Home Office

NON-TECHNICAL SUMMARY

# Development, Optimisation and Validation of Small Animal Imaging

**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**

In vivo Imaging, Refinement, Development and Validation

| Animal types | Life stages |
|---|---|
| Mice | adult, aged |
| Rats | adult, aged |

## 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?**

This project aims to develop, optimise, and validate in-vivo small animal imaging techniques as biomarkers to evaluate physiology, detect pathology, and monitor the effects of therapeutics

**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?**

Medical imaging techniques such as magnetic resonance imaging (MRI), computed tomography (CT), and ultrasound (US) are fundamental to modern healthcare systems. They provide doctors with diagnostic information informing on the presence of disease, its severity, its location and involvement with surrounding tissues. These techniques can provide anatomical and/or functional information about the organ of interest. Anatomical scans are pictures of organs that display the different structures of interest, for example a tumour or stroke. Functional scans are pictures of the tissue of interest that display a functional tissue property such as blood flow to the organ.

The process of developing a new imaging technique for human benefit is a lengthy one. New techniques must be tested to ensure they produce reliable results that do not depend on the type of scanner, day, or conditions of the scan. Preclinical testing provides the ideal platform for this type of development activity, due to the availability of highly specific rodent models of disease and ability to perform tightly controlled experiments for assessment of reliability.

Further to providing new diagnostic tools for clinical medicine, medical imaging techniques are useful in rodent studies to understand disease mechanisms and/or to monitor the effects of novel therapeutics. Medical imaging techniques also beneficial for animal welfare as they provide a non-invasive way of studying the function of tissues.

This project aims to develop new techniques to measure damage to the blood vessels and brain tissue associated with dementia and cancer treatments. These conditions affect huge numbers of elderly people, creating an enormous economic burden on society. Developing new clinically translatable techniques capable of diagnosing these conditions will help improve outcomes for these patients.

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

The primary output will be improved imaging capability through establishment of multiple new imaging techniques. We will aim to share these imaging techniques with other users at our institution and further afield to avoid duplication of efforts, helping to reduce animal use.

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

The following groups will benefit from these outputs:

- The animals. Development of new imaging techniques will replace more invasive techniques and reduce scan durations.
- Human patients: Imaging techniques developed in this project will eventually be translated to diagnose human disease.
- Researchers and clinicians studying vascular and tissue damage. The project will provide new methods for more sensitive and specific study of these properties.

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

We will maximise outputs through sharing of imaging techniques with other researchers at our establishment and further afield including other universities and industry. We will disseminate our methods via regular presentations at national and international conferences.

**Species and numbers of animals expected to be used**

- Mice: 1150
- Rats: 1150

# 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 and rats have a developed nervous system that has sufficient similarity to human anatomy. Adult rats and mice will be used as opposed young animals because organ structure and function is fully developed. Rats are particularly advantageous since they have larger organs and will tend to produce higher quality data than mice, thus we will use rats instead of mice when developing techniques that are limited in signal strength. Development of medical imaging techniques using lower species (e.g. zebrafish) is not possible due to the much smaller size of organs.

Genetically modified animals or animals with inherent or induced cerebrovascular disease will be used when we need to test if new imaging techniques are sensitive to specific pathology.

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

Animals may receive radiotherapy to the brain or other organs prior to imaging.

Genetically altered animals may be bred and aged. Hypertensive animals may be purchased and aged until they develop high blood pressure.

We may surgically place a clear window at the surface of the skull to enable live microscopy of the surface of the brain (<5% of procedures). We may introduce needle into the tail vein (50-80%) or directly into the brain (<5% of procedures) in order to administer imaging contrast agents.

Imaging sessions will typically last between 30 mins and 3 hours in duration. Before or during the scan, animals will be injected with contrast agents or dyes or compounds to alter physiology. The mixture of gases used may be altered during the scan to change physiology for validatory purposes or as a contrast agent. Animals may be repeat scanned up to a maximum of 10  times with a minimum of 24h between scans. Blood sampling may be performed.

Following imaging, animals may undergo behavioural testing then will be humanely killed for ex-vivo analyses.

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

Radiotherapy may lead to transient reddening of tissue.

Implantation of clear windows in the skull may result in transient localised inflammation.

No adverse effects are expected from imaging apart from those associated rarely with general anaesthesia including death due to anaesthetic intolerance (~5-10 in 1000) or repeated imaging which may result in weight loss (5-10%) due to stress and repeated prolonged anaesthesia.

Injection of contrast agents or compounds may occasionally lead to adverse reactions (possibly resulting in animal death), but this will be limited via use of published dosing, preparation, and injection protocols where available. Repeated tail vein cannulation may damage the tail, leading to necrosis.

Behavioural tests may induce mild stress for a short period.

**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)?**

The expected severity is moderate for all animals.

**What will happen to animals at the end of 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?**

Animal experimentation provides the ideal platform for development of imaging techniques, due to the availability of highly specific rodent models of disease and ability to perform tightly controlled experiments for assessment of measurement reliability.

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

There is a certain amount of non-animal work that can and will done (e.g. on non-animal test objects made of gelatin or from cultured cells) to carry out initial scan optimisation, and we will aim to replace the use of animals wherever possible. Test objects can be constructed that mimic flow through a blood vessel and will be used to provide early testing of flow measurements. Use of cell cultures will be used to test measurements of cell permeability where practicable.  However, these test objects lack the complexity needed to fully test new techniques and eventually animals will be required.

**Why were they not suitable?**

These non-animal systems are useful for very early and basic testing of new methods prior to applying in live animals but fundamentally cannot provide the required level of complexity to accurately mimic the function and structure of live tissues.

# 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?**

The number of mice and rats to be used throughout the project is calculated based on considering the type of animals needed, the expected number of experiments and the expected number of animals per experiment.

Up to 300 genetically altered mice or 300 genetically altered rat will be needed to validation purposes.

We will develop approximately 10 new imaging techniques and validate 6, per year per species, across all imaging modalities.

For development experiments, we will use approximately 3-5 animals. For validation experiments, we will estimate approximately 5-10 animals per group.

Together, these considerations lead to estimates of 1150 mice and 1150 rat.

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

We will always aim to use the minimum number of animals required to for the purpose being investigated. The specific experiments to be undertaken will be guided by the imaging technique being developed, and appropriate sample size calculations with be conducted based on prior data either from publications. When data does not exist, we will acquire pilot data to inform sample sizes. We will seek expert statistical input and utilise rigorous experimental design (e.g. NC3R's EDA) in the conduct of all experiments.

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

Dynamic imaging before and after injection of a contrast agent or compound will be used where possible to remove the need for a control group.  Where possible, historic data will be used to inform sample sizes. Otherwise, an initial pilot/exploratory study will be used to generate data to test feasibility of the approach and provide initial estimates of effect sizes and data variability . Important experimental results will be repeated and validated via follow-up experiment to minimise the likelihood of spurious nonreplicable results by other groups. Numbers of animals used in each study will be monitored to capture drop-outs or any requirement for a positive control group. Required numbers of animals per protocol will be updated as more recent data becomes available.

# 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.**

Where possible, radiotherapy using gamma-radiation given under restraint rather than anaesthesia. Image-guidance will be used to target the radiation field, avoiding unnecessary radiation dose to normal tissue effects.

For all experimentation, we will use naive mice or rat where it does not impact the scientific objective. For studies requiring use of genetically altered, experimental models of cerebrovascular disease, or irradiated animals, the models that will provide the necessary pathological changes at the youngest age and with mildest phenotype will be used. All imaging and injections will be performed under anaesthesia. During imaging, respiration and temperature will be monitored. Injections will be given using an appropriate sized needle.

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

When setting up and optimising imaging techniques for the first time which may require long scan durations, we will use animals that are terminally anesthetised. Adult mice and rats will be used, including genetically modified animals when necessary. Adult rats and mice will be used as opposed young animals because organ structure and function is fully developed. Use of less sentient species (e.g. zebrafish) is not possible due to the much smaller size of organs.

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

A key objective of this licence is to optimise imaging techniques. In practice, this results in shorter anaesthesia durations for the animal but will also result in other benefits such as reductions in total injected volume (for example by using the shortest injection line possible).

Imaging procedures will be regularly reviewed (monthly) and areas of potential refinement identified. Animals will be closely monitored for adverse effects and appropriate steps will be taken to minimise suffering, pain or distress. Use of tools such as the grimace scale will be used to assess the condition of the animals. Wherever necessary appropriate anaesthesia will be employed to reduce the suffering and distress caused to experimental animals. Appropriate analgesic regimes (as advised by the NVS) will always be used for pain relief and for all protocols the earliest endpoints will be used where possible.

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

The NC3Rs website contains the most up to date resource and will be regularly checked for updates to best practice. When injecting contrast agents or pharmacological agents, we will consult the joint consortium guidance (see protocol steps) for the most appropriate dosing and injection routes.

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

I will remain in constant contact with our establishments animals facility personnel including the Named Training and Competency Officer (NTCO), Named Information Officer (NIO), and Home Office Liaison Contact (HOLC) to ensure I am as up to date as possible with changes in regulations and procedures. Any opportunities for additional training or CPD will undertaken without delay. We will also regularly consult with the NC3Rs Regional Programme Manager and check the NC3Rs website on a regular basis for updates to best practice concerning injections and refinements to procedures.