Home Office

NON-TECHNICAL SUMMARY

Calcium-permeable channels and mechanobiology in health, disease and therapeutic development

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Heart disease, Vascular disease, Liver disease, Exercise, Therapy

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

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?

The aim is to advance understanding of calcium-permeable channels, associated calcium-regulated mechanisms and other related mechanobiology in health and disease and to investigate ways in which this information might be used to improve the lives of people who have conditions caused by defective calcium-permeable channels, associated calcium-regulated mechanisms or other related mechanobiology manifesting in heart and cardiovascular system diseases.

A retrospective assessment of these aims will be due by 04 February 2027

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's 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?

It is important because it has possibilities to help humanity address common problems such as heart and blood vessel diseases (cardiovascular disease) and associated conditions such as diabetes and liver disease. These problems are major reasons for premature death, reduced quality of life, costs to health services and reduced work efficiency and work lifetime. Globally, cardiovascular disease is the most common cause of death. Physical inactivity, which is a failure to sufficiently stimulate normal mechanobiology mechanisms, is the 4th leading cause of mortality globally (World Health Organisation) and responsible for 1 in 6 deaths in the UK (UK Government). It often leads to cardiovascular disease and related conditions such as diabetes.

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

Our ultimate goal is to provide health service personnel new therapeutics for treating and managing cardiovascular diseases. We expect numerous original high-quality research discovery and translational papers to be published in competitive peer-review journals under open access licence agreements, open access review articles on this topic in peer-review journals, advancement of new scientific careers and new fruitful clinical research partnerships and international research

collaborations. We expect to see new small-molecule modulators of these mechanisms (i.e., new drug-like molecules) with demonstrated effectiveness that enter licence agreements with pharmaceutical and other commercial partners so that they can be developed as new therapeutic drugs.

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

During the period of the licence and beyond, beneficiaries will be other scientists, research funding agencies including charities, our own establishment, other universities, university undergraduate and postgraduate students, postdoctoral researchers and research technicians. There will similarly be benefit to scientific knowledge and therefore to deep knowledge available to humanity for improvements to and protection of life. Potential beneficiaries in the period of the licence and beyond will be commercial partners and associated investors working with us to develop new therapeutic agents. There may be benefit to patients in the life-time of this licence but it is more likely that benefits of this type will be evident in the longer term, such as 10 years; because sufficient time needs to be allowed for drug safety and efficacy trials in large patient cohorts.

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

We will look to maximise outputs through collaboration with basic science and clinical partners at our own establishment and partner establishments.

We will look to publish all findings, including unsuccessful approaches, wherever possible. We will deposit complex data sets such as 'omic' data (e.g., transcriptome results from RNAseq studies) in public repositories.

Species and numbers of animals expected to be used

- Mice: We estimate using 12,500 mice in this project (i.e., 2,500 per year), with about 3,000 of these mice used for experiments (i.e., 600 per year).

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice have been selected because they have established suitability for gene modification experiments and subsequent detailed investigation of in vivo and ex vivo phenotypes. Many of the modified mice we need have already been engineered by us or are available from repositories. Mice are the smallest mammal in which it is currently possible to combine studies of gene modification and invasive approaches for measurement of the relevant biology, including cardiovascular, metabolic, hepatic functions and exercise responses. The breeding cycle is relatively rapid and the lifespan short, enabling studies of the impact of gene modification through to old age within a 5-year programme of research. The widespread use of mice as a model of humans will facilitate comparisons of our data with

data arising from other research groups and enable independent cross-validation of our findings (e.g., by sharing genetically modified mice and studying them using common analysis systems). While all mammals have differences (e.g., mice are much smaller and shorter-lived than humans and have a faster heartbeat), mice are widely accepted as a suitable first step for exploring the in vivo relevance and therapeutic potential of mechanisms. Like humans, mice have strong desire and capability for voluntary long-distance running, which is important for our research proposals to transform understanding of the health benefits of exercise through determination of the molecular detectors of the mechanical forces of exercise; importantly, prior studies show that exercise is protective against disease in mice and that they are a good model for many fundamental aspects of the benefits of exercise seen in humans.

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

The first action will be to breed existing genetically modified mice or introduce new genetic modifications aimed at subtle gene changes that do not threaten life but alter physiological responses and may cause a disease-like phenotype. A typical aim is to disrupt a specific gene in a specific cell type to determine the functional role of this gene/ cell in whole animal (mouse) physiology. We will use standard techniques that usually achieve conditional disruptions in the genome, so that the mice develop to adult stage normally and then the gene is modified; e.g., by tamoxifen injection to induce cre-mediated disruption. Such an approach minimizes unwanted effects that would otherwise be a risk to embryonic development, resulting either in embryonic or neonatal death. Another typical genetic aim is to recapitulate in mice a mutation identified in humans through association with disease. We identify such mutations, for example, in collaboration with research partners and in our UK Biobank (https://www.ukbiobank.ac.uk/) data analysis, as shown by our recent study of COVID-19 fatality. The murine version of the mutation is then generated in a new mouse line using CRISPR/Cas9 technology. We did this recently for a mutation that causes mild anaemia associated with malarial resistance. We showed excellent recapitulation of the human disease, previously unrecognized mechanisms underlying this disease and a novel therapeutic strategy. In a paper in preparation for publication we identified cardiometabolic abnormalities in these mice that we anticipate will usefully inform the medical care of people with such mutations, which have been estimated to occur in about half of people of African descent.

The next step is phenotyping of the genetically-modified mice and their matched control (unmodified) mice. We do this by observing the physiology of the mice, for example by collecting and analyzing blood from the mice at 8-22 weeks of age, by remote telemetric observation of blood pressure in conscious mice during physical exercise at 14-18 weeks old, measurement of heart anatomy and function by non-invasive echocardiography in mice at 12-16 weeks of age or by phenotyping in specialized metabolic cages for indirect calorimetry. In some experiments we seek to further improve the relevance to people who have cardiovascular disease by increasing the fat content of the diet and allowing the mice to grow old (2 years), at which point we would, for example, collect blood to determine concentrations of lipids such as cholesterol.

A next step may involve surgical intervention in the mice to induce a condition that models human disease states such as abdominal aortic aneurysms. Risk factors for this life threatening condition are well documented to be high blood pressure, high blood cholesterol and chronic obstructive pulmonary disease (COPD).

A next step may involve administration of a small-molecule (drug-like substance) that is commercially-supplied or synthesized at Leeds as part of our medicinal chemistry drug discovery programme. Such molecules would be inhibitors or activators of the mechanisms under investigation (e.g., of a specific type of calcium-permeable channel).

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

Most often the impact on the mice will be no more than the captivity and associated experience that may be compared with mice kept as pets in peoples' homes but most likely under more consistently high quality conditions. This is classed as subthreshold.

When impact is more than subthreshold, it will most often be mild impact. A typical expected effect is reduced exercise performance on a running wheel (e.g., 50% fewer rotations of the running wheel during observation for 4 days in mice at 20 weeks of age). Another expected effect is reduced blood pressure elevation during running wheel exercise in mice at 14-18 weeks of age. These are not behavioural changes as far as we can tell, although we plan to study the changes in more detail using remote observation methods to determine any psychological effects. We think instead that they are mild physical effects that are comparable to a person being relatively physically unfit. From some mice we will collect blood and in some mice we will inject substances (e.g., tamoxifen) and this impact can be considered similar to that experienced by a person whose blood is collected or who has a substance injected.

In some instances we will perform recovery surgery, for example to insert a telemetry probe to measure blood pressure, or to implant a device either under the skin or in the perineal cavity of the animal. These devices enable us to constantly administer substances relevant to our research aims, thus eliminating need for frequent handling and repeated injections to animals and are viable for up to one month. In such cases there is suffering that may be compared to what a person experiences when undergoing surgery for a moderately severe condition, although it is not possible to explain the situation to the mouse or justify the intervention in the interests of the mouse's health. These surgical protocols are completed under balanced general anaesthesia, including pain-relief procedures to minimize suffering prior to, during and after the surgery, and sufficient time is allowed for recovery prior to any measurements from the mice.

We do not expect unusual pain or weight loss, tumours, abnormal behaviour or any other such concerning effects in our genetically modified mice or in the mice during any of our experimental protocols.

In some instances, a genetic mutation we engineer in the mice will be expected to cause disease similar to that seen in some people with a similar mutation. We will, however, select mutations that are likely to cause only mild disease in order to minimize suffering. However, if we observe mice with genetic modifications showing more than expected adverse effects, manifested in neonatal mortality or poor growth rate, we will take necessary steps to minimize suffering.

One of our protocols creates aneurysms in mice that have a risk of rupturing and causing sudden death, as in people who have such a condition. We take special precautions with these experiments and only use this approach when necessary.

Very occasionally a mouse may die unexpectedly from unknown causes that seem unrelated to any of our actions. This is a severe event, but we think such events are rare natural events and we will make effort to minimize the risks of such occurrences. Any such events are reported under a PPL Standard Condition 18 Notification.

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)?

Subthreshold, Mild, Moderate and Severe.

Based on experience under my current licence, I could expect there to be 26.5% Subthreshold, 65% Mild, 8.2% Moderate and 0.3% Severe. I anticipate, however, that the proportions will be left-shifted (i.e., less severe) because of 3R implementations described in this proposal.

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 04 February 2027

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?

We need to use mice to advance understanding of calcium-permeable channels, associated mechanisms and other related mechanobiology mechanisms that contribute to natural physiological processes in whole animals such as mice and humans. The mechanisms are important for body homeostasis in physiology that is often disturbed in disease and modulated by physical exercise. If we want to understand animal biology and treat diseases in people and animals, we need to advance knowledge in part through whole animal studies. The homeostasis (steady-state of the living system) and its disturbances are whole body interorgan system effects that cannot be reproduced in cell culture environments or artificial or stored organs in the laboratory; this is because animal physiology and diseases depend on integrations across cell and organ types in the living, moving body as a whole; i.e., a human heart is not a human. Other non-animal approaches are not currently available or possible for generating insight into such whole animal systems. For example, how could we study the health

benefits of physical exercise (a key topic of this research) in cells or model organs cultured in the lab when such entities do not run around or exercise in any way that relates to our whole body exercise? In vitro data have already been generated and more will be generated to justify and optimize steps in the in vivo animal studies. Such data help us design the best possible animal studies and minimize risk of wasted life or unnecessary suffering. Moreover, a key technical approach for determining the role of a gene is to modify the gene so that its function is decreased or increased. We cannot do this in humans, although we can observe what happens when genes are modified naturally in people: and we do use such information to guide our mouse studies; it helps us to know what to target and to predict what might happen.

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

We have considered humans, human tissues, human cells and human induced pluripotent stem cell systems to recreate parts of organs (e.g., the heart) in tissue culture. We have considered ‘lower’ species such as flies and worms.

Why were they not suitable?

We will study humans, human tissues and human cells and such studies will be used in preference to animals whenever possible. However, the animal studies are still essential because in mice we can: (1) accurately compare control (non-genetically modified) and test (genetically modified) mice at the same age and in the same sex, matched living conditions and sufficient numbers to enable statistical comparison; and (2) accurately phenotype the mice using a range of powerful techniques, the application of which to humans would almost certainly be impractical and prohibitively expensive and may be unethical.

We are working with collaborators to establish and study human induced pluripotent stem cell systems to recreate parts of organs (e.g., the heart) in tissue culture. The primary motivation here is to create models systems that can inform drug discovery programmes. It is doubtful that they recapitulate normal physiology however, so the arising data are of questionable value for understanding physiology. No systems currently exist for recreating whole animals using such technology, so complex interorgan systems biology and exercise biology cannot be studied using such methods.

We have considered ‘lower’ species such as flies and worms but concluded that their biology is too distant from the biology we want to understand and too remote as a basis for translation to problems of disease in people, such as those caused by insufficient physical activity and calorie-rich diet.

A retrospective assessment of replacement will be due by 04 February 2027

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?

We have estimated the numbers based on experience in studies of this type under our current licence, which is similar in many ways (but updated, improved and simplified). We will use related approaches in the proposed new licence and are working to achieve similar investments in our research (i.e., volume of peer-reviewed grant funding).

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

We are working to reduce the number of animals being used by initiating new human cell, human tissue and patient data analysis studies to answer questions. We are doing this by establishing new partnerships with clinicians in the NHS and by studying the UK Biobank and other databases. Such research is the basis of grant proposals we have submitted or are in the process of submitting to the Medical Research Council, British Heart Foundation and other such agencies; these proposals do not include requests for animal studies.

We are working to reduce the number of animals being used by initiating computational projects and collaborations that model calcium-permeable channels and mechanobiology. Such models are increasingly sophisticated. We are using these models to understand human mechanisms and working towards predicting the effect of human disease genetic mutations on the mechanisms and their downstream consequences for cell and organ function. These are ambitious projects but we hope that they will eventually avoid or further reduce the need for animal studies.

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

Our institute employs an expert technologist trained to PhD level in genetics to oversee and optimise our animal breeding programmes to maximise efficiency, maximise value for the science and minimise use of animals. This individual supports our animal studies and those of other investigators in the institute who work on other but related disease challenges. This support system provides a consistent approach and encourages and facilitates sharing and teamwork to maximize scientific benefit from each animal.

We will work with a professional biostatistics collaborator to maximise the quality of experimental design and maximize the value of the arising data. Two statistical approaches will be used as we previously described in work with our statistics collaborator.

We will use small-scale pilot studies to indicate if a particular experiment is promising in terms of the value of the outcome, if the overall experimental design can be improved (e.g., are there any unnecessary steps, are we missing any opportunities to collect important information?) and if the

estimate of variance can be improved to inform the power calculation. Only then will the full study be conducted using blinded and randomized experimental processes in the protocol.

We will use telemetric techniques that are available for sequential measurement of blood pressure, cardiac electrical activity and breathing in conscious animals. In addition, use of imaging modalities now available to us can also pick up progression of pathological changes in longitudinal study of one animal. Both these techniques produce reliable data and help reduce the number of animals used.

We are seeking external funding for a new remote phenotyping system that we hope will enable us to further improve the quality of data collection and thereby reduce variance in the data collected from the animals and justify use of fewer animals. We already submitted a grant proposal for this equipment and it has been short-listed for funding (equipment cost £828,745). The system is the TSE-Systems PhenoWorld Multi-Arena for quantification of the physical activity of the mice and matched exercise in knockout and wild-type groups over long periods of time; high-quality standardized physiological phenotyping of multiple parameters including behavioural and cognitive parameters in social groups with minimum human interference; continuous assessments and the potential for interim evaluation of data during long protocols. The equipment is for remote metabolic, physiological, cognitive and behavioural phenotyping of individual mice in social groups of 10 with total capacity for 60 mice to enable sufficient statistical power. In this system, each mouse is tagged, recognized at strategic positions for data acquisition and monitored for controlled access and evaluation in tunnels and compartments including for indirect calorimetry, monitoring of feeding/ drinking and excretion, cognitive assessment and in-cage voluntary running wheel analysis with workload control and motor skill testing. There is detailed tracking of movement for maximum detail on total physical activity and telemetry for remote monitoring of parameters such as blood pressure. This is part of our long-term vision to implement increasingly sophisticated phenotyping and genetic approaches and offer training, standardization and collaborative opportunities worldwide. Nevertheless, even if we obtain funding for this new phenotyping system, there will still be substantial breeding of new genetically modified mice because our plans include increasingly sophisticated genetic approaches to generate more informative insight and less overall modification to the animal (e.g., by disrupting a gene in endothelium of only one organ, rather than throughout the body as we and others do currently).

A retrospective assessment of reduction will be due by 04 February 2027

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.

Mice have well-established suitability for gene-modification experiments and are the smallest mammal in which it is possible to combine studies of gene modification and invasive approaches for measurement and induction of disease. Mice are used as a model of the human because they are mammals and fundamental mechanisms are likely to be similar across the mammals. While all mammals have differences, mice are widely accepted as a suitable first step for exploring the in vivo relevance and potential of mechanisms. Progress cannot currently be made directly from in vitro ‘test tube’ studies to human in vivo studies.

Suffering will be minimized through use of inducible gene-modification systems as described in more detail in other sections.

Suffering will also be minimized by use of the appropriate doses of chemicals for observation of effects. Before testing such modulators in vivo we will test them for toxicity in cell viability assays in vitro to anticipate if toxicity might arise in vivo and at what dose. For modulators which remain potentially interesting, we will test them in vivo in mice. We will start by administering the modulator by osmotic mini-pump or i.p. injection at a dose expected to be sufficient to modulate the target and with the Named Veterinary Surgeon (NVS) known to be available for consultation. Where repeated administration of substances is required by subcutaneous or intra-peritoneal (i.p.) injections to achieve the desired effect, we will instead use mini-osmotic pumps to deliver continuous infusion if possible. These pumps are implanted either in subcutaneous or intraperitoneal space under balanced general anaesthesia using aseptic surgical techniques and are well tolerated by mice.

To induce exercise related cardiometabolic effects we will provide free access to a running wheel as part of the home cage environment.

We use acclimatization to phenotype monitoring systems to reduce stress and improve the quality of the data collected.

Sequential imaging of animals in longitudinal studies can pick up pathological changes at an early stage, thus implementation of humane end-points can be implemented more accurately and result in refinement of experiments.

For aneurysm studies we have already refined the dose of the agent used to induce aneurysms and therefore minimize the risk of aneurysm rupture (i.e., to minimize the risk of a lethal event) and have increasingly moved towards use of models that have a very low risk of rupture (especially peri-adventitial elastase application to the aorta) combined with mutations pertinent to the human disease and calcium channel mechanisms we study. We only use the angiotensin II (AngII) infusion model (with rupture risk) to confirm findings from the refined model.

Throughout, a single-use needle policy will apply to all injections.

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

We are working to understand animal physiology and the responses of this physiology to external factors such as diet and exercise and to injury events such as damage to the vascular wall (e.g.,

leading to aneurysm formation). We are working to develop novel agents that can hopefully be taken forward to achieve new medicines to treat disease problems in people (usually adult people). In order to do this, we need to study animals that are as similar as possible to the human situation. This means using an adult mammal when it isn't possible or appropriate to advance the research using humans or cell culture systems alone. We need to understand whole body physiology in the living moving state, for example to understand exercise responses, which require whole body movement. We cannot do such research using immature life stages, species that are less sentient or animals under anaesthesia, although we will use terminal anaesthesia strategies where appropriate.

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

As mentioned elsewhere, we are seeking external funding for a custom TSE-Systems PhenoWorld Multi-Arena. In addition to scientific advantages, such systems should substantially improve animal welfare by enabling more remote observation of mouse phenotypes without human interference. Importantly, the system enables study of individual mice in social groups of 10 in enriched environments with natural-type tunnel systems and free voluntary access to running wheels. We think this will greatly improve the living environment for the mice and thereby further improve animal welfare.

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

https://www.nc3rs.org.uk/

https://www.lasa.co.uk/

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

We are part of an emerging mouse genetics and phenotyping consortium with a group at another university to refine mouse study approaches. A major purpose of this consortium is to share best-practice and work together to optimise and improve methodologies, and thereby refine.

We are part of the National Centre for the Replacement, Refinement and Reduction of Animals in Research mailing list and receive regular Newsletters and updates on the 3Rs initiative.

We are working increasingly closely with clinical research groups and learning about databases for research such as UK Biobank and to find ways to replace animals in research (e.g., machine organ perfusion systems for studies of human organs such as liver and using observations of human genetic variation to understand gene function in the natural environment).

We are working with other groups to collaborate in the development of 2D and 3D human induced pluripotent stem cell approaches and thereby potentially reduce and replace animal usage.

A retrospective assessment of refinement will be due by 04 February 2027

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?