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NON-TECHNICAL SUMMARY

Mechanisms regulating neural stem cell activity and neuronal production

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Stem cells, Neurogenesis, Quiescence, Ageing

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

To identify mechanisms that control the activity of neural stem cells and the process of neurogenesis in the embryonic, adult and ageing brain.

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?

Neural stem cells play a central role in the generation of neurons and glial cells during brain development and in the adult brain. Defects in neural stem cell activity in the embryo has been linked to neurodevelopmental disorders and defects in adults and in particular in ageing and diseased individuals, to cognitive impairments and mood disorders. Identifying the mechanisms that control the activity of neural stem cells and the process of neurogenesis is essential to elucidate the aetiology of these disorders and understand how the brain is formed and maintained.

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

The project will result in an improvement of our understanding of the mechanisms that control the activity of neural stem cells and the process of neurogenesis, and how these mechanisms change between embryonic and postnatal development, adulthood and ageing. The outputs will take the form of publications in peer-reviewed journals and presentations at academic conferences but will also include dissemination to the lay public via popular science initiatives. Materials, data and methods will also, where appropriate, be accessible online.

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

The main beneficiaries of our research in the short and medium term will be other scientists working in the fields of brain development, brain plasticity and brain ageing. For example, scientists may investigate whether the mechanisms we uncover are deficient in neurodevelopmental pathologies (e.g. lissencephaly), neurodegenerative pathologies (e.g. Akzheimer’s disease) and mood disorders (e.g. depression). In the longer term, these studies may lead to the identification of new targets for the development of new pharmaceutical and therapeutic interventions in these pathologies.

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

We will share our data, analysis tools and resources with other scientists, using one to one interactions and collaborations, repositories and our lab webpage, which will reduce the number of replicated experiments in the field. We will disseminate our research by publishing results in peer reviewed journals. We will also present our work to academic peers at scientific conferences (national and international) and engage with public partners to disseminate our 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 will perform our study in mice because of the number of available mouse lines that are genetically suitable, the ease of generating new mutations or manipulating gene expression acutely in this species, and the comparison with previous studies in the scientific literature. Also the brain of mice is sufficiently similar to that of humans to make its study worthwhile to learn about mechanisms of brain development and function in humans. We will use embryonic, postnatal, adult and aged mice, since we are investigating the changes that occur in neural stem cell activity and neurogenesis during the lifetime.

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

Some animals will receive injections of substances to induce gene deletions or measure cell proliferation. Some animals will receive several injections with the same or several substances, at variable intervals. They will then be humanely killed at various times after these manipulations in order to study their brain tissues.

Some animals will undergo surgery where a window is opened in their skull for injection of substances into the brain to manipulate gene expression locally. Adverse effects after the surgery may reach moderate levels of severity for a short period of time, but all animals will receive pain relief and will be closely monitored until they recover completely. At variable time after this, they will be humanely killed in order to study their brain tissues.

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

Some animals will undergo surgery of moderate severity. After recovering from general anaesthesia, mice will be less active for a day or two after surgery. Animals might lose some weight but will typically regain that weight within two to three days. Some animals will also be allowed to age (up to 24 months) and as a result may experience certain discomforts associated with the ageing process. At the end of experiments, or if mice show signs of ill health, distress or suffering that are not improved or resolved within a timeframe approved by the veterinary surgeon, they will be humanely killed.

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)?

60% of mice will experience a severity category 'Mild'

40% of mice will experience a severity category 'Moderate'

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

- Killed
- 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?

Mice will be used in this project. Neural stem cells can be maintained in culture and we perform such experiments, but their behaviour in vitro is substantially different from what it is normally, as the cells in vivo are embedded in a tissue comprising multiple cell types that interact with stem cells and influence their behaviour. This stem cell niche is too complex and not sufficiently characterised to be reconstituted in culture, and in vivo experimentation in whole animals is therefore unavoidable to reach a thorough understanding of how neural stem cell behaviour is regulated.

How ageing affects neural stem cells and neurogenesis can only be studied in vivo as there is strong evidence that the ageing process, both healthy and pathological, involves interactions between brain cells and the immune and vascular systems, which again have not been reconstituted ex vivo.

The proposed studies could not be undertaken in non-mammalian species because their neural stem cells and niche environment are too different from neural stem cells in mammals and particularly humans (for example, non-mammalian species do not have restricted stem cell niches in the parts of the brain called dentate gyrus and subventricular zone as both humans and mice do).

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

This project will be done in parallel with studies using non-animal alternatives, including

Mouse Neural Stem Cell (NSC) cultures.

Human Induced pluripotent stem cells (iPSC)-derived neural cultures, including brain organoids

Human foetal brain material from the Human Developmental Biology Resource.

Why were they not suitable?

The cell culture models will provide us with useful information. Mouse NSC cultures will be used to get insights into the intracellular mechanisms (e.g. transcription factors, chromatin remodelers) that regulate neural stem cell behaviour. NSC cultures are a reductionist model that allow access to fundamental properties and constituents of neural stem cells that are maintained in this artificial setting (e.g. transcriptional and epigenetic mechanisms controlling NSC activity and quiescence). Other NSC properties differ between culture and in vivo settings, including their interactions with other niche

components (absent in culture), their behaviour (e.g. NSC divide asymmetrically in vivo but symmetrically in vitro) or population dynamics (NSC numbers and activity rates change with age in vivo while NSC cultures cannot be maintained long enough to assess these changes), and must therefore be studied in vivo. Human Induced pluripotent stem cells (iPSC)-derived neural cultures will be used to determine how much similarity or divergence exist between human and mouse neural stem cell and the mechanisms that regulate their activity. However any in vitro result will need to be validated by studies of neural stem cells in mice in vivo because the cells are substantially different in the two environments.

Moreover, in vitro experiments do not allow the study of interactions between neural stem cells and their niche environment, crucial for the regulation of neural stem cell activity, and interactions with the immune and vascular systems, crucial in brain ageing.

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 have been estimated based on previous experience when dealing with complex genetic crosses in mouse breeding as well as estimates on the number of experimental animals required based on expected effect sizes and variablity (SD) from the literature, past work, and in vitro pilot experiments.

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

Several factors will lead to a reduction of animal numbers, including reducing variation and good experimental design involving the use of appropriate statistics. In particular, statistical tests will be used to ensure that we use the minimum number of animals possible to reliably interpret our data.

We will design our mouse breeding strategies carefully to minimize the number of generations necessary to reach the desired combination of transgenes/ mutations, and to maximise the proportion of offspring carrying this combination of transgenes in the litter. Where appropriate, we will use otherwise unwanted offspring as negative controls.

We will also interrogate gene function by performing as much as possible acute manipulations of gene expression, e.g. by injecting viruses or CAS9/gRNAS to avoid the extensive breeding required to generate and maintain mouse mutant lines.

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

Efficient breeding and holding lines as frozen down embryos and sperm will be used to minimise the number of mice being produced for these studies. However, generation of surplus GA mice is sometimes difficult to avoid, e.g. when the genotype of interest is homozygote and breeding generates heterozygotes in addition to homozygotes, in numbers too large to be all used as breeders. Where possible, genetically altered lines will be maintained in a homozygous state, thereby obviating the generation of a large excess of offspring with inappropriate genotypes. In other cases, homozygotes will be generated from heterozygote intercrosses, with littermates genotyped as heterozygous or wild type used as age-matched controls. Genetically modified lines will be sourced from repositories to avoid remaking of lines whenever possible. Pilot studies will be undertaken to ensure the correct time course and experimental paradigms for our experimental purposes before larger scale studies are undertaken. Tissues sampled from the animals used in this project will be shared among lab members and with researchers in other labs when applicable.

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.

Mice have been chosen for this work as the least sentient species to model the human nervous system well enough for our work to be relevant to understanding of human brain development and adult neurogenesis. Mice are essential because the experiments require the use of the latest and most refined gene manipulation technologies to identify and label cell subtypes and allow cell specific gene overexpression and downregulation approaches, and these approaches are available in this species.

Our transgenic mouse models are not expected to exhibit any harmful phenotype. Acute manipulation of gene expression in the brain by stereotaxic injection of various substances (viruses, shRNA, gRNA) will require opening of a cranial window under general anaesthesia. For this surgical step, appropriate anaesthetic/analgesic regimens and post-operative care will be used to minimise pain and surgery will be performed following aseptic technique to reduce the risk of infections. The initial surgery is the only step in which any pain may occur. Any surgical procedures will undergo regular review to identify further refinements to minimise animal suffering including optimisation of implants and anaesthesia/analgesia regimens.

Some of the mice will be analysed by perfusion of the brain tissue under terminal anaesthesia followed by histological analysis. Other mice will be analysed by harvesting the fresh brain tissue after schedule 1 killing, followed by biochemical analysis. The analysis will therefore generate minimal suffering.

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

Mice will be used for this project as they represent the least sentient species appropriate for this type of work. Other species which are less sentient (such as fish or amphibians) cannot be used because they do not show sufficient similarities to humans (e.g. they have large adult neural stem cell populations that are significantly different from those found in mammals as their brains continue to grow throughout life).

Some experiments will be conducted in animals at embryonic or early postnatal stages, but other experiments will require using adult and ageing animals because part of the project focuses on stem cell activity and neurogenesis during adulthood and in old age.

Some of our experiments will be done using tissues from animals that have been euthanised or terminally anaesthesised. However, in order to examine the impact of gene expression manipulations, other experiments will be performed on anaesthesised animals followed by recovery for various duration until the time of analysis.

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

The animal facility staff run a comprehensive health-monitoring programme whereby animals health and welfare are checked and recorded on a daily basis. The animals will be maintained under conditions where their health status can be protected as far as is reasonably practicable. We use refined holding techniques for the animals, as well as group housing and enrichment. We use appropriate anaesthetic/analgesic regimens (including pre- and post-operative analgesia) and aseptic technique to minimise pain and will refine these with advice from the Named Veterinary Surgeon to ensure that we are using the best possible option given our experiments.

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

Resources hosted on the NC3Rs website, in particular:

- ARRIVE guidelines on experimental design and reporting results.

- PREPARE guidelines for planning animal experiments

- 'Procedures with Care': 'Aseptic Technique in Rodent Surgery'.

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

We will stay informed about advances in the 3Rs from several sources:

- Liaison with our animal care staff and NC3Rs representative

- Technological advances in the published scientific literature

- The NC3Rs website