Home Office

NON-TECHNICAL SUMMARY

Investigating the role of physical cues in nervous system function in wild type and transgenic animals

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

CNS development, neural disorders, mechanotransduction, mechanobiology, atomic force microscopy

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Our brain is the most complex organ in our body. How nerve cells know where to send their long extensions called 'axons' to connect to distant cells is currently still poorly understood. To investigate how the growth of nerve cells is controlled during embryonic development and after injury, we combine approaches from biophysics and engineering with state-of-the-art cell and molecular biology. We are mainly interested in how the local stiffness of the surrounding tissue regulates neuronal behaviour, and how nerve cells sense the stiffness of their environment. The long-term goal of this research is to discover new mechanisms controlling the growth of nerve cells. This is important for fundamental research and might help us to understand how the brain develops. It might also help to develop novel strategies to promote neuronal regeneration after, for example, spinal cord injuries.

The questions that we want to answer within the lifetime of this project licence are:

- What are the mechanical properties of tissue and cells in health and disease?
- What is the role of physical cues in shaping the development of the nervous system?
- Do physical forces affect the behaviour of single cells?
- Does the stiffness of the surrounding environment affect intracellular mechanisms that direct cellular functions?
- Do different pathological processes affect neural functions through changes in the physical properties of their surrounding tissue?

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.

What are the potential benefits that will derive from this project?

During the development of the nervous system, the nerve cells build long extensions (axons) which connect different areas in the brain. Only if the correct areas are connected with one another will the brain work properly. Previous work has shown that axon growth is strongly influenced by the mechanical properties of the surrounding tissue. We now aim to understand how tissue stiffness regulates neuronal function. By answering the questions above, we will contribute to a better understanding of brain development and neuronal growth. Our fundamental research may also lead to break-throughs in our understanding of different pathological processes in the nervous system such as, for example, neurodegenerative diseases or spinal cord injuries, which currently bind patients to wheelchairs for the rest of their lives.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

4760 mice

1150 rats

2650 Xenopus (frog)

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

We will need to breed and maintain genetically modified (mice, Xenopus) and non-modified animals (rats and mice, Xenopus). These protocols are expected to have minimal or no impact on the health and welfare of the animals.

In exceptional cases, a moderate impact on their health and welfare might be reached due to the nature of the genetic alteration. Specifically, in the wobbler mice used to investigate the impact of tissue stiffness on the onset and progression of a neurodegenerative disease, the degeneration of nerve cells may result in symptoms which strongly resemble amyotrophic lateral sclerosis (ALS). Clinical signs include reduced bodyweight, unsteady, wobbly gait, and reduced muscle strength of the head, neck and forelimbs. These animals will be closely monitored and humanely killed when weight loss reaches or is likely to exceed 15% of their initial weight. Furthermore, wobbler mice will be regularly assessed for both paw condition and gait impairment and be humanely killed as soon as either reaches a predefined severity level or if the mobility of more than two limbs is affected. Occasionally, wobbler mice may show eye infections due to grooming problems (< 5%). If the infection persists for more than three days despite treatment, the animal will be killed. Very rarely, wobbler mice may have seizures or show signs of laboured breathing (< 1%). These animals will be killed immediately.

We will induce superovulation in female frogs and mice. This means that we will administer a specific drug to females, which induces a stronger production of egg-cells and therefore a larger number of offspring. Minimal or no impact on the health and welfare of the animals is expected.

In order to breed some of the genetically altered mouse strains, we will need to order embryos which will be implanted into pseudo-pregnant mothers which will eventually give birth to the offspring, which is later used for breeding and maintaining the rest of the mouse colony. Since one method used requires a surgery this can affect the health and welfare of the animal. However we minimise this by using good technique and pain relief after surgery.

In order to induce a pseudo-pregnancy in a female mouse, we need to sterilize male mice that will be held with the females. This is essential because the female hormonal system changes in the presence of the male, resulting in the acceptance of the embryos in the female. Since the sterilisation method

used requires a surgery this can affect the health and welfare of the animal. However we minimise this by using good technique and pain relief after surgery.

We will use genetically altered mice as well as genetically normal (wild type) rats and mice on this licence. Where it is necessary to use immature cells, pregnant dams will be killed after they have been anaesthetised before the pups are removed from the uterus and killed for use in our experiments.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

Our work is set up to minimise the number of experiments with living animals whenever possible. We will make extensive use of cell culture when observations in the living animals will not be crucial. Unfortunately, we cannot replace our animal work completely with cell cultures (i.e. cells that are grown in dishes in the laboratory), due to the lack of available cell lines that are relevant to neuroscience studies. Our studies in living animals and in cell cultures will be accompanied by in silico models (i.e. computer-based studies and simulations that do not require animal use), which will replace some experiments, and help us make informative predictions to only pursue promising experimental conditions.

Reduction

Explain how you will assure the use of minimum numbers of animals.

Experiments will first be run as a pilot study with a minimum number of animals, taking into consideration good experimental design principles, including randomisation (random allocation of animals or tissues for experiments) and blinding (keeping researchers unaware of the experimental treatments until the study is over) to prevent potential bias of researchers and increase confidence in the conclusions of our studies. Successful pilot studies are the basis for standardising the protocol which reduces variability and mistakes, by using biostatistics approaches and principles of good experimental design. Importantly, in all our experiments we try to retain tissues for later use, and to share tissues among researchers whenever possible. Finally, in the context of frog experiments, our researchers arrange their experiments on the same days to minimise the numbers of frogs undergoing procedures as frogs lay thousands of eggs at a time.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

We will always use the animal species of the lowest possible complexity. Wherever possible, we will work with frogs, an excellent animal model for studying the development of the nervous system,

especially in the context of the optic pathway. The Xenopus optic pathway, where neurons from the retina grow along the brain to connect with the optic tectum (the analogous of the visual cortex in humans), is one of the best understood systems in the field of axon growth and guidance. Hence, much knowledge exists already, minimizing the number of required experiments. Then, we will test whether the findings which result from the frog embryo experiments can be reproduced in higher animals, we use rodents because their neurobiology is similar to humans'.

Mice and Rats will be housed in groups whenever possible. Environmental enrichment material will be provided (chew sticks and play tunnels). In order to reduce stress, non-tail capturing methods are used (cupping/picking-up the animals using a tunnel). Wobbler mice will always be housed with at least one non-wobbler littermate who can help them groom. They will be housed on soft bedding. To facilitate feeding, extra-long nipples will be provided on the water bottles and they will be served pellets from the floor.

The frogs are kept in a temperature-controlled aquatic environment. They are handled as little as possible as this induces stress in the animals. Instead of invasive methods for identification of the frogs, pictures are taken of each frog and used for identification to reduce handling-induced stress as well as inflammation and injuries. The frogs are provided with enrichment tunnels for refuge so they can display natural behaviours. The dedicated Named Animal Care & Welfare Officer (NACWO) attends and contributes to National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) events, and has provided a significant welfare improvement using visual welfare methods of diseases and clinical signs in photographs.

Breeding and maintenance are accomplished with standard husbandry practices. Pilot studies are run on a few animals at a time and discussed on a case by case basis.

Surgical procedures will be carried according to the LASA "Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017)".

Furthermore, we will plan our experiments, maintain our animals, and monitor the components of our study according to the PREPARE guidelines.

We consider and apply ARRIVE guidelines for improving the reporting of our experiments. This allows us to maximise the published information and can therefore help to reduce unnecessary studies.

Everyone working with animals will be supervised will be supervised and trained by experienced members of the lab, and when people with the required expertise are not available internally, we will ask our animal facilities or our collaborators for training and support, either directly by their staff, or by providing the appropriate contacts (e.g. using Training and competency databases).