Home Office

NON-TECHNICAL SUMMARY

Genes and mechanisms involved in genome regulation

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Stem cells, Chromatin, Mitochondria, Brain development, Neurodegeneration

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?

To study how genes affect the nuclear and mitochondrial genome in stem cells and their progeny

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?

The DNA in our cells (also known as our genome) is separated in two different places within our cells: the nuclear genome is found in the nucleus, and the mitochondrial genome resides in the energy-producing parts of our cells called the mitochondria. The main function of many of our genes is to organise these genomes, control their replication, repair damage and regulate the expression of other genes.

In this project, we plan to study how genes affect replication, stability and transcription of the DNA (from either genome) in neural stem cells and their progeny (nerve cells and glial cells) in the brain. Many neurodevelopmental and neurodegenerative disorders are caused by changes in gene expression and by the accumulation of mutations in the nuclear and mitochondrial genomes. However, it is not always known which genes are responsible for these changes. We aim to uncover how changes in our genomes arise, by studying how disease genes as well as novel genes with unknown functions may impact on genome regulation and expression, both in the nucleus and the mitochondria, during normal development and aging of the brain.

By studying how genomes are regulated in normal circumstances, we can learn about what might go wrong, for example in cancer and neurodegenerative disorders. In addition, studying these processes in cells and animals with specific mutations involved in developmental and neurodegenerative diseases will advance our understanding of pathology in humans and eventually lead to novel therapies.

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

The main output of this project will be an advanced understanding of the processes involved in development and aging of the brain, both in healthy conditions as well as in disease. A first part of the project will lead to a better understanding of whether and how genes that are known to be involved in brain development, aging and neurodegenerative diseases, impact on regulation of gene activity in the brain. In a second part we will identify novel genes and mechanisms that specifically regulate genes in the mitochondria, the energy factories of our cells. This second part will provide new hypotheses and research avenues for future research projects about mitochondrial and neurodegenerative diseases.

The results from our research will be communicated primarily through publications in open-access peer-reviewed journals, with details of the research methods according to the ARRIVE guidelines. These publications will be archived in Europe PubMed Central. When appropriate, we will deposit papers to open-access preprint servers like BioRxiv upon initial submission. Together, this will ensure early and broad dissemination of the data, but at the same time provide rigorous peer-review to guarantee the quality of the results and the interpretations. All forms of raw data will be made fully accessible with unrestricted access once published. This will provide other researchers the opportunity

to benefit from the data, and to reproduce results and interpretations. Data generated from this project will form the basis for new funding applications, and may eventually inform future clinical trials.

Throughout the research, novel tools will be developed. These will also be described in open-access peer-reviewed journals and be made fully accessible to other researchers, either by distributing them upon request, or by depositing tools and protocols in public repositories. When appropriate, intellectual property may lead to patent applications.

In addition to scientific publications, we will engage directly with other researchers through participation in meetings, which provide an opportunity to share non-published results at an early stage during the project. As a clinician-scientist, it is important to me that clinicians and patients remain aware of progress made through basic research. For the general public, output will be disseminated and discussed through active public engagement and outreach activities.

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

This proposal constitutes mainly fundamental research into a biomedical question about normal development and functioning of our developing and aging brain.

The short-term impact of this research will mostly be for academic and clinical researchers with an interest in brain development and regulation of the nuclear and mitochondrial genomes. The molecular tools we have previously developed, the further improvements we plan to make, and the approach we use by first verifying our tools and hypotheses in non-regulated model systems (e.g. in fruit flies or in cell culture) before translating our findings into the mammalian brain, will benefit a wide range of researchers. The findings and tools we generate will be freely shared with other researchers through open-access publications, depositing data in public repositories and presentations at scientific meetings.

We anticipate that on the longer term, this research will benefit patients and their family members, primarily those with neurodevelopmental and inherited neurodegenerative and mitochondrial diseases. Once published, our data will be available to pharmaceutical companies who may use the results to guide development of new treatments. When obvious targets emerge from our research, we will actively pursue these further and explore possibilities to collaborate with expert labs and pharmaceutical industry to maximise the potential therapeutic impact of the findings.

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

Apart from the novel biological insight, a major anticipated benefit from our previous work was related to development of novel molecular tools that could open up research opportunities in other fields. The molecular tools are freely available from the lab, and already prior to publication of our tools, we have set up several collaborations with groups in other research fields to apply our tools in mammalian cell culture systems and other organisms. Two of these collaborative projects have recently been published.

This open sharing approach will continue to be a major feature of our future research projects and culture, and will allow us to maximise the outputs of our work. The environment where this research project will primarily be conducted is highly interactive and fosters collaborations with world-leading experts in the fields of nuclear and mitochondrial genome regulation in the brain. We aim to share non-

published results at an early stage during the project, through oral and poster presentations at conferences, and regular informal interactions with other scientists within and outside our institute where we can discuss scientific progress, preliminary data and share ideas, tools and reagents.

Species and numbers of animals expected to be used

- Mice: 4200

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.

Most of the research carried out in mice will be based on data obtained from studies in the brain of fruit flies (Drosophila melanogaster), which is the main focus of the laboratory. In a first part of the project, known disease genes and homologs of factors that we previously showed to play a role in development of the Drosophila brain will be tested for their role during mammalian brain development and aging. Development of the outermost layer of the brain (the cerebral cortex) in the mouse embryo is the most widely used model system of mammalian (and human) neurogenesis. Embryos between 11 and 14 days (E11-E14) after conception are mostly used, because these stages precede and overlap with the peak of neurogenesis in the mouse cerebral cortex, which is the biological process we are interested in. In addition, well-established protocols exist that allow targeted genetic manipulation of specific cell types in the brain during these developmental stages, without affecting overall development, wellbeing and survival of the mouse embryos.

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

This project will rely on the maintenance and breeding of both wild-type and transgenic mice. Transgenic mice have small modifications in their genome that allow us to activate or prevent the expression of specific genes in particular cells of the brain. These modifications do not affect the normal development and physiology of the mice and the mice are essentially healthy. Some of these modifications only become functional (i.e. start or stop to express a specific gene) upon injection of specific substances. Some mice will therefore receive injections with these substances at doses that are otherwise harmless and usually do not cause any side-effects.

Some wild-type or transgenic mice will also undergo a surgical procedure called 'in utero injection and electroporation'. In this procedure, pregnant female mice will be subjected to laparotomy (a surgical procedure that involves opening the abdomen of the mouse). Embryos in the second half of pregnancy will receive injections into the fluid-filled cavities of the brain, through the wall of the uterus which otherwise remains intact. These injections contain DNA or RNA that will modulate the expression of specific genes in a subset of the cells in the brain. After injection, electrodes may be placed onto the uterus, across the brain of the embryos, and small currents delivered through these electrodes allow injected DNA or RNA to enter the stem cells of the brain (a process called 'electroporation').

Alternatively, RNA can be embedded in a virus that will enter the stem cells of the brain without prior electroporation. The total procedure lasts about 30 minutes (about 5 minutes per single embryo), and the majority of embryos and mice are expected to recover uneventfully. In a minority of the embryos, the injection may cause damage, either to the brain or to the amniotic sacs that wrap around the embryo. This will lead to death and resorption of the embryo by the mother.

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

The majority of animals that are routinely bred in the context of this project are not expected to exhibit any harmful phenotype or show signs of adverse effects that impact materially on their general well-being. Injection of substances is also not expected to cause harm or adverse effects. Of those animals that will undergo surgical procedures, the majority of the animals are expected to recover uneventfully from surgery. Distress before, during and after the surgery will be kept to a minimum by good surgical practice and the use of pain relief medication. Animals exhibiting any unexpected harmful phenotypes will be killed humanely.

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)?

Mice, mild, 85%.

Mice, moderate, 15%.

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?

Although there are currently no reliable methods that allow us to answer our key questions without use of animals, we have previously fully exploited other possible resources, and will continue doing so in the future. In the past, we have been heavily involved in the development of both invertebrate and non-animal model systems that strikingly recapitulate many events that occur during mammalian brain development and that improve our understanding of nuclear and mitochondrial genome regulation. Moreover, we regularly collaborate with research groups both within and outside the institute that are experienced in embryonic stem cell-derived models of brain development. Nevertheless, validation of these findings in the context of a living animal, either in genetically modified mice or through surgery on

mouse embryos during their development in the uterus, remains central to the study of brain development and essential to allow future clinical translation.

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

Most of the research carried out in mice will be based on data obtained from studies in fruit flies, which are the main focus of the laboratory. In addition, we heavily rely on cell culture to complement fruit fly research, and to optimise tools and techniques prior to applying them in vivo in mice. We will continue following this approach and will continue to collaborate with research groups both within and outside the institute that are experienced in embryonic stem cell-derived models of brain development and aging.

We are aware of recent developments in human and mouse cerebral organoid models, also as recognised by the NC3Rs. However, the current models still have limitations (cost, required technical expertise, variability, etc) that prevent us from applying this on a routine basis in our lab. Nevertheless, we actively explore these possibilities, which might result in future collaborations to extend the findings from our work, both in mouse, cell culture and Drosophila.

Why were they not suitable?

Non-animal alternatives are highly suitable to meet part of our Objectives. This clear focus on replacement has previously led to substantial reduction in animal usage for our research, and allowed us to answer some of the basic scientific questions we are studying in other non-regulated model systems. Nevertheless, brain development and aging in fruit flies or even in cells cultured in a dish does not recapitulate everything that occurs in a living human brain. In addition, several aspects of how our nuclear and mitochondrial genomes are regulated are specific to mammals, or can only be studied when cells are in the context of a living animal. Therefore, relevant findings from these other models will need to be confirmed in mice before we can consider translating them to a clinical context.

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?

For routine breeding of transgenic mice and maintenance of mouse colonies, we plan to use 3600 mice (9 transgenic strains, each 2 cages of breeding stocks with 8 animals, replaced every 8 weeks over maximum of 5 years). The number of animals that will undergo surgery will depend on the number of factors that we plan to test over the course of the project, but we envisage to use 600 adult mice. This will allow us to study 20 genes in several embryos per experiment, and conduct at least 10 replicates in 3 different cell types of the brain).

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

With the approaches described above, which combine experiments in Drosophila, cell culture and mice, we are able to design the best and most efficient experiments and use only a minimum number of animals, but with the prospect to generate information of the highest quality. Experiments are designed according to the PREPARE guidelines, taking advantage of the experimental design checklist (https://norecopa.no/PREPARE), following NC3Rs guidance (https://nc3rs.org.uk/3rs-advice-project-licence-applicants-reduction and https://www.nc3rs.org.uk/experimental-design-assistant-eda).

In addition, the technique we plan to use (DNA adenine methylation identification, DamID) to determine whether and where proteins associate with the nuclear and mitochondrial genomes is highly sensitive and specific, and has previously been extensively optimised. Its major strength lies in the need for very little biological material, in contrast to many of the other currently employed techniques that generate the same types of data. We are now able to obtain meaningful data about where specific proteins bind to the genomes from single mouse embryos and as little as 10,000 cells. This technical advantage will help to design the best and most efficient experiments and use only a minimum number of animals to allow substantial reduction.

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

Apart from good experimental design, the major optimisation lies in the prior validation of our experimental approaches and genes of interest in non-regulated model systems. In addition, since many experiments will rely on animals that have not undergone genetic modifications and we will only use the offspring, we will actively engage with tissue-sharing schemes at our institute.

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.

When studying development and aging of the brain, mice are currently the most heavily studied and best understood animal that closely resembles human development. In addition, they offer the possibility for genetic manipulation. All animals, and particularly those that have undergone surgery, are monitored to ensure that they receive appropriate care and to minimise suffering.

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

Although a major focus of our research is on the use of less sentient animals (in particular fruit flies), brain development and aging in these flies obviously does not recapitulate everything that occurs in a living human brain. Several aspects of brain development and gene regulation are also specific to mammals, and do not occur in other less sentient animals. Therefore, relevant findings from these other models will need to be confirmed in mice before we can consider translating them to a clinical context.

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

Optimisation of the surgery procedure (in utero injection and electroporation) has been an important part of our previous work and this will remain the case. We have previously introduced several refinements compared to standard working practices, inspired by the Home Office regulations and extensive discussions with the veterinary surgeon and technical support team. This included optimisation of pain relief during and after surgery; change of anaesthetic agents from long-lasting injected agents to inhaled isoflurane with less side-effects upon recovery; optimisation of sterile technique according to Home Office and LASA guidance; improved post-operative care, bedding and food as suggested by the technical team from the animal facility.

All animals will be monitored to prevent them from being distressed or experiencing harmful phenotypes and adverse effects and their bodyweight will be followed to ensure that humane endpoints are adhered to. Pain will be managed adequately through use of pain relief medication and anaesthesia during and after surgical procedures.

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

Surgery will be conducted according to LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery. We will take the PREPARE and ARRIVE guidelines into account, and regularly consult the advice and guidelines from the NC3Rs (https://nc3rs.org.uk/3rs-advice-project-licence-applicants-refinement).

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

I regularly receive and read the information that is distributed by the Named Information Officer. The facility where the animal experiments are conducted has an active training and education programme to ensure that researchers remain updated about advances in 3Rs, and I plan to discuss my research, approaches and techniques regularly with scientists, animal technicians and Named People (in particular Animal Care and Welfare Officers and Veterinary Surgeons) within and outside the institute. External resources that will be regularly consulted are The National Centre for the 3Rs (https://nc3rs.org.uk/resource-hubs), the Laboratory Animal Science Association (LASA), the Institute of Animal Technology (IAT), and the Royal Society for the Prevention of Cruelty to Animals (RSPCA). Norecopa (https://norecopa.no/databases-guidelines) provides an up-to-date database and guidelines regarding the 3Rs that will be used as an easily accessible online resource.