Home Office

NON-TECHNICAL SUMMARY

Elucidating sources of contrast in quantitative brain MRI

Project duration

2 years 0 months

Project purpose

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

Key words

Magnetic Resonance Imaging, brain, histology, multiple sclerosis

Animal types	Life stages
Mice	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to investigate how different components of the brain tissue contribute to the observed contrast in magnetic resonance images.

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?

Magnetic Resonance Imaging (MRI) is a type of scan that uses strong magnetic fields and radio waves to produce detailed images of the inside of the body. It is for example routinely applied to image the brain of healthy people and of patients. The characterization of one particular type of tissue in the brain, i.e. the white matter, is important for diagnosing disease (such as for example Multiple Sclerosis). While MRI is very good in depicting lesions in the brain ("**qualitative assessment**"), clinicians are now interested in **quantitative information** such as length or volume of brain cells to provide a more refined diagnosis. The structure of white matter, however, is complex, which makes it challenging to interpret the MRI images in an accurate and quantitative manner. We therefore need to study the relationship between imaging findings and anatomical features in an animal model such as the mouse, where we can easily compare our MRI findings with histology. This would not be possible in humans. Histology can then be related to the MR images in a novel way by spanning a range from nanometer to millimeter.

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

To date, quantitative readouts ('imaging biomarkers') from MRI are indirect, meaning we are measuring **physical** properties (such as relaxation times or diffusion parameters etc.) from which we infer **physiological or anatomical** properties. The estimated parameters will be affected by tissue properties **AND** potentially by the experimental configuration. It is therefore important to understand the influence and contribution of both parts. This process becomes a fundamental aspect for developing these imaging biomarkers and it will be ultimately essential for their application on patients in the clinics. The output of this project will therefore lay the foundation for answering major open questions in multi-scale imaging (i.e. covering resolution from µm- to mm-range) and in MRI.

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

The proposed experimental design (i.e. manipulation with the cuprizone model) together with the cutting edge MRI and electron-microscopy technologies used in the present study will generate an extremely valuable multimodal dataset. We expect the outputs of our study to be beneficial on both short and long terms. Initially, throughout the duration of the research project, methods, analyses and findings will be presented internally and externally trough conference reports for the benefit of the scientific community to support collaboration and design of new research studies. Furthermore, we will use a combination of approaches providing complementary information to gain a deeper understanding

of the biophysical origins of differences in the estimated MRI parameters thus enhancing the interpretation of quantitative MRI. Hence, on the long term, we hope outputs from the present study will help devising biophysical models capable of achieving a better biological accuracy and perhaps, enhanced diagnostic power boosting MRI clinical utility.

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

Throughout the duration of the research project, study results will be reported using peer reviewed journals and conference reports. Where possible original, peer-reviewed research publications will be made freely available and open access to research data will be supported. Furthermore, collaboration among research groups will be promoted. Data analysis will be reported and made available to enhance reproducibility and replicability. Finally, the PhD thesis will be made freely available from the EThOS repository

Species and numbers of animals expected to be used

- Mice: 70

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.

The project will use adult wild type C57Bl/6 mice as they are one of the predominantly used species in biomedical research. Furthermore, C57Bl/6 is the strain where cuprizone induced demyelination has been consistently reported. It is therefore the most utilized strain in studies using the cuprizone diet.

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

The project will use two groups of mice, only one of which will be fed with a Cuprizone supplemented diet for a duration of six weeks. Both groups however will be subjected to two MRI scans, six weeks apart, typically lasting 2-3h. In rare circumstances, due to technical difficulties with equipment, a third MRI scan may be performed. In either case, the second or third scanning session will be performed under terminal anaesthesia. A clinically approved MRI contrast agent might be given during the imaging session.

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

- *Cuprizone feeding* has been associated with weight loss. This is expected to happen especially in the first two weeks of diet followed then by a gradual increase in body weight, which however remains below the baseline. Behavioural phenotype associated to cuprizone feeding include motor limitations such as tremors, abnormal walking and reduced coordination, spatial memory,

reduction in social interaction with peers, environment exploration and spontaneous motility. Administration of cuprizone supplemented diet will be limited to a short period of time therefore preventing permanent damage.

- *MR imaging* is performed under general anaesthesia. While the adverse effects of general anaesthesia are rare, the mice will typically undergo two MRI sessions six weeks apart under general anaesthesia. The animals will not be recovered after the second imaging session. If for technical reasons a third scan is required, it will be conducted under terminal anaesthesia.

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 for all animals will be *moderate*.

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?

Animals are essential to understand the observed imaging contrast under in vivo conditions as mimicks the clinical scenario. Furthermore, the ability to compare the in vivo measurements with histological “ground truth” analyses in a controlled setting is a fundamental aspect and would not be possible or ethical to perform in humans.

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

We will always make use of non-sentient phantoms and/or cadavers and ex vivo tissue in the first phase of the development.

Why were they not suitable?

None of these alternative settings reflect the complexity of the in vivo situation (as also found in patients), where there is interplay between motion (i.e. cardiac and respiratory motion, blood flow) and complex (magnetic) properties associated to living brain tissue.

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?

Authority is being sought for a defined study, for which the number of required animals was determined using power calculations (n=20). An additional group (n=20) has been added for validation after the developmental phase on non-sentient phantoms / under ex vivo conditions.

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

Minimizing the number of animals will be achieved by moving to in vivo testing / validation only once all imaging sequence are fully tested on non-sentient phantoms / under ex vivo conditions. We will adhere to a strict workflow to achieve our scientific goals while minimizing animal usage. The NC3R's Experimental Design Assistant has been used to design the study while minimizing the total number of animals.

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

As mentioned before, we will always make use of non-sentient phantoms and/or cadavers and ex vivo tissue to ensure that all imaging techniques are working before the in vivo application.

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 mice because this species provides sufficient similarity in health and disease to man. The proposed in vivo imaging is non-invasive in nature but requires for the animal to be anaesthetized. The dietary intervention is required to generate the disease model but will be limited to a short period of time therefore preventing permanent damage.

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

Mice are the mainstay of biomedical research. In order to elucidate the imaging contrast observed in MRI **and** to perform histological validation, these models are essential.

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

As mentioned, the proposed in vivo imaging is non-invasive in nature, but requires for the animal to be anaesthetized. All animals will receive additional heat and fluid support during and after the imaging examination to aid recovery from anaesthesia and will be closely monitored until fully recovered. During imaging sessions, ECG, respiration and body temperature are continuously monitored. All cages include environmental enrichment and animals are kept in social groups whenever this is compatible with our objective. Cuprizone feeding has been associated with weight loss. This is expected to happen especially in the first two weeks of diet followed then by a gradual increase of body weight, which however remains below the baseline. We will therefore weigh the animals at least three times a week and document it.

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

The NC3Rs website has a repository on topic-specific resources, including anaesthesia and analgesia, which are the most relevant topics. In addition, regular updates on training and best practice guidance is disseminated via the mailing lists mentioned above.

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

We have university-wide mailing lists informing on advances in the 3Rs and on new training opportunities in this area. Furthermore, we are in close and regular contact with the University Veterinary Officer, with whom we regularly review our procedures. This represents an effective mechanism to convey and translate any new advances into code of practice.