Home Office

NON-TECHNICAL SUMMARY

Molecular and Genetic Regulation of Glial Development and Function

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

No answer provided

Animal types	Life stages
Zebra fish	adult, juvenile, neonate, embryo

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?

Our work aims to understand what genes allow glia to develop properly and provide support to a healthy 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?

As life expectancy increases, there are a growing number of brain diseases affecting people. Researchers need to continue figuring out how the brain develops and functions so that these diseases can be treated or cured. Our brains are made up of two types of cells — some of these produce electrical signals (neurons), and others provide support (glia). For centuries, research has focused on neurons being the cause of brain disease. In many conditions, including Parkinson's and Alzheimer's, the supportive glial cells are abnormal and lose their ability to function. This research shows us that glia are very important in normal brain function. In fact, we now know that glia are often the underlying cause of these diseases. However, we still don't know much about how glia develop, and we don't understand how they protect our brains. Our studies try to start breaking the ice on how glia develop and function at a very basic level. We hope this will help find new ways to treat or prevent brain disease.

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

The goal of this project is to learn more about the fundamental principles giving rise to an appropriately shaped and functioning cell. This work will provide a valuable foundation for other research aimed at understanding the support cells(glia) of the brain. Some of the genetically altered animals generated in this research will also serve as valuable methods for designing new drugs used in the treatment of brain disorders. Throughout the project, we will share our findings with other researchers (at conferences or in collaborations) and will publish our results in open access journals.

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

We currently collaborate with other laboratories in the UK and internationally, who will mutually benefit from our findings to improve their experimental designs and establish new research programs aimed at understanding neural diseases. In the future, our results will likely enhance the development of drugs and/or methods that prevent, diagnose or treat human disease.

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

We already have active collaborations with basic and translational research labs that mutually rely on our research findings. We actively publish all of our results and openly share our methods and

resources with anyone who requests them. As is required by most funding bodies and within the University, we publish our findings in open source platforms. Peer-review publication is often time-consuming and is often missing many negative results or unsuccessful approaches. We do and will continue to share our findings on open source un-reviewed platforms such as Researchgate and Bio Archives, which includes details of adverse outcomes and strategies in a public forum.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 12950

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.

Zebrafish are a classical developmental model that has been successfully used to understand many critical biological and disease-related questions in science. They are the best model organism for our research because they give us the ability to perform much of our experiments before they are sentient animals that can feel any pain. During these stages, zebrafish embryos are small and translucent which means that a microscope can be used to noninvasively observe normal and abnormal development.

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

Typically, adult wild type, mutant or transgenic zebrafish males and females are placed in a tank together and fertilised eggs are passively collected. We will also generate new mutant and transgenic zebrafish lines by injecting genetic material into fertilised embryos and allowing them to grow to adulthood. These stocks will enable us to visualise the effects of mutations or collect physiological (i.e. normal living function) data in non-sentient embryos (i.e. embryos which are life stages that cannot feel pain). To preserve genetically modified zebrafish lines that are not being actively used, we will occasionally collect and store gametes (eggs and sperm) so that these lines can be re-established at a later date.

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

In general, mutations with severe defects or malformations do not survive to sentient ages (i.e. in embryos up to 5 days after the eggs were fertilised). We do not expect that any of our mutation or transgenic modifications will adversely affect animals in any way. However, should any abnormal behaviour or clinical signs develop in growing or adult fish, 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)?

Mild in all cases

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

- Used in other projects
- Kept alive

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?

Unfortunately, we know so little about how glia develop, there are currently no alternatives to animals for our research. We are always working to replace some of our animal methods with other techniques. For example, we recently developed a CRISPR-based (a gene editing) method that allows us to screen genetic changes in early stage fish embryos before they can feel pain. This new method allows us to edit genes more accurately. It replaces older methods where large numbers of genetically modified adult animals where used and gives us the same results. We also actively collaborate with insect and cell culture labs. By sharing our ideas with these groups, we continuously evaluate other methods, including organoid culture methods. These efforts will allow us to continue replacing our animal methods as our research into glia progresses.

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

Cell culture (individual cells growing in a dish) and organoid development (a three-dimensional laboratory based culture method that enables the growth and analysis of whole tissues).

Why were they not suitable?

Cell and organoid cultures are being developed to study glia. However, these techniques have failed to replicate the mature cell shapes and functional qualities needed for our research. As we build our understanding of how glia mature and use this knowledge to adapt organoid culturing techniques, we will likely enable such studies in the future.

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 number of animals estimated for use in this project is predominately made up of breeding, maintaining, generating and storing genetically altered (GA) animals. We obtained these numbers by drawing on our experience carrying out similar studies. These estimates allow us to produce at least the amount of embryos we need for statically valid results.

Breeding and maintenance of GA animals: Breeding results in significant energy loss for the adult fish breeders. We, therefore, must ensure enough tanks of fish and prevent repeated use of breeders over a week. Some GA lines will be used more depending on the experimental design. However, we estimate that we will need up to 10 2.8L tanks per line (depending on the number of staff using each line). Overall, we expect to maintain up to 18 GA fish lines, expressing fluorescent markers or gene mutations, at any given time. Thus, the total number bred will be 18 GA lines x 10 tanks x 12 fish per tank. Stocks of breeders will be renewed every year, so the total fish number amounts to 18 lines X 10 tanks x 12 fish x 5 years= 10500 GA fish.

Generation of F0 founders: For our research, we plan to generate 18 transgenic and mutant lines. From experience, we know that the generation of genetically altered (GA) lines has relatively low efficiency and the presence of genetic modifications are not always passed to F1 offspring. To ensure that we successfully capture the desired mutation or transgenes, we will generate 5 F0 lines (with 2 tanks of each line) for each GA line we have proposed to make. Therefore, our usage is estimated as follows: 18 lines X 10 F0 tanks x 12 fish per tank = 2160.

Obtaining zebrafish gametes: For, obtaining gametes (sperm and eggs) it will be necessary to maintain separate stocks of males with optimal breeding performance temporarily. We will perform this for lines that need to be archived which from previous experience amounts to on average 6 GA lines per year. For a target of 2 tanks of 6 male-only fish (a multiplying factor of 2 has to be considered assuming a 1:1 ratio of males and females per clutch), we estimate 360 fish over 5 years.

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

All of our experiments are carefully thought out and follow the appropriate steps of the PREPARE guidelines checklist (https://norecopa.no/media/7864/prepare_checklist_english.pdf). Consequently, our experimental design relies on distinct hypotheses or questions. We use carefully selected negative and positive controls to ensure the validity of our findings. For example, phenotypes that we find in genetically altered embryos are confirmed by direct comparisons to control (un-altered) animals following the same test conditions. In genetic sequencing experiments, we extract both types of cells from the eye (the neurons- as a positive control, and glia – experimental cells). Most of our methods are already established and commonly used in zebrafish labs all over the world. However, when we develop new techniques, we carefully consider the literature and seek expert advice before starting. There are instances where we use blinding and randomisation to achieve unbiased results. For example, in reverse genetic screens, we use a random number generator to keep track of each gene we are testing. This way, the person imaging and quantifying phenotypes is unaware of which gene it relates to until the analysis is complete. All of our experiments are designed to use only the number of animals needed to generate enough embryos for each test and reach a robust statistical power. If we

are uncertain about the analytical approach or power calculations, we always seek the advice of an experienced bio-statistician.

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

We also reduce the number of adult animals we use by collaborating and sharing some of our fish with other users at the university. We also maximise the outcomes of our findings by making our genomic data available on public databases. In turn, these open-source platforms provide other researchers with data and prevent replication of our work in other labs. We reduce the breeding of transgenic lines by using wild type embryos wherever feasible. We collect gametes from genetically altered adults and store them for later use to reduce the number of animals we maintain at any given time.

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 be using non-genetically altered and genetically altered zebrafish lines. The latter were either previously made or will be generated by the proposed work. The purpose of this licence is strictly to create, house, maintain and store the zebrafish lines. There are no methods proposed that are likely to cause pain suffering distress of lasting harm to these animals.

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

All experiments that are outlined in this proposal will be conducted on non-sentient stages of development.

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

The maintenance and care of adult animals are carried out by highly trained bio-facility staff. Together with our animal facility staff and other zebrafish researchers at the university, we frequently discuss ways to improve animal welfare in the facility. This includes discussions about additional precautions for preventing diseases, enhancing the viability of embryos through diet and improving water quality by replacing old filtration equipment. Our bio-facility staff also work closely with us and train our research staff to maintain training records for all regulated procedures carefully. Furthermore, our staff are working to develop better handling skills and improve methods of regulated procedures. For instance, years of refining the genotyping methods we use now allows us to only take the tiniest amount of fin tissue required to obtain valid data. We have shown that removing this very small amount of tissue

causes no pain to our fish. This means our fish do not need to be held in a tank containing water to which an analgesic (pain killing drug) has been added and so enables us to return our fish to tanks where they can be fed and allowed to interact with others in normal water (see https://www.liebertpub.com/doi/full/10.1089/zeb.2015.1165). We will continue to work closely with these staff to refine our methods for our research.

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

We ensure our publications conform to the ARRIVE guidelines: https://www.nc3rs.org.uk/arrive-guidelines. We also make use of 3Rs tools that are specific to fish and continuously updated by the university biomedical services team: https://www.ubs.admin.cam.ac.uk/3rs/search/category/species-specific/topic/fish

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

Our animal facility officers and staff continuously inform us of advances in the 3Rs as it relates to our work. All project holders are also required to have a member of their group attend quarterly facility meeting where these advances are discussed in more depth. Any implementation of these advances is done under the strict guidance of a named veterinary surgeon.