



Home Office

NON-TECHNICAL SUMMARY

CNS Neuron-glia interactions, myelin plasticity, and regeneration, in health and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Myelin, Regeneration, Glia, Stem cells, Brain circuits

Animal types

Life stages

Mice	adult, pregnant, embryo, neonate, juvenile, aged
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Rats	adult, embryo, neonate, juvenile, pregnant, aged
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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?

This project aims to understand how the brain develops and repairs itself in health and disease. We study how stem cells in the brain can become cells that make myelin (a fatty membrane that insulates neurons) throughout life. We want to know the importance of correctly made myelin for brain function, in health and disease. We are looking at how different cells in the brain communicate between each other to control development, and repair, throughout life. The ultimate aim is to learn how to enhance myelin repair or make new myelin as a therapy for several brain disorders.

A retrospective assessment of these aims will be due by 10 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?

Neurological disorders are conditions that affect the brain or spinal cord, and are one of the largest groups of conditions without many treatments. They have a large social and economic cost and it is important that we find new ways to treat them.

This research will help us learn more about how the brain grows and works, and how it can sometimes fix itself – like spontaneous formation of new myelin in the white matter of the brain. We will learn more about the pathways and factors that help the brain repair itself. This could lead to new treatments for neurological disorders, such as:

- (i) developmental brain disorders; like cerebral palsy, where there is damage to the brain around the time of birth (~17 million affected in the world) and the genetic disorders that are collectively called leukodystrophies, where the white matter doesn't form properly or isn't maintained properly leading to severe disability and death;
- (ii) acquired disorders such as schizophrenia and multiple sclerosis (~2.5 million affected in the world); and

(iii) age-related disorders such as dementia (~50 million worldwide; inc. Alzheimer's disease, frontotemporal dementia and vascular dementia).

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

The aim of this project is to understand how, throughout our lives, stem cells in the brain turn into cells that make myelin, and in disease replace myelin when it is lost or damaged. In disease it becomes clear that myelin is essential for normal brain function, as when damaged or lost it can cause both mental and physical disability.

The fact that myelin can be regenerated provides an exciting therapeutic target for a wide variety of neurological conditions. In fact, the MS (Multiple Sclerosis) society has set this as one of the main therapeutic targets for the next generation of combined treatments along with disease modifying drugs.

The outputs of this licence will likely result in a new information and publications of the following:

1. Better understanding of how myelin is formed during development, and an insight on how dysregulated developmental myelination can alter brain function. This is important for understanding some neurodevelopmental disorders, and conditions, like cerebral palsy, autism and leukodystrophies.
2. Understanding on how myelin changes during our lifespan, whether myelination is regulated differently at different ages, and potentially explain why myelination stops with old age – and why myelin is lost with age. Researching these lifelong myelin changes using approaches that focus on brain function will give us clarity on how myelin influences learning and memory, behaviour and potentially dementia.
3. One of the main aims of this project is to understand how to promote myelin regeneration, to aid development of new therapies for several white matter disorders like multiple sclerosis. We expect to uncover a pathway to promote myelin regeneration, and test its therapeutic potential.

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

Our ultimate aim is to take the fundamental knowledge we gain in this project and use it to influence potential new therapies that will directly benefit people. This is a very challenging, but the first step is identifying the processes that underlie myelin regeneration and understand how myelin influences brain function. With this knowledge gained we can identify new testable pathways for augmenting regeneration. Some of these pathways may be targeted by using repurposed drugs – facilitating their path to clinical trials.

People with MS, in particular, will benefit from this research by the potential provision of cheap and safe drugs to promote remyelination and prevent progressive disability.

The myelin research field will also benefit from this work as we will advance the knowledge within the field.

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

In order to maximise the output of this project, we plan to undertake the following measures:

We will share all our experiments and findings (both successful and unsuccessful) to the scientific community through publications, conference presentations, and workshops. We aim to upload our results on open access platforms, either before or simultaneously with their publication, so that they can be promptly available to other scientists and to prevent unnecessary duplication of effort.

We will make the results of our project is accessible to the public, enabling those interested to learn more about this field. We have established platforms to actively engage with the public.

We will collaborate with other scientists and/or research and development companies, sharing our knowledge, skills, and techniques to facilitate scientific discoveries.

Species and numbers of animals expected to be used

- Mice: 19000
- Rats: 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.

Myelination only occurs in vertebrates (animals with a backbone) and so non-vertebrate species are inappropriate for understanding myelination and myelin regeneration/repair. There are no myelin regenerative models that have been developed in birds, reptiles, amphibians, or fish. Thus, we use rats and mice because they are the least aware species that can model interaction between brain cells (neurons and glia cells), myelination, cognition (thinking) and behaviour in neurological disorders. The brain circuitry implicated in many neurological disorders is very similar between rodents and humans.

As we are interested in myelin throughout the lifespan we will be using all ages of rats and mice, including older animals so that we can investigate the role of ageing in repair of myelin problems. We are also trialling the use of new-born animals (from birth to 5 days old) for some of our brain injection surgeries as this procedure is less invasive (no drilling of the skull) and requires fewer injection sites in order to get wide coverage of the brain. Another advantage of using the very young animals is that the brain structures needed for the sensation of pain are not yet fully developed before 5 days of age, reducing the overall severity of the discomfort that they will feel.

These experiments will largely be performed on mice, which are essential to this project, as it centres on using the power of genetic modification to highlight mechanisms of how neuronal activity can regulate myelination. However, a substantial proportion of the experiments suggested will be conducted using rats as, due their size, rats are the ideal animal model for toxin-induced myelin damage, in addition to conventional cell culture methods being well established for rat tissue. In some cases genetically altered rats will also be needed for experiments, these modifications will also be

used to highlight mechanisms of myelination in experiments which require the increased size of rats and are currently not possible to perform in mice.

There are no demyelination/remyelination models that have been developed in birds, reptiles, amphibians or fish. An overwhelming majority of studies on the biology of CNS regeneration have been undertaken using laboratory rodents, especially mice and rats. Thus, for this work to contribute to mainstream translation-relevant research it is necessary to use mice and rats. These are also the mammals of the lowest neurophysiological sensitivity likely to produce satisfactory results. Much of the proposed work will use tissues after the death of the animal and/or laboratory based methods, however due to the genetic manipulations of the animals this tissue is not generally available from established tissue banks.

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

A variety of different procedures will be performed as part of this license, in most cases animals will recover quickly and without incident (mild severity) or occasionally will be non-recovery, where animals are anaesthetised for the procedure and are killed under anaesthesia. Procedures that fall under the mild category include; injections, drug administration in food or water, blood sampling, breeding and maintenance of genetically altered animals where the alteration is not harmful, behavioural tests, or labelling (with dyes or fluorescent markers) the central nervous system under non recovery anaesthesia. We use drugs or dyes, either injected or fed to the animals, for several different reasons; to activate genetic modifications, to provide pain relief before or after procedures, to label certain cells so that we can identify them, or to change the responses of certain cells to different stimuli e.g. so that we can boost or inhibit myelin formation or maintenance. Once procedures are completed animals will be humanely killed, the methods used to do this will vary but will be carefully considered to balance the experience of the animal with the usefulness of the tissues that we can obtain after death.

Animals may be aged before or after any of the procedures, they are housed normally and kept until they are the age required for the experiment, this may vary from a few weeks old up to 2 years old.

Injections into the eye are used to investigate how myelin is normally made and ways in which it might go wrong. This is a relatively quick procedure performed under anaesthesia. Repeated eye injections are not typically performed and the total number is limited to two injections.

We use neural implants to either shine light into the brain to activate certain cells, to take pictures of the brain, to record brain activity, or to have a slow release of drugs into the brain over a long period of time so that we don't have to do lots of repeated injections into the brain. We use injections into the brain for 3 main purposes; to change the activity of the brain cells, to label specific cells, or to generate a small demyelinated injury (or lesion, like in multiple sclerosis).

The most severe work involves causing damage to the brain so that we can see how it heals, damage is initiated by injecting a chemical into the area of the brain that we want to have the injury. We have three protocols which involve causing damage to the brain. These protocols are split into the number of brain surgeries that the animal will undergo. Our main protocol involves one surgery but can involve several procedures occurring within the same surgery. Some animals will undergo brain surgery on more than one occasion (maximum of three occasions). In these cases, one surgery will be an injection into the brain, another will be implantation of either a recording device or a cannula attached

to a minipump to deliver substances to either prevent or improve repair, and the final surgery would be the injection to generate an area of damage. When animals have multiple major surgeries, they have time to heal and recover before the next surgery, and if they respond poorly and have the most severe side effects then they are not used for subsequent surgeries. Before or after these surgeries animals may receive substances to control the repair, either by injection, infusion, or in their food or drinking water. We may also use the implanted devices to record brain activity or image labelled cells in the brain so that we can see more of the repair process and what cells are responsible for it. We can also use behavioural tests to see how these procedures affect the learning, movement, and capabilities of the animal. Some of these behavioural tests need to keep animals singly housed so that the right animal is recorded (e.g. a running wheel in their cage), some of the behavioural tests need the animals to be slightly hungry to perform the learning task, so they will be food restricted – they do still get fed everyday, just a smaller amount, as this ensures they will like their treats they receive as award for correct answer on a behavioural task. At the end of the experiment the animals are killed by various methods, such as fracturing the neck, perfusion fixation under anaesthesia, or decapitation. Where decapitation is used this is usually in alive and alert adults using a guillotine to ensure a simultaneous separation of the head.

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

Some of the work under this license will lead to moderate adverse effects, these include; genetic alterations which can lead to tremors, maintaining animals as they age so that they show signs of ageing, eye injections (under anaesthesia) which could cause irritation, brain injections (under anaesthesia) and neural implants (under anaesthesia) which can lead to temporary functional difficulties such as low level problems with walking or head tilts.

For lesioned animals, in most cases the worst clinical sign is a head tilt and some difficulties in balance when moving around that resolves in 72 hours or less. In less than 10% of cases this damage to the brain can lead to spontaneous rolling of the animal or rolling when stressed, or the animal being temporarily unable to right itself. Animals that continuously roll will be humanely killed immediately, and those that cannot right themselves and show consistent reactive rolling will be killed if they do not improve within 8 hours. In up to 15% of animals injected with one of the substances, animals may show a side effect in which they uncontrollably extend their limbs, as this can stop them from being able to move around freely they will be killed as soon as this is seen, this is like a condition called dystonia which is seen in some diseases in humans.

Painkillers will be provided to animals so that they are not in pain from any of these procedures, and during the recovery period they will be kept warm and in a low stress environment, we will also help them to eat and drink if they need it by giving them easily accessible soft food and long nozzled water bottles. Animals will be closely monitored at all times and will be checked on with increased frequency during the recovery period. Surgical procedures will be performed under general anaesthesia and sometimes multiple separate general anaesthetics will need to be used, typically after anaesthesia animals are somewhat disorientated but will be receiving painkillers to minimise any post-operative pain. Animals will only undergo multiple general anaesthetics if there is a scientific need for additional surgery, for example, in one surgery we may need to inject a substance that makes certain types of cells in the area respond to light, allow the animals to recover and the substance time to work, then perform a second surgery in which we make an injury then shine light onto the cells to see if that leads to faster healing. Animals will only be used for multiple surgical procedures if they recovered quickly

and completely from the first procedure. In general recovery from anaesthesia should be quick and animals can return to normal behaviour without much intervention. The cumulative effect of any procedures will be considered and kept to the minimum required to yield statistically meaningful data. All animals used in this license are cared for by dedicated staff who follow detailed instructions regarding their care. Humane endpoints have been set throughout this license to minimise suffering, ultimately all animals will be humanely killed and their tissues harvested for further analysis. Procedures are continually reviewed and refined whenever possible to improve the overall welfare of the animals.

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)?

	Non recovery	Mild	Moderate	Severe
Mouse	1%	86%	12%	1%
Rat	6%	44%	43%	6%

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

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 10 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 use of animals is necessary for the current project which addresses several major outstanding questions about the role of myelin in the brain and how to regenerate myelin (the fatty coating around nerve cells) and achieve full functional recovery. The results of this project may have significant clinical relevance to a number of white matter disorders.

Existing data show that for most purposes the rodent nervous system is a good model of the human one, both for normal brain function and for the disease processes with which this project is concerned. As the brain is very complex with many different cell types interacting with each other, as well as different environments within the brain, it is not possible to model in a dish (in vitro).

A large part of experiments will be conducted using mice, which are essential to this project, as it centres on using the power of genetic modification to highlight mechanisms of how myelin is formed and its effect on brain function. However, a substantial proportion of the experiments will be conducted using rats as due to their size they are the ideal animal model for toxin-induced lesion.

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

We have considered cell culture based assays; both rodent and human based.

Whenever possible culture experiments, using human central nervous system cells derived from skin cells, will be conducted in specific cell culture petri dishes in the laboratory to reduce animal use. We will also use tissue from dead animals for culture assays to reduce the invasive procedures as much as possible.

Why were they not suitable?

Cell culture based assays do not always represent an appropriate alternative to animal use, thus animal models are needed, especially to study complex environments with different cell types interacting, or to study behaviour, and are essential to understand complex disease mechanisms such as myelin regeneration and changes in the central nervous system with ageing. We know that many brain disorders worsen with ageing and replicating this process is not currently possible in a non animal model.

Though our human cell model is constantly improving we still cannot replicate the complexity of a live brain, such as in learning and memory, or the factors affecting myelin formation, damage, or repair such as in diseases like multiple sclerosis.

Existing data show that for most purposes the rodent nervous system is a good model of the human one, both for normal function and for the disease processes that we are investigating in this project. Thus, making it our best choice to investigate these complex interactions.

We use cell culture models in conjunction with animal models to provide a hierarchy of models from which a full and detailed understanding will emerge. Where possible, if cell culture models will suffice to answer the question, then they will be used in preference to animal work. As cell culture models invariably involve the use of neonatal tissue, they do not fully replicate the effect of adult ageing that are critical to achieving the programmes objectives so cannot replace all the animal work. This holds true for human cell culture models as cells derived from induced pluripotent cells also mimic foetal cells, more than mature brain cells.

A retrospective assessment of replacement will be due by 10 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?

For establishing and maintaining each transgenic rodent line, we will follow available guidance on good breeding practices. We estimate a minimum of 5 cages per line: 2 cages for the breeding pairs, and 2-3 cages for offspring and breeding stock animals (separated males and females after weaning). Equalling a total of 10-15 animals per line per week. This is the minimum number that allows for an uninterrupted flow of offspring (taking into account the <100% mating success, and the fact that at any time some of the colony will be below breeding age). To keep breeding and maintenance to a minimum for the duration of the project licence, we will cryopreserve each line and only keep lines active that are being used for experiments. Additionally, we have used our annual return of procedures data from our previous licence to estimate the numbers of animals we will need for breeding.

Experimental groups, and the number of animals required for statistically significant results, will be determined from our previous work, published data from others, and non-animal work used to identify potential mechanisms or pathways of interest. Routes, dosage volumes, frequencies and durations will be obtained from published literature where available. Pilot studies will be used to confirm that the chosen parameters are well tolerated by our specific models and genetic backgrounds, and to provide estimates of the expected variability and the size of the effect so that we can set the group sizes appropriately. Robust control groups are required to ensure validity of data – our surgical models can themselves cause responses not related to the experimental question being tested, but as a consequence of damage to the central nervous system from the implant or injection. In experiments where we implant a device for monitoring animals over multiple timepoints, we will use the initial recordings from before additional procedures to act as a control.

We will use randomisation tools in order to assign animals to experimental or control groups, and analysis will be performed using computer automation or with experimenters blinded to the conditions.

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

In all experiments we aim to use the minimum number of animals compatible with obtaining scientifically and statistically significant results. Experimental groups are chosen to contain age, sex and strain matched animals as far as is possible (on occasions where, for example, poorly breeding transgenic lines are used then groups may have mixed sexes in equal numbers).

To minimise variability control animals will be obtained at the same time and from the same supplier (or littermates if bred in house) as the animals under experimental protocols where possible, housed in the same conditions, and procedures performed by either the same researcher or with conditions divided evenly between researchers. Within the same experiment the same batch of drugs or reagents will be used for all groups.

Wherever possible data will be analysed blinded to experimental treatments until after the final statistical analysis.

The animal numbers used in experiments will be based on the number of different manipulations being carried out, and guided by animal usage that restricts animal numbers to the minimum. Preliminary experiments and existing literature will be used to calculate, using reputable statistical experimental design tools, required sample sizes prior to the any full experiments being carried out, to ensure that we generate statistically meaningful data.

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

In order to further reduce overall animal usage in this licence, where possible we design experiments across experimenters in the laboratory in such a way that tissue from an animal can be used for other experiments, for example one researcher using the brain, and another the optic nerve, or sharing of embryonic tissues so that the full litter can be used. Furthermore, we bank tissue from our transgenic animals for a few years, so these tissues can be used for future experiments instead of needing to set up a new experimental procedure.

Where cross fostering of pups is required, foster mothers will be preferably sourced from within our ongoing breeding colonies rather than having additional matings set up as potential foster mothers.

We have established and will further explore, replacing some cross-sectional studies (where animals are killed at different timepoints) with longitudinal studies (where the same animal can be used to give results at different timepoints), to both reduce animal number but also improve the scientific output of those studies.

Whenever possible culture experiments will be conducted, however, cell culture based assays do not always represent an appropriate alternative to animal experiments. Thus, animal models are needed, especially to study complex environments with different cell types interacting, or to study behaviour. From our preliminary cell culture work using rodent myelinating co-culture, and primary oligodendrocyte cultures we have already identified candidate pathways and genes that may regulate myelination, this preliminary work reduces the numbers of animals used for the animal work as we are only taking forward promising candidates from our preliminary data.

We have access to a recently set up user run email list, where surplus wildtype animals generated from breeding and maintenance protocols can be shared. We have used this list to offer and obtain animals for control tissues, with the aim to minimise the purchase of wildtype animals where possible.

When our GA rats have reached the end of their breeding and maintenance protocol, we have coordinated with other labs to provide them with tissues or blood after sch1 killing. We also do this with our wildtype rats bought in for tissue culture whenever possible.

A retrospective assessment of reduction will be due by 10 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.

These experiments will largely be conducted using genetically altered mice. However, a substantial proportion of the experiments suggested will be conducted using rats as, due their size, rats are the ideal animal model for toxin-induced lesion, in addition to cell culture methods being well established for rat tissue. In some cases genetically altered rats will also be needed for experiments, these modifications will also be used to highlight mechanisms of myelination, in experiments which require the increased size of rats and are currently not possible to perform in mice. We use rats and mice because they are the least sentient species that can model glia-neuron interaction, myelination, cognition and behaviour in neurological disorders.

For ageing experiments, animals will only be kept if they are showing no, or minor and controllable, ill effects from growing older. Teeth checks and general health monitoring ensures that animal suffering is kept to a minimum.

To induce gene expression or to modulate or deplete specific cells, some animals will be given substances by mouth, injection, oral gavage, or in their drinking water or food. We also use behavioural tests in some animals to see the functional effect of our modifications. The behavioural tests chosen are mostly observations of movement or teaching animals to use a touch screen and 'punishments' are in the form of lights or noises rather than electric shocks or other painful punishments.

Injections into the eye are used because the optic nerve is normally fully myelinated with a well known timeline of myelination and this makes it an ideal model system that allows us to investigate how myelin is normally made and ways in which it might go wrong. Eye injections are safe and are routinely used in human patients. The eye injections take place under general anaesthesia (human patients receiving eye injections generally just have local anaesthesia) to ensure that the distress of the animals is kept to a minimum.

For some experiments we will use injections into the brain or spinal cord, with or without implanting a device to record or stimulate the area, this is the most refined method to provide a local effect on the

brain. Targeted manipulations in this way are more refined than disrupting the whole brain or spinal cord. We also use injections to transplant cells into the central nervous system.

In our most severe work, we will use brain lesion models in order to investigate the myelin regenerative (healing) process. Our lesion models create small areas of myelin damage and have been chosen partly because of the minimal behavioural and movement side effects. For example, our primary model does not cause progressively worsening symptoms, i.e. the animals are able to move freely and perform normal physiological functions, like feeding, drinking, grooming and movement after the initial surgical recovery period. Our lesion model is carried out by performing an injection under anaesthesia into a specific area of the central nervous system, animals get better over time and recover back to their pre-lesion state of fitness within 1-3 days, though the symptoms immediately post lesion surgery can be severe and distressing to the animal. Other lesion models can cause lasting or progressive symptoms such as paralysis or movement problems.

In undertaking these procedures, we are continually assessing how the procedures can be refined in order to minimise the discomfort that the animals may experience and seeking to replace procedures using live animals (in vivo) with procedures using tissues after death (ex vivo) where possible.

We take the welfare of the animals very seriously and routinely use pain relief and provide appropriate supportive care during surgical procedures, we familiarise animals to handling for procedures where handling is necessary, and increase monitoring of animals that have undergone invasive procedures so that we can quickly identify and treat or minimise any adverse effects arising. Stress can also affect the healing process that we are investigating so it is important for the animals to be in the best possible condition not just from a welfare perspective, but also to make sure that we get good data. The humane end points proposed are a balance between the suffering of one animal and the potential need for a replacement animal to undergo procedure. Due to the nature of the processes that we are investigating, some clinical signs associated with myelin deficits are to be expected, we have already set our humane endpoints to be prior to the onset of more severe clinical signs.

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

Myelin only occurs in vertebrates (animals with a backbone) and so non-vertebrate species are inappropriate for myelination and myelin regenerative studies.

We have tried to establish a model to study developmental myelination and its role on brain development in a frog larvae. However, experiments are confined to the early developmental period, and experimental methods to study myelination are not well adaptable to this model making it hard to use for some of the fundamental questions on myelination. Furthermore, it is not suitable as a model to study the role of myelin and glia on neuronal circuit function with age and myelin regeneration. Indeed, there are no demyelination/remyelination models that have been developed in birds, reptiles, amphibians or fish.

An overwhelming majority of studies on the biology of CNS regeneration have been undertaken using laboratory rodents, especially mice and rats. Thus, for this work to contribute to mainstream translation-relevant research it is necessary to use mice and rats. These are also the mammals lowest on the evolutionary tree likely to produce satisfactory results. Much of the proposed work will use ex

vivo tissue and/or in vitro methods, however due to the genetic manipulations of the animals this tissue is not generally available from established tissue banks.

Currently the rodent nervous system is the most suitable model of the human one, both for normal brain function and for the disease processes with which this project is concerned.

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

We have refined many steps in our procedures to minimise welfare cost to animals, and we will continue to do so for the duration of this licence. Wherever possible we will use the least invasive method of administration and the minimal number of surgical interventions required to generate meaningful data.

Over the course of this license we will seek out potential refinements by;

1. Evaluation of alternative forms of tamoxifen (a gene inducing agent) to minimise the ill effects associated with its use.
2. Frequent monitoring of animals during recovery periods.
3. Personal license holders working under this license will meet regularly to review the methods used and identify any issues or areas of potential improvement.
4. Attendance at locally run method workshops, such as tamoxifen workshops, where the different methods of administration are discussed in terms of how well they work and animal experience. Where the conclusions of the workshop represent an improvement on current methods without compromising ongoing experiments we will switch to the new method if covered under this PPL and/or amend this license if required.
5. Monitoring the field for new developments and technologies to improve procedures. For example, we will test the use of powered minipumps with a larger reservoir rather than needing to replace osmotic minipumps.

The refinements we have established under our previous licence or within this new license are as follows:

1. We have refined our methods to minimise stress to the animals by administration of post-operative pain relief in jelly – this means that the animals often do not need to be restrained in the recovery period post-surgery and do not need analgesia injection (other than the pre-operative analgesia). This refinement has been widely shared and implemented in other labs.
2. We have further extended our jelly refinement to cover administration of experimental test substances in jelly/chocolate spread wherever possible, thus minimising the need for daily injections or minipump implantations.
3. We have incorporated the use of minipumps to replace large numbers of injections, to reduce the handling stress to the animals and minimise the risk of needle injuries, to be used when

administration in the food or drinking water is not a suitable route for dosing animals.

4. We have introduced the use of an analgesic cream on the ear bars of the stereotactic frame to minimise post-operative discomfort.
5. We have refined the grading system from previous licenses based on our increased surgical experience and introduced some additional grades and clarification of how animals will be graded to maximise replicability of severity reporting between users, further detail on the grading system is found within relevant protocols. Animals that are not showing signs of improvement will be killed, but those animals showing consistent improvements over time without reaching the humane end points detailed within the license will be kept such that future surgeries are minimised.
6. For our most severe protocols involving multiple stereotactic surgeries, we have introduced limits so that animals experiencing severe side effects after the initial surgery are not used for any subsequent surgeries, to ensure that animals do not experience severe suffering twice.
7. We have implemented a non-recovery protocol for injection of fast-acting-labelling substances into the brain to minimise the overall severity experienced by the animals and avoiding the adverse effects associated with brain injections and surgery.
8. We have developed body condition score sheets and/or clear grading systems based on input from the NVS, guidance on actual severity reporting, the adverse effects described in the literature and adverse effects encountered in our own experience. These score sheets and grading systems will be made available to animal technicians as well as personal license holders to ensure that there is reproducible identification of potential problems as well as recognition of subtle combinations of effects which could impact on animal welfare. These score sheets and grading systems will be regularly reviewed to ensure relevance and accuracy.
9. We have generated a new mouse model an alternative to the shiverer mouse, this has numerous advantages over the shiverer mouse as they are overtly normal and do not show seizures, tremor, or the reduced lifespan that is seen in shiverer mice. This will be shared with the scientific community to minimise the overall use of the shiverer mouse worldwide.

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

We aim to follow the best practice guidance to ensure experiments are performed in the best possible way. To achieve this, we do the following;

1. We adhere to the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) and Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.
2. We have and will continue to adopt the Laboratory Animal Science Association (LASA) Guidance on Preparing for and Undertaking Aseptic Surgery (2017) and the Home Office Minimum Standards of Aseptic Surgery. We participate in workshops relating to surgical procedures and follow the literature on how to keep our methods up to date with the best possible practise.

3. For breeding we observe the guidelines provided by the Home Office and the NC3Rs Resources on 'Genetically altered mice' and reputable laboratories for best practices to breed genetically altered rodents.

4. We have refined methods to administer drugs, and chemicals, we always seek the least invasive method, such as minimising injections of painkillers after surgical procedures by administering in jelly or chocolate spread. We will continue to develop new methods, and follow new developments in the field, on refining procedures for the administration of substances. Again, we will refer to the NC3Rs website for guidance.

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

We receive the NC3R newsletter that highlights latest NC3R publications and advances. We will regularly attend available workshops on welfare. We regularly discuss our work with the animal facility staff, and named veterinary surgeons, as they are aware of new developments and have good guidance.

We are active users of our 3Rs search tool, that is regularly updated. This database for example contains helpful information on ways to reduce the harm to animals used in research. In addition, it has helpful advice on how to reduce the number of animals we use in experiments. 3Rs updates and suggestions are regularly shared with license holders.

Within the neuroscience field, when new technologies become available they are often presented at conferences or published in scientific journals, we attend numerous conferences and frequently check available publications for advances in the field. Where these new technologies offer the opportunity for reduction or refinement of animal procedures, in a way not covered by our license, then we will apply for an amendment to allow pilot studies of their use. Where these new advances offer the chance of replacement of animals then we will compare them to our existing animal models to validate them for our research questions prior to adopting the new technology or technique.

A retrospective assessment of refinement will be due by 10 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?