Home Office

NON-TECHNICAL SUMMARY

Development and Homeostasis of the Enteric Nervous System

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Gut, enteric nervous system, gut-brain axis, neural stem cells, gut inflammation

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 aim is to characterise genetic, molecular and cellular mechanisms implicated in the assembly and maintenance of complex neural systems in animals. To address these questions we study the nervous system that controls digestive function and how it interacts with other tissues of the gut and the brain.

Neural networks throughout the body are receiving and processing information regarding the outside world or the internal state of the body (sensory system), are planning and co-ordinating movements (motor system), modulate the activity of various organs (autonomic nervous system) and support cognitive activity (brain). The neural system that controls gut physiology (called enteric nervous system-ENS) is critical for growth, digestion and metabolism and although far from the head, it is anatomically and functionally connected with the brain. Defects in the ENS result in severe and potentially fatal conditions and have been associated with chronic gastrointestinal disorders, such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). Despite the importance of the ENS, we still know very little about the mechanisms that control its development and organisation and how it responds to gastrointestinal disorders, such as infections and chronic inflammation. We also have a limited understanding as to how defects in the ENS and gut function affect brain activity and behaviour.

Our aim is to fill this knowledge gap by identifying pathways associated with the development and function of the ENS, examine its response to pathological conditions and understand how it communicates and affects brain activity.

Specifically, our work has two objectives:

1. To discover genetic and molecular pathways that control the development and organisation of the nervous system of the gut and

Characterise the response of this neural system to physiological changes or pathological conditions.

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?

Our work will advance fundamental biomedical science in an area that has great physiological importance but remains relatively underdeveloped. By advancing the understanding of basic and disease mechanisms, our work will promote the development of novel therapeutic strategies. All data, reagents and animal models developed by this work will be made available to the wider scientific community.

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?

Our programme of works used mice (27000) and zebrafish (9000).

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?

Prior to animal work, we carry out biochemical and cell culture experiments which, in combination with information obtained from publicly available databases, identify candidate genes that are essential for the formation and function of the nervous system. This information is then used to generate transgenic animals in which the expression of selected genes is modified. One of the main experimental approach involves histological and microscopic analysis of post-mortem tissues and physiological analysis of organs isolated from transgenic animals. Therefore, in the majority of cases, there will be no further intervention other than that required for breeding and genotyping. However, in certain cases we will need to inject substances or other agents in order to modify gene expression and mark cells. Substances will be administered by the most appropriate route, selected for the minimal invasiveness compatible with efficient delivery to the target tissue. To understand how the neural system of the gut functions and regenerates and how it interacts with the brain, we will manipulate these organs by altering the expression of genes and (in the case of gut) inducing pathology (inflammation) using chemical or infectious agents. A small number of animals may be analysed using behavioural tests. It is expected that most animals will experience none or only mild adverse effects.

Replacement

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

To understand how genes and cells control the development and function of the nervous system in the context of whole animal and in a way that is meaningful for clinical studies, it is necessary to carry out animal experiments. For example, gene function may be influenced by nutrients, oxygen, circulating hormones and other aspects of the complex physiological environment inside the body. It is not yet possible to recapitulate all of these parameters in vitro, nor to mimic the metabolic crosstalk between different populations of the ENS and gut-brain axis..

Nevertheless, our current and future research makes extensive use of alternatives to animals. In vitro cultures of mammalian cells/tissues can be used for many of our studies and are undoubtedly an important source of replacement.

Before embarking on any animal experiments, we will collect as much evidence as possible to determine whether a candidate genetic or environmental manipulation has a reasonable chance of success and is relevant to *in vivo* systems. Evidence will be collected from our own experiences and previous results as well as by surveying the mammalian and other literature. In addition, we will use non-regulated procedures to collect expression data from fixed non-GM mammalian tissues and functional/expression data from genetically and/or environmentally manipulated cell lines and/or early fish embryos.

Reduction

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

other cases, homozygotes will be generated from heterozygote intercrosses, with littermates genotyped as heterozygous or wild type used as age and gender matched controls. Whilst most (~80-90%) of the experimental work will be *ex vivo* following breeding where any physiological or other interventions are required, we expect that 5-6 animals per treatment group will usually be sufficient to obtain robust results. For most of the quantitative experiments, design will be based on ARRIVE guidelines and sample sizes may be set using power analysis, generally using a significance level of 5%, a power of 80%, and a least practicable difference between groups of 20%. Otherwise, we will use the minimum number of animals to provide an adequate description, generally on the basis of previous experience (our own, or from the literature).

This programme of work will make optimal use of several tissues, fluids and cell types per individual mouse. We will aim to collect organ samples from multiple body sites and to provide other affected tissues to appropriate scientists, so that they do not have to breed mice specifically for their experiments. This highly integrative approach will maximise the information obtained from the minimum resources. Cryopreservation of gametes, embryos, tissues and cells is routine and will ensure that the minimum number of mice is bred.

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 are using refined GA mouse and zebrafish models, employing conditional and inducible technology where appropriate.

To minimise stress during breeding and maintenance, we will follow best practice guidelines and follow local refinements of husbandry such as cage enrichment and sufficient amounts of nesting material. On receipt or generation of a new line, we will minimize suffering by ensuring increased observation and monitoring until a detailed phenotypic analysis for each line is accomplished. If any welfare implications are identified, they will be acted upon and refinements considered in consultation with the NVS and NACWO.

The majority (~95%) of animals produced under the breeding protocol are not expected to exhibit phenotypes beyond a mild classification but a small proportion may exhibit a moderate phenotype - particularly if they are modelling a human disease. However, it is not possible in all cases (such as newly generated lines) to predict fully the nature or severity of any potential defect and for that reason the limit has been set at moderate. For all types of mice, however, there will be careful monitoring of strain characteristics and the information will be collated and regularly reviewed to ensure that phenotypes to not exceed their usual features.

For all manipulations we will adhere to local or national guidelines that aim to minimize suffering. Most of the work as well as the administrations of gene inducers/repressors or other agents are standard and previous refinements from our own experience and from the literature will be used. If, however, there is insufficient information available, new manipulations will be pre-screened in small-scale pilot studies to

obtain indications of the minimum dose and exposure time that is likely to be effective, thereby minimising any potential suffering.

Unless otherwise specified, all surgical work in this project will be undertaken in accordance with the principles set out in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2010) or other such publication promoting best practice. Analgesia will be provided according to contemporary best practice and advice from the NVS/NACWO.