Home Office

NON-TECHNICAL SUMMARY

Neurovascular breakdown in dementia risk and progression

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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

brain, vasculature, Alzheimer's disease, neuron, menopause

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult, Aged animal

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?

We want to understand how the brain keeps its blood supply working well. Usually, when brain cells become active, nearby blood vessels open up to deliver more oxygen and nutrients. This partnership between brain cells and blood vessels is called neurovascular coupling. We will study how this process changes as the body ages, during menopause, and as diseases such as dementia develop. When this system stops working properly, the brain becomes more vulnerable to damage. By finding out what goes wrong, we hope to develop ways to protect the brain. This could include simple behavioural changes or repurposing clinically available medicines that help keep blood flow healthy and support brain function.

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?

Understanding how brain cells control their blood supply helps us learn how the brain gets the energy it needs to function properly. In many brain diseases, including Alzheimer’s disease and dementia, the blood vessels in the brain begin to change, often before any damage to brain cells can be seen. These early changes may play a key role in how the disease develops and progresses, but we still don’t fully understand how problems with blood and energy supply affect brain health over time. There are several known risk factors for dementia (such as ageing, carrying the APOE4 gene, or going through menopause) that also impact the brain’s blood supply. By studying early changes in blood vessels in the context of disease risk and progression, we hope to find new ways to protect the brain and prevent Alzheimer’s disease from developing.

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

I expect to generate new knowledge about how the brain regulates its blood supply during healthy ageing, menopause and early disease, especially when several risk factors occur together. This reflects real human experience and should make the findings more relevant. I aim to identify the biological pathways that change during these stages and highlight those that may offer the best opportunities for future prevention or treatment.

A key output will be a new dataset from RNA sequencing. This technique shows which genes are switched on or off in cells by measuring the RNA “messages” that control how a cell behaves. By examining these signals, you can see how brain blood vessels alter their activity with age, menopause, or different APOE genotypes. APOE is a gene that comes in several common versions (APOE2,

APOE3 and APOE4), and these versions can influence a person's risk of conditions such as Alzheimer's disease. Studying how these risk factors affect RNA signals will help reveal which biological pathways are changed and could be targeted by new therapies.

I will publish the results in scientific journals and present them at conferences, and I will make the datasets openly available so that other researchers can build on this work. The project will also create well-characterised animal models that can support further research in this field.

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

This work will first benefit the research community by filling a major gap in how ageing, menopause and genetic risk factors influence the brain's blood supply. By refining and standardising the mouse menopause model, the project will improve animal welfare and provide clearer guidance for other laboratories. The datasets, methods and openly shared resources will help other researchers design better experiments, reduce unnecessary animal use, and develop new ideas more quickly.

The findings should also help shape clearer health messages for the public. Showing how heart and blood vessel health supports brain function can reinforce practical steps people can take in midlife to lower their risk of later cognitive decline. I will also compare the pathways identified in mice with data from local human tissue banks, including peri- and post-menopausal donors, to strengthen links between laboratory research and clinical understanding.

Longer term, the project aims to highlight the biological pathways most likely to matter for prevention or early treatment. This could guide future therapeutic development and support new clinical collaborations. Even before any new treatment emerges, better information and clearer public guidance can help people make informed choices that support their long-term brain health.

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

We will maximise the effects of outputs of this work by ensuring we have engaged most fully with those who will benefit from this approach. We aim to reach patient groups and members of the public via clinical links available through clinician colleagues or partnerships supported through local dedicated centres. Collaboration is a very important part of almost all of our projects to maximise the success by linking with experts who can help us to achieve ambitious experimental aims in novel directions to answer our important questions. By collaborating with others on these projects, and sharing our own expertise in other collaborations, the number and impact of our outputs will be maximised.

Our publishing strategy focuses on sharing coherent research narratives in high-impact journals, ensuring each output communicates a clear message. Where this is not possible, due to incomplete datasets or limited resources, we will still publish findings in appropriate formats. This includes negative or unexpected results, which are scientifically valuable when they challenge assumptions or support null hypotheses. We are committed to transparency and believe that all results, even those outside the main narrative, should be shared to support progress across the field.

We will also, as detailed above, aim to communicate our results widely in academic and lay circles, so that the impacts are broadly felt.

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.

We use mice to study how the brain controls its blood flow, because their brain and blood vessel systems work in very similar ways to ours. Many useful mouse strains already exist, including ones that carry human genes linked to Alzheimer’s disease. For example, some of our mice have the human APOE4 gene, which increases a person’s chance of developing Alzheimer’s. Others have the APP/PS1 mutation, which causes the brain to form amyloid plaques—tiny clumps of protein that build up between nerve cells and are a key feature of Alzheimer’s. These models help us study how early disease changes begin long before symptoms appear. We will also bring on menopause in mice in a way that reflects the stages seen in humans, including an important transition phase before periods fully stop called 'perimenopause'.

Mice are especially useful for imaging because their small size allows us to inject fluorescent dyes into the bloodstream and use microscopes to watch their brain and blood flow. We can even do this while the mice are awake and moving on a treadmill, which avoids the effects that anaesthetic can have on blood vessels. Imaging awake animals causes minimal suffering when coupled with prior training of the mice. We train each mouse slowly and gently to get used to the system where they are head restrained under our imaging microscope. This is done over several days by slowly increasing the mouse's exposure and time on the system, and by giving them positive rewards afterwards, such as giving them a fruit juice soaked wooden block or some low-fat seeds in their home cage. This approach helps to keep the animals calm and relaxed during imaging.

Most of our work uses young to middle-aged mice so we can study how blood vessels and brain cells work in healthy conditions, and how early risk factors for Alzheimer’s begin to change these systems. In some cases however, we do include older mice to understand how ageing (the biggest risk factor for dementia) affects the brain’s blood supply.

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

Most mice in this project will have a small window inserted in the skull and a round metal headplate with horizontal arms to fix them under our microscope while they are awake and behaving. In the same procedure, or a later one, we may also place a tiny pump under the skin called an osmotic minipump. This pump slowly releases chemicals that model conditions such as high blood pressure. If the amount of a chemical, or how long it needs to be given, is not suitable for delivery using a small implanted pump (for example when inducing menopause), it will instead be given as repeated injections into the abdomen. To reduce discomfort, the injection side and exact location will be alternated each day. After

surgery, each mouse is given time to recover and is gently habituated to sit comfortably under the microscope while standing on a mobile homecage locomotion tracker. The mobile homecage locomotion tracker is suspended on a cushion of air with no motor. Instead, the mouse sets the cage in motion with its paws. Due to the cage's low weight, the effort required to move the cage is minimal. The animal has an illusion of exploring the cage, while, in reality, it stays firmly head-fixed during imaging and recording.

Habituation to head restraint involves exposing the mouse to the experimenter and imaging equipment (session 1), before gradually increasing the amount of the time the mouse is restrained (e.g. session 2: 1 minute; session 3: 5 minutes; session 4: 10 minutes; session 5: 20 minutes; session 6: 30 minutes). The mouse behaviour will be assessed during habituation to head restraint to monitor stress levels using a 'habituation to head restraint scale', and animals which show signs of stress will be removed from restraint and given at least 24 hours before any further head fixation.

At least two weeks before our imaging protocol starts, we may also inject a harmless fluorescent marker so we can see nerve cell activity. Each mouse is imaged several times before, during and after menopause by fixing them under the relevant imaging equipment through head fixation. The imaging techniques we use allow us to record the activity of brain cells and/or blood vessels. Across all our imaging techniques, we may gently stimulate the whiskers with a puff of air or mechanical brush to cause the nerve cells, and subsequently the blood vessels, to become active. For each multiphoton microscopy session which uses fluorescence imaging, we inject a dye intravenously that lights up the blood vessels immediately before imaging so that we can record brain activity and blood vessel dilations. And, if the mouse carries genes linked to Alzheimer's disease, we can also label amyloid plaques (the protein clumps seen in the disease) with a single injection the day before so we can also see them under our microscope. Alongside imaging, we run simple behaviour tests to test their memory and activity levels. Our behaviour tests may be used to assess the impact of genetic, surgical, pharmacological or lifestyle (e.g. exercise or diet) interventions on cognitive function. During our behaviour tests we will typically use video monitoring in arenas with objects, escape routes or mazes, as well as recording behaviour within the home cage.

After all imaging is finished, the mouse is humanely culled so we can study the brain in more detail. This allows us to examine blood vessels, signs of inflammation, leakage in the blood–brain barrier and areas that may not have received enough oxygen.

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

Mice that undergo surgery may experience pain during recovery, though this is managed with pain relief drugs. Mice usually recover very quickly from surgery but can be subdued for a few hours. We can treat them with more pain relief drugs and also give them soft food to help them recover. We monitor the animals closely after surgery to see if they need extra care. If they do not get better after giving them more care, then we cull them by 4 hours post pain relief administration, so they do not continue to suffer, or immediately if their suffering is prolonged and/or judged likely to exceed moderate.

Mice may experience a very short discomfort during injections that are needed to control protein expressions, or to deliver drugs or dyes into blood vessels. However this discomfort would typically be mild and transient.

Mice may be initially stressed when getting used to being held in a head restraint under the microscope, but we accustom them to this gradually so that they only suffer a very short amount of stress (~1 min).

Some mice, mimicking aspects of Alzheimer's disease, may develop some memory problems but we do not expect this to impact on their welfare.

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)?

Protocol 1: Breeding. 3000 mice, 100% mild.

Protocol 2: Breeding. 1000 mice, 75% mild, 25% moderate.

Protocol 3: Cranial window surgery. 1800 mice, 100% moderate.

Protocol 4: Micro LED implant surgery. 250 mice, 100% moderate.

Protocol 5: Electrophysiological implant surgery. 250 mice, 100% moderate.

Protocol 6: Behaviour testing without surgery. 500 mice, 75% mild (375 mice), and 25% moderate (125 mice) where transgenic animals developing Alzheimer's pathology are aged or experimental manipulations are applied to induce sickness (e.g. 4 mg/ml LPS).

What will happen to animals used in this project?

- Killed

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 the most suitable model for this work because they are living animals with a full cardiovascular system and an intact brain, which allows us to study how factors such as menopause, oestrogen treatment, and lifestyle affect brain health and cognition. These processes are highly complex and currently cannot be replicated in vitro. Alternatives such as ex vivo tissue culture or microfluidic “organ-on-a-chip” devices are limited. Post-mortem tissue lacks active blood flow, preventing the study of dynamic neurovascular responses. Organ-on-a-chip devices can model specific cellular processes but cannot reproduce real blood with oxygen-carrying red blood cells, the full branching vascular network from pial arteries to capillaries, regional differences across brain areas, or long-term systemic conditions such as menopause, ageing, chronic hypertension, or sedentary

lifestyle. Neuronal circuits in chips are simplified, so complex brain activity and behaviour, including memory, cannot be studied. For these reasons, while organ-on-a-chip systems are useful for reductionist questions, they cannot replace live mice for studying chronic, whole-organism interactions between blood flow, hormones, genetics, and cognition.

Cell cultures or isolated vessels are also insufficient because neurovascular cells behave differently outside the intact brain, and the spatial organisation of cells, which is critical for proper function, is lost. Simplified or acute systems can exaggerate disease-related effects because the neurodegenerative brain is highly sensitive to stress or invasive procedures. By using chronically prepared mice and allowing recovery before imaging while awake, we can capture gradual, physiologically relevant changes rather than artefacts caused by acute stress.

We specifically use mice because genetically modified strains are readily available, allowing inclusion of human genes relevant to Alzheimer’s disease, such as APOE4 or APP/PS1. Their small size allows gentle head fixation under a microscope to record activity from both neurons and blood vessels, providing detailed, physiologically relevant data. In vivo data from mice can also inform computational models, for example by measuring blood oxygen to simulate vulnerability across brain regions, and RNA sequencing can reveal vascular pathways altered by ageing, menopause, or genotype, guiding targeted analysis of human tissue and bio-bank datasets. Where possible, we plan to extend our findings to computer simulations and hypothesis-driven studies of human data, minimising unnecessary duplication and maximising translational relevance.

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

Non-protected species such as drosophila were considered. Searches of PubMed, Google Scholar and Web of Science searching terms like “neurovascular coupling,” “menopause,” and “APOE” alongside "drosophila", show that while APOE flies exist and some cognitive testing is possible, it is not possible to induce menopause or study neurovascular coupling in the brain while awake animals are head-fixed beneath high resolution microscopes. Because our questions specifically require intact blood flow, genetic risk factors, and menopause, mice remain the most appropriate model.

We use several non-animal approaches where appropriate. For example, we employ cell culture models to screen multiple pharmacological interventions in high-throughput experiments, typically using tissue from a single animal. Only the most promising candidates are then tested in vivo, where neuronal–vascular communication remains intact. We also use post-mortem tissue to minimise procedures on live animals, and computational modelling to simulate cell activity and nutrient diffusion, extending findings from ex vivo data.

Once key mechanisms have been identified under controlled preclinical conditions, we are also considering translating these findings to human data. This will include assessing corresponding features in post-mortem brain tissue from peri- and post-menopausal women held in the local brain bank, and examining large-scale imaging data from UK Biobank participants. Such integration ensures that mechanistic discoveries made in animal models are cross-validated against human physiology and population-level patterns, strengthening their translational relevance.

Why were they not suitable?

Cells can change their nature in culture, and tissue slices do not preserve the intact vascular system, so it is important to also conduct experiments on alive animals that have nervous and vascular systems intact. Computational modelling is only informative if you have sufficient information about the system you are modelling, and can complement well-designed experiments on animals to maximise data and understanding from these experiments.

The animal work is essential to guide meaningful translation to human datasets. Large-scale resources such as brain banks and UK Biobank contain vast amounts of data, but without specific mechanistic targets these datasets are often too broad to yield interpretable insights. Controlled preclinical experiments allow us to identify precise cellular and vascular mechanisms of interest, such as specific signalling pathways, vessel types or oscillatory features that change with menopause. These findings then inform targeted analyses in human tissue and imaging data, ensuring that the questions we ask of these complex datasets are biologically grounded and hypothesis-driven rather than exploratory.

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?

Based on our experience from closely comparable experiments in previous work, we have a good understanding of the likely number of mice required. To date, similar studies have consistently used fewer animals than the maximum requested here. The numbers proposed are therefore intended as a conservative upper limit, set to avoid underestimation while remaining consistent with the principle of Reduction. Actual animal use will be reviewed throughout the project and adjusted downward where possible as data accumulate.

We will have a team of four researchers initially (myself (principal investigator), postdoctoral research associate, research technician and PhD student, but will adjust numbers on the licence with amendments if more members join the team and it becomes apparent that our estimates are inaccurate.

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

During the design stage, we planned the project so that each animal gives the maximum amount of useful information. We used the NC3Rs Experimental Design Assistant to map out all procedures and time points, and to ensure the design was efficient and avoided unnecessary use of animals. The study plan was reviewed by expert panels and external referees during the funding process, which confirmed that the design was scientifically sound and ethically justified.

Group sizes were chosen using guidance from a statistician and based on data from previous studies, so that numbers are high enough to give reliable results but not higher than needed. These sample sizes will continue to be reviewed and adjusted as new data emerge.

We also reduce numbers by collecting several types of complementary data from the same animal. This allows us to link changes in blood flow, oxygenation, behaviour and tissue measures within individuals by applying advanced statistics (e.g. linear mixed-effects models), which reduces variability and lowers the total number of animals required.

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

We aim to predict the number of mice we need to breed to maintain the optimal number of breeding pairs at any one time. Wherever possible, we also use non-experimental genotypes to sustain our colonies. Pilot studies with a small number of mice are conducted to test new experimental ideas, estimate effect sizes, and ensure that only the optimal number of animals are used to detect meaningful effects. This also helps to refine data collection and avoid using mice in experiments unlikely to produce usable results.

We will limit animal variation by using strains of the same background, age, and weight; limit nuisance variables by standardising the time-of-day procedures are performed, or data are collected; and use standard protocols in experimental procedures and data analysis.

In our data analysis, we maximise the data collected from each mouse, using tissue and imaging from one experiment to serve as a control group and/or generate preliminary data for another project where appropriate. For example, APOE3 or APOE4 mice without additional interventions (such as menopause induction, HRT, exercise, or ageing) can serve as shared control groups across multiple planned studies. This approach allows us to reduce animal numbers while obtaining robust and reproducible data across experiments.

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 genetically-altered mice expressing human proteins to mimic aspects of human disease, and we will inject substances to mimic human menopause and/or label different neurovascular cells allowing us to monitor their function. Our most common form of menopause induction is chemical, avoiding the more invasive surgical method of removing the ovaries. The chemical induction of menopause involves 21 days of daily dosing with 4-Vinylcyclohexene diepoxide (VCD), which can be delivered through injections where the experimenter will alternate injection sites to minimise

discomfort. Mice typically recover well from this, although there may be some skin discomfort associated with repeated injection or surgical implantation, which may be treated with topical ointment and/or analgesia if necessary. We use mice because there are lots of genetic tools available, but also it is possible to do the experiments we need while maintaining a good standard of welfare in mice. Mice are small, which means they can be housed in cages which give them enough space to be active and to socialise. They are able to fit under our microscopes and we can see far enough into their brains for our experiments to work. They can be handled regularly to get them used to us and the experimental process.

The genetic alterations in our mice generally do not cause them any harmful effects. The most damaging manipulation we use models some aspects of Alzheimer's disease by expressing a human genetic risk factor and the human protein that is broken down to produce beta amyloid, a peptide that aggregates to damage nerve cells in human Alzheimer's disease. However, in our disease-risk (APOE) mice we have not previously observed any memory or physical problems, and in our disease-onset (APP/PS1) mice whilst cognitive deficits in spatial learning and memory have been reported in the literature at 7 months, we predict only mild memory problems in this model during ageing which we do not expect to have any welfare implications, as we have not observed object recognition or spatial memory problems (vs wild-type) on the novel object recognition task or Barnes maze by 12 months. We do plan to age some of our disease mice up to 24 months, and so aged mice will be monitored more closely to assess for adverse effects (e.g. weight loss).

Many of our experiments involve performing surgery on mice to insert a small window in the skull. Mice usually recover very well from this surgery, and are grooming, eating and drinking within a few minutes of coming round from the anaesthetic. In accordance with the named veterinary surgeon (NVS), following surgery we will use analgesia to reduce any associated pain, and we will generate study plans ahead of surgery which will be shared with relevant team members, technical staff, and the NVS to summarise how animals will be monitored. We handle the mice regularly so they are used to human contact. We then get them used to our recording apparatus very gradually, increasing time on the apparatus only when the mice are no longer stressed by it. This means that they are relaxed during our recording sessions, minimising their distress.

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

We need to use animals that are sufficiently similar to humans to be informative about how human physiology and disease work. Mice have a similarly organised brain and vascular system and we can use measurements, such as the level of blood oxygen, that are also used in humans to compare and check the validity of our experiments for understanding humans. Our experiments on live mice are necessary to study the interactions between the nervous and vascular systems, but where possible we use other experiments on cultured cells, or brain tissue from mice that are no longer alive, to minimise the level of any harm, as well as computer simulations. These experiments are good for learning about specific signalling pathways, and how simple systems work, but we need the experiments on more complex systems to understand how these simple systems interact in real life. In mice there are also many genetically modified strains which allow us to study the impact of disease relevant genes (e.g. APOE4 or APP/PS1) on brain health or behaviour.

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

Throughout my research career, I have actively refined experimental methods to reduce pain, distress and the risk of harm to animals.

Previous refinements have focused on surgical techniques and post-operative outcomes. For example, we modified cranial window implantation by emphasising careful preparation of the skull surface before attaching the head plate. This improves implant stability, reduces the likelihood of implant failure, and allows early intervention if loosening is detected. As a result, this refinement reduces the risk of discomfort, repeated anaesthesia, or premature euthanasia before the planned study endpoint.

We have also refined behavioural and recording procedures to minimise stress. Recording sessions were adjusted to be shorter but more frequent, which improves habituation to head restraint and reduces the duration of each restraint episode. This allows animals to return more quickly to normal behaviours such as grooming and resting. Welfare observations, including video monitoring and scoring, were carried out when introducing these changes to confirm that they did not increase stress or discomfort, and findings were shared with the Biological Services team.

Environmental enrichment is used as an additional refinement to support normal behaviour and reduce stress. In the home cages, animals will be provided with nesting material, shelters for resting, and wooden blocks for gnawing, and will be group-housed where compatible. For animals undergoing habituation or training (e.g. to head restraint), positive reinforcement will be used, such as small food rewards (e.g. seeds or squash-soaked wooden blocks). These measures encourage species-typical behaviours, improve coping with experimental procedures, and help minimise anxiety and distress.

Further refinements have reduced invasiveness where possible. For multiphoton imaging, we now use subcutaneous injection of lower-molecular-weight fluorescent dyes when appropriate, as this avoids the need for intravenous injection. Intravenous delivery is only used when scientifically necessary, such as in aged or disease models where vascular leakiness means subcutaneous dyes are unsuitable.

All procedures described in this licence incorporate current refinements. However, refinement is treated as an ongoing process. Animal welfare is reviewed continuously, and methods are updated as new evidence, experience, or best practice guidance becomes available, in consultation with Named Persons and relevant experts.

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

We use a range of resources, including academic papers using similar methods or mouse lines. We have also contributed ourselves to the academic conversation regarding best practice for in vivo imaging, for instance we published papers highlighting the importance of good anaesthetic regime, gradual habituation to head restraint and/or monitoring locomotion during awake in vivo recordings. We also refer to resources and publications provided by organisations including NC3Rs and LASA, including the LASA/RSPCA report on avoiding animal mortality, the LASA guide to aseptic technique, the NC3Rs newsletter and the ARRIVE 2.0 guidelines for reporting of animal experiments.

Other useful resources we will use include RSPCA Animals in Science department resources, Replacing Animal Research for 3Rs updates, Mousewelfareterms.org for a list of terms that are useful for assessing and describing mouse welfare, and the information provided by vendors of genetically

altered mouse lines to plan for known phenotypic effects found in those strains (e.g. references and information on www.jax.org). As well as the Morton et al. (2001) guide to 'refining procedures for the administration of substances'. We have included a table of maximum dose volumes per route to our protocols in conjunction with the NVS and based on various sources. We will also implement standardised scoring measures such as weight loss (no greater than 15%), mouse grimace scale (MGS) and/ body condition scoring to monitor mouse welfare and humane endpoints were needed (e.g. for post-surgery recovery).

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

Advancing and refining our methods is an ongoing process. We regularly receive updates from the Laboratory Animal Science Association (LASA) and the NC3Rs, which provide valuable information on training and new approaches. We also hold regular meetings with our Biological Research Facility, who can suggest refinements based on practical experience. While learning about developments in other research groups is useful, it is by critically evaluating and reflecting on our own experiments that we can most effectively implement improvements to enhance animal welfare. For example, in a previous project I refined our post-surgery protocols to improve recovery by delivering analgesics in wet mash, which mice consume more readily, transferring them into a heat box immediately after surgery, and keeping them in a warmer room for the three days following recovery. These refinements were developed iteratively through advice from other researchers, the Named Animal Care and Welfare Officer, and the Named Veterinary Surgeon, demonstrating how small changes can meaningfully improve welfare outcomes.