c) Collaboration: We will share the genetically altered animals with other laboratories.

To reduce the number of animals used in experiments:

a) Pilot studies: We will do studies with a small number of animals in order to plan a more conclusive study.

b) Computer modelling: We will use simulations on computer to better understand our experimental observations.

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 will use mice to study mechanisms of learning and memory in the brain. In order to record and control activity in the brain, we will use a technique called 'optogenetics'. This technique allows us to label specific cell types, record their activity using miniature microscopes, and stimulate or silence them while an animal learns a behavioural task. This technique will also enable us to target the same cell types in brain tissue taken from the animal after it has been humanely killed and in nerve cells grown in cell culture. Optogenetics is both more specific than conventional electrical stimulation and less destructive than conventional lesioning techniques, and opens up for entirely new types of experiments to study the causal relationship between neural activity and behaviour.

Some animals will be genetically altered to model human brain disease. Most of these animals will show only mild signs of disease. In a mouse model of Rett syndrome, in which a faulty gene can cause movement problems and epileptic seizures, most animals will be killed before they develop clinical signs, but a very small number of animals will be followed to a moderate degree of impairment, including abnormal gait, hindlimb clasping, tremors, and reduced coordination to allow tests of possible treatments.

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

We will use cell culture prepared from baby mice and brain tissue from mice of any age to study neural mechanisms. However, mechanisms involved in natural learning and memory can only be studied in awake behaving animals. The mouse is the least sentient model species that display memory mechanisms of proven relevance to human memory.

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

We will monitor animals carefully in all breeding procedures. For animals with progressive disease, we will only use young breeders, and replace breeders if they show signs of disease, for example movement problems in Rett syndrome.

We will follow the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2nd edition, 2017) for all surgical procedures in adult animals and ensure appropriate pain management. To assist with animal welfare, we will use monitoring and observation charts to follow the recovery of each animal. Recovery is expected to be uneventful, but the monitoring would enable us to humanely kill the animal if the animal is suffering.

All surgery will take place under general anaesthesia and additional pain suppressing medication will be given both before and after surgery. Introduction of substances into the brain of newborn mice will also be done under anaesthesia. To ensure pups are not rejected by their mother following surgery in

newborn mice, we will minimize the time the pups are away from their mother and a small amount of their nest bedding will be rubbed over the pups to cover any foreign surgical, adhesive, or human smells before they are returned to the mother.

The investigators who study mouse behaviour will be trained for this purpose and we will use rewards, rather than punishments, to encourage learning in behavioural tasks. All animals will be gradually acclimatised to the behavioural apparatus. Some animals will have a small bracket attached to their head. This will be used to restrain their heads in some of the experiments while mounting a recording device.

To reduce the inescapable harms arising from using animals in research, we have considered the following points:

1. To reduce the transportation of animals, we will import the minimum number of breeding pairs and continue a breeding programme in house. We will consider importing frozen embryos instead of adult animals when possible.

2. To the extent possible, we will co-house animals. In rare cases, we have to single-house animals because of sensitive implantation devices but, even in those cases, we will try to keep them at least in pairs.

3. To the extent possible, we will improve the husbandry of mice in behavioural experiments by using enrichment cages where mice have access to two floors, running wheels, and cardboard houses and tunnels and where they can be housed up to ten in each cage.

4. To reduce the number of animals required for breeding we have moved the breeding procedures to an animal facility with a lower mouse pup mortality than at the one we used previously.

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

In planning experiments, we will consider the PREPARE Guidelines (Smith et al., Laboratory Animals, 2017) and the RSPCA and LASA, 2015, Guiding Principles on Good Practice for Animal Welfare and Ethical Review Bodies. A report by the RSPCA Research Animals Department and LASA Education, Training and Ethics Section. (M. Jennings ed.). We will consult position papers from LASA (https://www.lasa.co.uk/current_publications/). Specifically, for surgeries, we will follow the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2nd edition, 2017). We will be guided by the updated ARRIVE Guidelines 2.0 (Percie du Sert et al., PLoS Biol 2020) when reporting results from our research.

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

We will seek information at the web pages of NC3Rs (https://www.nc3rs.org.uk), LASA (https://www.lasa.co.uk), and Norecopa (https://norecopa.no). Moreover, our institution's web pages provide regular updates on new developments in the 3Rs. We will consider these advances carefully to

see whether they can be implemented effectively in this project. We will also engage with the Named Information Officer for new 3Rs information.