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NON-TECHNICAL SUMMARY

Breeding and Maintenance of Genetically Altered Animals for Regenerative Neuroimmunology Research

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Mouse Breeding, Genetically altered mice, Colony Maintenance

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 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?

The breeding, generation, and maintenance of mice with genetic modifications. These mice will supply project licenses within our group with the appropriate mice needed to carry out the experimental work.

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?

When mice are used in the study of human diseases, they are frequently selected because of their similarity to humans in terms of genes, as well as brain structure and function. Additionally, mice are the most widely used animal species in experimental research. This is because it is relatively easy to induce disease models like human diseases, and to manipulate their genes (i.e., genetic modification) in a way that allows studying specific mechanisms of disease. Here, genetically-manipulated mice are produced for the experimental licenses in our group. These will help us understand how cells of the immune system can affect the healthy, diseased, or injured brain's structure and function.

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

Outputs will be a steady supply of appropriate mice for studies on other projects.

Mice produced in this licence will be used to study aspects of the human disease multiple sclerosis and human spinal cord injury. These mice will help us understand how cells of the immune system and the brain talk to each other. The mouse behaviour will be studied.

New-born mice in this license will also be used to get immune system and brain cells to grow in lab dishes.

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

The projects receiving these mice will have a reliable source of mice to use for their experiments.

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

Lab members will meet and design a breeding plan to make sure the right number of mice are produced to match the needs of the experiment. Mice with genetic modifications will be made available to other labs we work with. All mice used on projects carrying out experiments within our group will be described in our publications. If we make a mouse with a genetic modification that does not work as expected (i.e., what is generally defined a negative result), we will look to also publish the negative results as appropriate. The licenses this work is supplying will benefit from good maintenance of the

colonies to supply the mice we need at the appropriate times. Lastly, we will look to share tissue (i.e., cells and tissue) with other groups as appropriate and as needed.

Species and numbers of animals expected to be used

- Mice: 3000

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 are using mice because currently they are the most commonly used animal in human disease research.

We are using adult mice as they need to be sexually mature for breeding purposes. Adult mice will supply project licenses within our group with the appropriate mouse models needed to carry out the experimental work.

We are using new-born mice to collect cells to grow and keep in a dish to do experiments.

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

Female mice will be given substances to make more eggs that are collected in the mouse ovary (i.e., superovulation). Substances will be given across two injections approximately 48 hours apart. Substances will be injected into the abdomen (i.e., intraperitoneal). Mice will be killed at the end.

Male and female mice will undergo surgical procedures using appropriate anaesthesia. For male mice, they will undergo a surgical procedure to render them sterile (i.e., vasectomy). For female mice, they will experience a single surgical procedure to place embryos, or fertilised eggs, into their reproductive tract. Female mice may also undergo non-surgical placement of embryos into their reproductive tract. This involves inserting a small thin tube into the uterus of the mouse to insert embryos directly.

Male and female mice will be housed together and allowed to naturally mate. This will produce mice with and without genetic modifications using standard breeding methods.

Some mice offspring (<50 total offspring per year) up to five days old will be collected and will be killed by removal of their head (i.e., decapitation). We will then collect cells of from the brain. These cells will then be grown in the laboratory and used for experiments.

Adult female or male mice will first be terminally anaesthetized. Then we will remove the blood by flushing it out of the mouse using a pump. Then we will collect the organs and store them in a solution that prevents the tissue from being destroyed. This allows us to store the tissue for long periods of time. Then, we will use the tissue for analysis.

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

Female mice that are breeding, receive injections for superovulation, or undergo a non-surgical procedure are likely to experience mild pain (i.e., that equates to the insertion of a needle beneath the skin), suffering, or distress. These individual procedures will not result in the significant impairment of the well-being or general condition of the mice.

Mice that undergo surgical procedures will experience short-lived (i.e., less than 24 hours) post-operative pain and discomfort. These impacts are not expected to last for more than 24 hours.

Mice undergoing terminal procedures will be completed under general anaesthesia. This will keep the mice in a state of sleep/unconsciousness for the length of the procedure.

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)?**

Mouse: Subthreshold 98%

Mouse: Mild 1%

Mouse: Moderate 1%

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

- Killed
- 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?

Mice are widely used in pre-clinical research. This is because their genes, biology, and behaviour are similar to humans. We can then use mice to study many of the causes and effects of disease or injury seen in human patients. Some things we can study are tissue damage and the presence of inflammatory immune cells. These are types of cells that release chemicals and molecules that prevent the damaged area from being fixed. We also use genetically modified mice. These mice allow us to turn on or turn off specific genes that are involved in the injury or disease. We can then study proteins and cellular activity and how they work in the injured or diseased brain.

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

Over the years our group has refined and improved our use of cells grown and maintained in plastic dishes. Using these cells, we have exposed them to experimental treatments to understand if they have a positive or negative response. This way we can test their safety before then testing them in a mouse.

Additionally, we have developed a new way to grow and maintain human cells in plastic dishes that does not involve the use of mice. We can then use these cells to perform experiments that test our ideas about how human cells respond to treatments without having to use cells from mice beforehand. This new way of testing cellular responses in plastic dishes is closer to the response of these cells in a living human. This will help us in being able to predict which aspects of the cell in humans are the most important to study using our mouse models.

Why were they not suitable?

Cells grown in plastic dishes are useful for studying some aspects of human disease. However, they do not fully capture the complex changes that happen to cells in a living mouse or human. Using mice allows us to study these complex changes in a setting that is more like the human disease or injury. This way, we can understand the true function of these cells in their normal setting.

Cells grown in plastic dishes behave differently to those found in a living mouse or human. Cells in plastic dishes lose their diversity and the ability to communicate with other cell types.

Therefore, it is necessary to use mice to assess the complex biological and behavioural responses of cells following an injury or disease. This is also important when testing a potential therapeutic treatment. There is also a requirement to demonstrate that a treatment is safe and effective in animals before progressing to human application.

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?

Mouse numbers were estimated based on a combination of the retrospective review, annual return of procedures, and the estimated animal usage needed to supply the experimental project licenses with our group for their duration.

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

The National Centre for the Replacement Refinement & Reduction of Animals in Research (NC3Rs) website provides excellent resources for implementing colony management best practices. This includes important considerations to create suitable breeding strategies to reduce the production of mice not carrying the genetic modification of interest for use on other projects. This resource is used as a reference to design breeding strategies prior to the mating of genetically modified animals. This will avoid the excessive waste of mice.

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

Breeding of mice colonies will be planned with care to avoid the creation of surplus stock by carefully and consistently tracking the number of mice.

Any line of genetically modified mice not actively being used in our experimental work will be removed from active breeding and the line frozen down to prevent the generation of surplus stock.

Breeding mice will be replaced before their reproductive performance declines. This will be done by maintaining breeder mice of various ages by replacing a percentage of them monthly.

Non-productive breeders, i.e. those female mice that have not produced a litter within 60 days of mating or since their last litter, will be replaced. Breeding mice will be young, sexually mature male and female mice as younger mice generally breed better than older ones.

Where possible, experienced males will be housed with size matched young females to improve breeding performance and prevent injury to the female mice during mating. Additionally, to produce offspring with the same age we will house females together in pairs to sync up their reproductive cycles. Then, these female mice will be housed with individual males which will result in the maximum number of pregnancies.

We will keep meticulous and accurate breeding records to evaluate the breeding performance of the mouse colony. This will allow us to detect problems sooner so that they can be corrected.

We are also coordinating with other groups to share animal tissues – including tissues from genetically modified mouse lines and post-mortem tissues - in order to further reduce overall animal numbers.

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 a non-surgical method to increase the number of viable embryos that are released from female mice to be available for fertilization.

We will be using a surgical method under anaesthesia in which mouse embryos are placed into the uterus of a female to establish a pregnancy.

We will use a non-surgical method in which mouse embryos are placed into the uterus of a female to establish a pregnancy.

We will be using a surgical method under anaesthesia in which male mice will undergo a vasectomy to render them sterile.

We will be mating and breeding male and female mice to produce offspring for use in our experimental licenses.

These methods are standard in the breeding and maintenance of mice for scientific purposes and have been routinely updated and optimised to cause the least pain, suffering, distress, and lasting harm to the mice.

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

We are extremely limited in the use of non-mammalian species (i.e., invertebrates (e.g., worms), fish, or amphibia). These non-mammalian species are not fully suitable for the study, development, and testing of treatments for use in humans (i.e., mammals). In fact, while some work is done in non-mammalian species, the complexity of the interactions in an organism can only be studied in mammals (e.g., mice). This is because they have similarities in organ, tissue, and cell structure with humans.

We need mice with cells that have reached a mature stage of development. This is to make sure it is similar to the cells present in adult humans. The use of mice during the beginning stages of life will be restricted to the collection of cells to grow and maintain on plastic dishes to perform experiments.

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

Female breeders will only be allowed to have six litters of newborn mice or only breed for six months, whichever comes first, before she is retired.

Stock mice that are required to maintain a live colony will be kept to a minimum to ensure appropriate mouse numbers for experimental purposes. This will avoid producing more mice than is necessary and reduce the wastage of mice.

If a strain of genetically modified mice will not be used for research purposes in the next six months, it will be archived through cryopreservation of female embryos and/or male sperm. This will preserve the genetic background of the strain until it is again needed for use in our experimental licenses. Cryopreservation involves the collection of non-implanted embryos from female mice and/or sperm from male mice followed by long-term storage in extremely cold temperatures (i.e., -80°C). This saves

significant space and mice care resources. Ultimately, it allows us to better manage our colonies being actively used to supply our experimental licenses.

We will select the most appropriate breeding strategy on a per strain basis. For example, if a manuscript is under review, and a colony is being maintained in the event that reviewers request further experiments, an intermittent breeding strategy will be used to avoid animals being wasted. This is where we would reduce the number of active breeding pairs. For example, if we needed 6 breeding pairs to supply our experiments, we would reduce this number to 2 breeding pairs while the research is under review.

Our breeding strategy for mice with genetic modifications will be to set up male and female mice either in pairs (i.e., one male and one female) or in trios (i.e., one male and two females). The use of trios increased breeding efficiency to produce the mice with the desired genetic modification. A well-designed breeding strategy will lead to the largest number of offspring with the expected genetic modification. This will reduce the wastage of mice that do not have the genetic modification.

We have also designed our breeding strategy to produce offspring that either have the genetic modification or do not have the genetic modification. When these mice are supplied to our experimental licenses, they allow us to determine if the changes we have made are real.

Female mice with offspring, called pups, will be provided soft nesting material to keep the pups warm and protected. As the mice develop and approach the weaning stage (i.e., the removal of the offspring from the mother), wet mash will be provided to ensure the pups do not suffer from malnutrition.

Mice undergoing surgical procedures will be administered pain medication immediately before and after the procedure. Mice will be kept under anaesthesia during the entirety of the procedure. They will also be placed on a heating pad to maintain normal body temperature for the entirety of the procedure. Following all surgical procedures, the mice will be placed into individual cages with a soft, grippable bedding to allow ease of movement. The mice will also be provided with access to a diet gel to maintain hydration. The cages will be kept in a heated chamber to maintain a stable body temperature. Mice will be monitored until they are awake, alert, and active (i.e., moving freely in the cage) and then returned to their home cage. Mice will be monitored daily for 7 days to ensure the surgical site remains closed, there are no development of clinical signs of distress and suffering, and to minimise the chance of post-surgical complications. Pain medications will be provided as needed to mice through either intraperitoneal (e.g., into the abdomen) injections or as a palatable substance (e.g., in the drinking water).

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

We will follow the guidance provided in the 'Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes' and the Jackson Laboratory. We will also use the resources available for colony management and breeding strategies on the 'National Centre for the Replacement Refinement & Reduction of Animals in Research' and 'Laboratory Animal Science Association' websites.

In addition, we will refer to the Jackson Laboratory resource manual on 'Breeding Strategies for Maintaining Colonies of Laboratory Mice' as well as the many manuals and guides available on their website for continued guidance.

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

The National Centre for the 3Rs (NC3Rs) will be the main reference to understand whether our experiments match the highest standards of 3Rs. We will adapt our protocols if the recommendations evolve throughout the duration of this project. Regular consultations on the latest practical guidance from Laboratory Animal Science Association (LASA), Institute of Animal Technology (IAT), and the Royal Society for the Prevention of Cruelty to Animals (RSPCA) will provide additional sources of new recommendations and advances in animal techniques and clinically applicable models.

As a license holder, it is my own responsibility to stay updated on published best practices. I will do this by consulting information for license-holders provided by our establishment and by speaking to other project license holders.