Home Office

NON-TECHNICAL SUMMARY

The diversity of glia in health, development and disease

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

Glial cells, Glial pathologies, Myelin, Gene therapy, Neurons

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

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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 project aims to investigate the various cell types in the brain (such as neurons and glial cells) and how they affect our well-being. We will prioritize understanding how genes impact the function of these cells, and use that knowledge to develop innovative therapies for brain diseases, specifically the rare Pelizaeus-Merzbacher Disease (PMD), which has an incidence rate of 1 in 200,000.

A retrospective assessment of these aims will be due by 28 February 2029

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

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 long "arms" of nerve cells are wrapped in a layer called myelin, which helps electrical signals travel quickly along the nerve cells. This myelin is made by special cells called oligodendrocytes in the brain and spinal cord. If the myelin breaks down, it can cause nerve cells to die, leading to serious problems like Pelizaeus-Merzbacher disease and multiple sclerosis. We want to learn more about the genes that are involved in making myelin and how they work, so we can develop treatments for these diseases. In this study, we will test potential treatments for Pelizaeus-Merzbacher disease, which currently has no cure.

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

Our research is focused on understanding how glial cells work. We want to learn about the different genes that are important for these cells to make a substance called myelin, which helps brain cells communicate with each other. We also want to understand how glial cells are organized in the brain and how they interact with other brain cells.

We will create a new model to study a specific disease called Pelizaeus-Merzbacher disease, which affects the myelin in the brain. We hope that this will help us develop new treatments for diseases that are caused by problems with myelin.

Our findings will be shared with other scientists by publishing them in peer-reviewed scientific journals.

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

We are creating for the first time a special type of mouse that has the same genetic mutation as humans with Pelizaeus-Merzbacher disease (PMD). This will be very important for scientists because it will help us understand the disease better and develop new treatments.

If the treatment we develop for PMD in this project works well, it could also be tested for other diseases that affect the same substance in the brain, like multiple sclerosis (MS).

In the future, if we find successful treatments in our animal tests, they might be used to treat humans with these diseases.

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

We will be presenting our ongoing research results, including any failed attempts, at scientific conferences like the European glia meeting. We'll also publish our findings on platforms like F1000research, so that other researchers can benefit from our work. By sharing our knowledge and collaborating with other research teams, we hope to advance the development of gene-editing therapies and promote the spread of new knowledge gained through our project.

Species and numbers of animals expected to be used

- Mice: 4000

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.

Mice have been the primary model for studying myelin biology, including the genes necessary for myelin production and brain development, as well as those involved in developmental diseases such as PMD. We plan to study these genes during the developmental stages of mice. Furthermore, we have found that isolating brain cells from mice at an early developmental stage for in vitro studies (using cells in a dish) is more effective and reliable than using adult mice. It's important to note that pre-clinical data obtained from mice is highly relevant for translational medicine, as it helps to inform and guide the development of potential treatments for human diseases.

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

- Generation of genetic altered mice: We will use breeding techniques to create a group of animals that have been modified in a particular way, such as turning off certain genes, increasing the expression of

other genes, or introducing specific genetic mutations.

- Substance administration: To study the effects of certain genes or drugs on mice, we need to introduce them artificially into the mouse's DNA. This is done through a process called genetic engineering, which creates what is called a "Transgene." We will also test drugs treatment that have been validated in a lab setting (in vitro). We will administer these Transgenes or drug candidates to the mice through injections, using different routes depending on the substance being tested. For example, Transgenes might be given to mice through an intravenous injection (into a vein) once, while drug candidates might be administered through an intraperitoneal injection (into the abdomen) daily for a week. We will follow established guidelines for animal welfare and make sure that the doses and frequency of injections are safe and appropriate.

- Behavior test: Assessment of cognitive, sensory and motor coordination might be done for mice developing phenotypes. For example, The rotarod behavior test is a commonly used test to evaluate the motor coordination and balance of mice. It involves placing a mouse on a rotating rod or drum, which gradually accelerates. The test measures how long the mouse can stay on the rod before falling off or being removed by the experimenter. During the test, the mouse needs to maintain its balance and walk or run on the rod to avoid falling off. This requires the mouse to use its coordination, balance, and motor skills. The longer the mouse can stay on the rod, the better its motor coordination and balance are considered. The test can be performed using different protocols, such as varying the speed of the rod or the duration of the test. It can also be used to assess the effects of different drugs or genetic manipulations on motor coordination and balance in mice.

- Health check: Daily health checks can be done if mice develop harmful phenotypes.

- At the end of the experiment all mice will either be humanely killed, or tissues and organs collected under deep, terminal anaesthetic unconsciousness. This process starts by first removing the blood by pumping a salt-containing liquid through the blood vessels, called perfusion, followed by tissue preservation in a fixative solution for follow-up analyses

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

In this license, the majority of animals being used (about 75%) are not expected to develop any significant changes in their physical or behavioral traits that will persist throughout their lives.

However, mice with mutations in their myelin genes may develop issues with their brain and nervous system, which can lead to problems with thinking, sensing their surroundings, and coordinating their movements. These symptoms may appear when the mice are about two weeks old and may persist throughout their lives.

In severe cases, the mice may also experience tremors and seizures by the time they reach three weeks of age, and may start losing weight as a result. These mice will be humanely killed if they develop seizures or if weight loss exceeds 15%.

When mice receive injections of transgenes or drugs, they usually only experience minor changes in their appearance or behavior. These changes are not higher than moderate in intensity. For example, some compounds may cause change in development leading to small pups compared with control or untreated littermates, or injecting Tamoxifen might cause the mice to temporarily lose weight.

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)?

Sub-threshold: 25%

Mild: 50%

Moderate: 20%

Severe: 5%

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

- Kept alive
- Killed

A retrospective assessment of these predicted harms will be due by 28 February 2029

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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?

The study of how glial cells are arranged and interact with other cells in the brain requires the use of animals. This is because the brain is too complex to be fully replicated in a lab setting. To learn how glial cells work in the brain and how they communicate with other brain cells, we need to study animals because the brain is very complex. It's impossible to replicate a whole brain in a lab that works the same way as a real one. To do experiments in the lab with brain cells, we have to take them from living animals. We also need to study live mice to see how neurological diseases affect them and if new treatments can help.

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

we considered using cell lines (e.g. HEK cells, Glioma stem cell lines...), stem cells-derived neural cells, cerebral organoids (Cerebral organoids are three-dimensional structures that are grown in the laboratory to resemble certain parts of the brain. They are made by culturing cells that can develop into different types of brain cells, which then self-organize and form structures that resemble the brain's

tissue) and in silico modelling (computer models are used to simulate the behavior of molecules or cells).

Why were they not suitable?

We currently do not have any cells lines that can make myelin, which is a substance in the brain that helps cells communicate with each other. Scientists have been trying to use different methods, such as growing special types of cells from stem cells or making mini brain-like structures called organoids, to study how myelin works in the brain. However, these methods are not very good yet and we haven't been able to see myelin being made in the organoids. These models are also not very useful for studying how glial cells are organized in the brain.

A retrospective assessment of replacement will be due by 28 February 2029

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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?

- This project involved using mice that had been genetically altered in specific ways. To study one particular gene, most of the mice needed to have an enzyme called "Cre recombinase" present alongside the gene. This meant that each mouse had to be bred with another mouse that had the Cre enzyme, in order to activate the mutation in the gene they were studying. Because of this, we needed to use a larger number of mice to carry out the research.

- Some genetically altered (GA) animal used in this project present recessive mutations in the chromosome X (A recessive mutation is a specific change in a gene that only has an effect if both copies of the gene carry the mutation. A recessive mutation located on the X chromosome means that the specific gene change is on one of the sex chromosomes. In males, it can cause phenotypes because they have only one X chromosome. In females, they may be carriers without symptoms).

PMD is a congenital leukodystrophy that presents a recessive mutation in a gene present on the X chromosome. Therefore, only boys develop the disease, and no cases of girls developing PMD have been reported in scientific literature. To ensure the most effective treatment for PMD, we will use male mice with PMD as our primary focus during the development process. This approach aims to make the treatment applicable and relevant to human patients. The females we will produce through these

breeding can be used for further breeding to generate more male PMD mice, as they will carry the mutation without developing the disease.

- The number of animals used in this project has been reached by carefull consideration and discussion with current PPL-holders for projects similar in character and scope to what we aim for and expect (protocols, number of mouse lines used).

- Based on these interations we have performed approximate power calculations in order to gauge a likely number of animals required for completion of the project. Balancing statistical power and reduction of animal use.

- To minimize the harm to animals, we will perform experiments using primary brain cells isolated from mice pups in a lab instead of using the whole animals. This means that most of the litters of mice pups will be used for these experiments instead of being raised as live animals.

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

- The NC3Rs Experimental Design Assistant (EDA) will be used to plan experiments in a way that uses the fewest possible number of animals while still achieving the scientific goals. Additionally, the EDA provides guidance on how to minimize any subjective bias in the experiment and how to conduct appropriate statistical analyses to ensure accurate results.

- PREPARE guidelines will be used for planning animal experiments.

- We will follow resources found on the NC3Rs website (e.g., to help us determine the appropriate number of animals to use in our experiments, plan pilot studies, plan breeding and colony management).

- Good breeding design will be implemented to minimize the number of unwanted genotypes.

- All researchers working under this license will have received basic training in statistics by attending courses. When needed, we will consult specialist statisticians regarding experimental design and statistics.

- We will maximize the use of experimental tissues/biological samples that are surplus to the original experiment by making them available to other researchers to address other biological questions that are within the scope of the current project aims.

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

- Pilot study will be design for each new GA animals and new protocols involving drug or transgene injection to test the feasibility and practicality of the experimental design before conducting a full-scale study. A small number of animals will be used to test the experimental procedures, refine the experimental design, identify any potential issues that may arise during the experiment and amend the licence if needed. This helps to reduce the risk of errors and increase the reliability of the results obtained from the main study. The data obtained from a pilot study can also be used to estimate the

sample size needed for the study and to determine whether the proposed study is likely to achieve its objectives.

- Computer modeling, power calculation, and sample size calculation will be used for each experimental design.

- Coordinated breeding between each user of this project will be done to minimize the number of breedings and animals used.

- Breedings will be optimized to acquire the maximum output.

- Mouse and tissue sharing, including tissues from genetically modified mouse lines and post-mortem tissues, will be done to further reduce the overall number of mice used.

- Several tissues from the same mice will be used.

- Mice will be placed in experimental groups randomly, and blinding will be applied to prevent any potential bias in the results obtained from the experiment. This ensures that the study is conducted in an objective and scientifically rigorous manner.

A retrospective assessment of reduction will be due by 28 February 2029

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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 use rodents as animal models because we have the most experience and technical expertise with them. Specifically, we will use mice instead of rats because mice are less sensitive to social stressors such as being separated from their mothers during weaning. This will help to ensure that the animals used in the project are as comfortable and stress-free as possible.

- Whenever possible, we will use genetic modifications that can be turned on and off (inducible) rather than ones that are always active (constitutive) to prevent any negative effects that are not directly related to the goals of the experiment. In this project, we are investigating the role of certain genes in adult mice. Therefore, we want to avoid causing any developmental problems in the mice, as our focus

is on understanding adult mechanisms. This approach is important to prevent unnecessary pain, suffering, and distress to the animals.

- We will prioritise non-invasive delivery for drug administration. E.g. Drugs administred in drinking water or jelly. Intraperitoneal injections rather than intracerebroventricular (into the fluid'filled space.s in the brain called ventricles).

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

- Mammals have highly developed central nervous systems. Glial cells are a crucial component of this system and have evolved in a unique way in mammals, setting them apart from other commonly used animal models such as worms, fruit flies, zebrafish, and reptiles. We will be studying glial cells to understand the mechanisms that are involved in human diseases related to the nervous system, such as myelin disorders like Pelizaeus-Merzbacher disease (PMD). This research will help us gain a better understanding of these conditions and how to treat them.

- There is as of yet no model of PMD in Zebrafish.

- The overwhelming majority of studies on myelin biology have been undertaken using laboratory rodents, especially mice.

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

- To improve the well-being of mice, all mice will have acces to enrischment and enhancements. This can involve housing them in groups to allow for social interaction, as well as adding materials like nesting materials, chew blocks, and wood shavings to their cages. Other enrichments like tunnels, climbing structures, and new objects can be added to encourage natural behavior. Finally, offering food in different locations within the cage can encourage foraging behaviors and make the mice feel more at home.

- Mice experiencing mild, moderate or severe symptoms (such as reduced motor and sensory function) or living in breeding cages where their pups will be collected, we will provide additional environmental enrichment (as listed above)

- For genetically altered animals potentially leading to severe phenotypes, we will establish intensive monitoring programme and clear endpoints (for example, a monitoring programme for experiments expected to cause neurological signs in animals will be created in form of a checklist, which covers general body condition and objective assessments of neurological function such as posture, movements, presence of tremor and seizure).

- In this project, we plan to change important genes using a method called inducible gene modification. We will use a common technique called drug-induced cre-lox mediated gene recombination, which involves giving the mice a drug called tamoxifen to activate the gene modification. However, we have noticed that tamoxifen can cause some temporary side effects, like weight loss, because it reduces the mice's appetite. To reduce this impact, we will give the drug at a time when the mice are less likely to

be affected, and we may use a flavored jelly instead of oral gavage. If we need to give the drug in their drinking water, we can add sweeteners to make it taste better.

- When possible, we will encourage voluntary treatment for experimental treatment as well as pain management using medicated palatable substances such as mediated chow (food that contains medication mixed in with the regular chow), medicated flavoured jelly/paste, medicated sweetened water.

- Whenever we are trying out a new procedure that might have obvious negative effects, we will begin with a small test to see how it goes (pilot test). We will also set up a system to keep track of what is happening and try to minimize any harm or discomfort.

- When possible, we will do a test on genetically altered mice to see if we can answer our scientific questions by looking at mice that only have one copy of the mutation (heterozygous mice) and show less harmful physical characteristics.

- For each mouse line, a detailed phenotypic assessment will be made.

- By minimizing the handling of mice that are likely to experience seizures, we aim to reduce the occurrence of seizures.

- In order to prevent seizures that can potentially result in the death of the mice, we will carefully evaluate and identify early clinical signs that indicate the likelihood of a seizure occurring in a pilot study. By identifying these early signs, we can intervene sooner and implement appropriate measures to reduce the occurrence of seizures. In humans, early symptoms associated with Pelizaeus-Merzbacher disease include nystagmus (rapid and involuntary eye movement), weakness (lack of strength or power in the muscles), spasticity (difficulty controlling muscles, resulting in stiffness and tightness), stridor (high-pitched sounds when breathing), dysphagia (swallowing difficulties), and walking difficulties. In mice that are likely to experience seizure, pilot study including Eye movement check, Grip strength behaviour test (the animal grasps a grid or another object, and the object and animal are pulled apart until the grip is released), Assessment of mouse posture (check abnormal hind limb posture and loss of hind limb extension reflex when mice are suspended by the tail), Mouse weight measurement, Evaluation of mouse walking behaviour and Intensity of shivering will be done to identify early clinical sign. During this pilot study, the mice will be checked three times a day. After completing the pilot study, we will refine and adjust the frequency of these check-ups. The goal is to be able to identify these early clinical signs while minimizing the amount of handling the mice undergo

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

We will follow technical updates on animal experimentation and welfare with articles published in "Lab animal" (https://www.nature.com/laban/) especially paper published by the NC3Rs team.

We will follow PREPARE guidelines for planning animal experimentation as well as ARRIVE guidelines when drafting manuscripts that detail animal research.

To make sure our experiments are done as accurately and ethically as possible, we will look to the advice and guidance provided by organizations like the NC3R, the Laboratory Animal Science

Association (LASA), and the International Mouse Phenotyping Consortium (IMPC)

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

The NC3R website can provide help and guidance to researchers working under this project to implement the 3Rs in experimental designs (https://nc3rs.org.uk).

We will seek guidance from both the Laboratory Animal Science Association (LASA) and the Royal Society for the Prevention of Cruelty to Animals (RSPCA) for the latest recommendations and advances in animal research techniques. Furthermore, we will use online resources provided by the Jackson Laboratory Resource Library, as well as the latest regulations, policies, guidelines, and databases provided by Norecopa, to implement the 3Rs in our work.

In undertaking procedures in this project, we are continuously assessing how the procedures can be refined in order to minimize the discomfort that the animals may experience. Members of the lab holding personal licenses who undertake procedures under this project license meet regularly to review operational procedures.

We maintain regular communication with animal facility staff in general and NACWOs and NVS in particular.

A retrospective assessment of refinement will be due by 28 February 2029

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?