Home Office

NON-TECHNICAL SUMMARY

Circuit mechanisms of learning and memory in the mammalian brain

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, circuit mechanisms, behaviour

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, 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 overall aim of this project is to understand how brain activity leads to changes in connections between cells, and how these changes in connections can explain memory.

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. It is believed that changes in the connections between cells in the brain underpin learning and memory. 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. 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. 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?

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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 as well as students. A longer-term benefit is better understanding of how defects in learning and memory mechanisms lead to disease. In particular, we will study developmental brain disorders and neurodegenerative diseases. The new 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.

Species and numbers of animals expected to be used

- Mice: 20,000

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 quite close to humans, evolutionary speaking. 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 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. Therefore, we need to do experiments at all life stages, from embryos through new-borns to adolescent and adult 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 to be used 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 problems with movement in a mouse model of motor neuron disease with dementia.

Some 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 injuries; 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. Afterwards, 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 or thirsty. The memory tasks may take up to three weeks, with testing every day.

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

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. Any mouse that shows more than mild motor impairment will be humanely killed. Any animal will be immediately humanely killed if it shows signs of suffering that is likely to exceed those detailed for the animal model.

Some animals will undergo a surgical procedure.

Some animals will be food restricted to achieve 80% of free-feeding weight for a period of up to 28 days and/or experience short periods up to 4 hours of water restriction before behavioural tests. They would take place no more than twice per day for up to 28 days.

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 - 85%

Moderate - 5%

What will happen to animals at the end of this project?

- Killed
- Kept alive
- 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 elucidate brain mechanisms of memory.

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

We will complement our studies using human neurons. This may better reflect mechanisms of human memory disorder.

We will also complement our animal studies with models on computer to better understand how our discoveries can explain memory.

Why were they not suitable?

These non-animal alternatives can complement animal experiments but can not directly investigate the circuit mechanisms of learning and memory in the mammalian brain.

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 (17,000). 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 1000 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 measure we use to reduce the number of experiments conducted on live animals, however, is to do experiments in brain tissue collected from animals after they have been humanely killed. This is then used to understand 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 nerve cells grown in cell culture.

Some animals will be genetically altered to model human brain disease. Almost all of these animals will show only mild signs of disease. A small number might develop problems with movement in a mouse model of motor neuron disease with dementia, but will then be immediately killed.

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 mechanisms. However, mechanisms involved in natural learning and memory can only be studied in awake behaving animals.

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 muscle weakness in motor neuron disease. .

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

The investigators who study mouse behaviour will be trained for this purpose and 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. 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.

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.