Home Office

NON-TECHNICAL SUMMARY

Mechanisms and therapeutics for brain disease

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

Nucleic acid therapy, Gene therapy, Epilepsy, Brain excitability, Ion channels

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

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 aim to develop powerful, safe and long-lasting treatments for brain diseases which include epilepsy and chronic pain, and to develop a better understanding of how and why these conditions happen.

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?

This work will give a new level of understanding of how brain activity changes between health and disease, and may lead to urgently needed therapies for people with lifelong brain diseases that cannot be treated with currently available medications.

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

-A new level of understanding precisely how genes are expressed in our brains and how they interact to give rise to healthy brain function.

-Full preclinical testing of novel genetic therapies which can treat and/or cure brain diseases, such as epilepsy and chronic pain, which cannot be cured with existing medications. This will include an estimated at least five promising therapies for each of chronic pain and epilepsy.

-Better understanding of how epilepsy medications pose neurodevelopmental risks to the foetus when taken during pregnancy.

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

Short-term: The main benefit will be to fundamental and pre-clinical researchers in neuroscience and molecular biology, as this project will generate a new level of insight into how gene networks shape brain function, and will generate pre-clinical data on new therapies for neurological diseases.

Medium/long-term: This work will provide enormous benefit to people who have currently untreatable neurological diseases, their families and the clinicians who care for them. This benefit will be seen in the longer term as the treatments discovered progress to clinical trials and into clinical use. The most immediate applications of the approaches in this PPL will be to epilepsy (>50 million patients worldwide) and chronic pain (~60 million worldwide). The genetic therapies being developed are designed to provide broad therapeutic benefit to a wide patient-base – regardless of the exact cause in

each individual patient – by treating a common pathological mechanism which is over-excitability in the brain.

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

The outputs of this work will be maximised by publishing all findings (positive and negative) in open access scientific journals, in order to disseminate the work as widely as possible within the scientific community. Findings will be presented regularly and openly at scientific conferences and, where appropriate, communicated to patient and public audiences, in order to maximise the reach of the work.

I also work closely with national epilepsy societies, which will maximise dissemination to directly interested individuals and, via these organisations, I regularly interact with people with epilepsy and patient groups. Finally, I am embedded within the local and regional epilepsy research networks comprising scientists and NHS clinicians. These clinical collaborations further increase the reach and relevance of all outputs generated within the project.

Species and numbers of animals expected to be used

- Mice: 1095

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.

This project will only use mice. Mice are an ideal model organism for epilepsy studies because their brain is a close approximation to humans, and many valid and reproducible models are available. The reproducibility means that smaller animal numbers are needed for statistical analyses, and the proximity to human brain supports predictive validity and translational potential of the work. Furthermore, mice are highly amenable to genetic manipulation, creating models of genetic brain diseases. For these reasons, mice are generally considered to be the gold-standard in epilepsy research. We will use adult mice for epilepsy studies as we are aiming to treat adult epilepsy.

For chronic pain study, we will use a well-established mouse model in which capsaicin in injected into one of the animal’s paws to induce local inflammatory chronic pain following, without the need for invasive surgery or more severe models. This model is appropriate because it recapitulates key features of human chronic pain, including over-activity in the brain, which we are aiming to treat with our novel therapies.

To study prenatal epilepsy medication exposure, epilepsy medications can be easily administered to pregnant mice without harm or distress, and then possible harm to the embryo and associated mechanisms can be studied in their offspring. Mice provide an ideal model because their relatively

short gestation period and relatively large litter size means that data can be generated quickly and in a reproducible manner. The use of inbred mice means that any effects that we observe will take place in the context of an identical genetic background, thereby minimising the variability of the approach.

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

In general, mice may (or may not) undergo a chronic neurological disease model (either epilepsy or chronic pain) and will then be treated with an experimental genetic therapy which aims to reduce the disease severity. Epilepsy models usually involve an aseptic surgical intervention to inject an epilepsy-causing chemical directly into the brain, whereas the chronic pain model in this project can be triggered by injecting a substance into one of the mouse's paws. The genetic therapies will all be delivered via aseptic surgical injection into the brain, which is currently the only well-characterised and reliable method to deliver them. In the case of epileptic mice, we will mount a cannula into the skull during the first surgery (where epilepsy is induced) so that the second surgery (genetic therapy) can be brief and does not require re-opening any wounds. In most cases, the epilepsy/pain will be allowed to develop for roughly 1 week to understand how the model presents in each mouse, and then the therapy will be administered. Gene therapies (which use harmless viruses to introduce DNA into the brain) will be allowed to express for up to 3 weeks during which time their impact on disease progression will be monitored. Antisense oligonucleotides (short DNA sequences which bind directly to other genetic material in the brain) typically act much faster, and their impact on disease will be monitored for up to 2 weeks after delivery. Some mice will also undergo behavioural testing. These tests are do not cause any suffering and assess the natural behaviours of rodents including seeking food and exploring novel objects.

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

-Mice may experience transient weight loss after aseptic surgery, although this typically only persists for 1-2 days and is mitigated by provision of wet food.

-Possible pain associated with aseptic surgical procedures will be manages using analgesics.

-Mice may experience minor irritation of wounds for 3 days post-surgery, and any animals experiencing this will receive appropriate analgesia.

-A small percentage of mice will experience re-opening of surgical wounds within 3 days of surgery, which will be repaired a maximum of one time.

-Epileptic mice may experience additional distress associated with the development of epilepsy and seizures. This is an unavoidable aspect of the disease which we are modelling. Mice will be monitored daily for any signs of pain/distress, and handling limited to avoid stress.

-Mice used for pain studies will experience moderate chronic pain, for a maximum limit of two weeks, as this is the clinical phenotype that we want to model and treat. The total time between initiating chronic pain, delivering a genetic therapy, and completing the experiment will be limited to two weeks.

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)?

Mice will experience different severities depending on what protocol they undergo.

-Non-recovery – ~10% of mice will be euthanised under general anaesthesia in order to perform a specialised methodology to dissect viable brain tissue for electrical recordings. These mice will never be conscious during or following any procedure.

-Mild (~5% of mice) – in the protocol to study prenatal medication exposure, the pregnant female mice will be treated chronically with antiseizure medications. These are known to have a risk of mild cognitive impacts, but are not expected to cause lasting harm or distress.

-Moderate (~85% of mice) - due to aseptic surgical procedures and the use of chronic disease models which can feature limited levels of pain/distress over a period of two weeks (chronic pain) or spontaneous recurrent seizures (chronic epilepsy). The most common adverse effects arise due to recovery from stereotaxic surgery. This can include a small temporary loss in body weight, or irritation as surgical wounds heal. Such models are commonly used in the research field and as such are continuously refined to minimise adverse effects as much as possible. At the end the animals will be humanely killed and then their brains dissected for further examination. This increases the information gained from each animal and also negates the need for more invasive in vivo recordings.

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?

We want to understand the mechanisms controlling the excitability of our brains, how these change in epilepsy and chronic pain, and how we can restore them to treat these diseases. Brains are highly complex systems made up of large numbers a different types of cells, each of which contains a huge number of different genes and which are intricately interconnected. Therefore the manipulation of individual gene(s) in individual cell types will have emergent effects on the entire brain system which cannot be captured in any other type of model system.

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

-Dissociated cell cultures

-Human-derived stem cell culture

-Organ on a chip (brain cell cultures grown directly onto a high density grid of electrodes)

-Long-term brain slice cultures

-Computational models

-Human brain tissue from epilepsy surgeries

Why were they not suitable?

-Dissociated and human stem cell cultures do not form physiologically realistic brain networks and therefore do not capture the complexity of the brain beyond the level of individual cells. They can however be used to measure effects at the single cell level and will be used as a replacement for certain scientific questions where this approach is suitable. Organ-on-a-chip is a similar approach in which cultures are seeded directly onto a dense grid of electrodes. This can give useful information about network interactions between all of the cells in the cultures, with the same substantial caveat that the cells do not form realistic networks which recapitulate those in the real brain.

-Brain slice cultures only represent isolated brain circuitry and they flatten and develop abnormal neural connections over time in culture, and ultimately generate spontaneous seizures themselves. They therefore lose realism over time and also complicate any scientific findings because the health of the brain slice itself is highly variable and changes over time. Slice cultures may be used as a replacement in specific cases where we want to express a genetic therapy in isolated tissue.

-Computational models are typically highly limited and capture brain cells/networks at a highly reduced level of complexity in order to make them computationally viable. In silico approaches such as the modelling of genetic networks will be used to generate hypotheses for animal work, but are unlikely to serve as a suitable replacement for any experiments within this PPL.

-Epileptic human tissue is difficult to obtain, and is often unhealthy and highly variable. In the case of testing genetic therapies for epilepsy, we will likely use this type of model in parallel with animal work to enhance the potential to move our strategies to clinical development.

In general, these models will be used to screen potential treatments and to generate hypotheses for in vivo work. Therefore, even though they cannot replace in vivo experiments, they can aid in reduction and refinement.

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?

In general, sample sizes are based on my own previous experience of using the relevant in vivo models, and published datasets were used to estimate relevant effect sizes and variability of datasets. From existing data, we know that mice undergoing our epilepsy model will experience an average of roughly 15 seizures per day, with each seizure lasting for roughly between 5-30 seconds. Mice typically lose consciousness during these seizures and so do not suffer during the seizure. Where possible, shared control groups have been used (i.e. comparing multiple treatment groups to the same vehicle control group) in order to reduce animal use. Studies will be blinded throughout experimentation and analysis, and animals will typically be assigned to treatment groups using a randomised block design. We will use a mixture of male and female animals and factorial experimental design to rule out any effect of sex on our experiments, without the need for the use of additional animals. Data output from each animal will be maximised through the use of ex vivo brain tissues, following in vivo work, for further biophysical/molecular/anatomical analyses.

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

-Use of a specific statistical design which allows us to assess sex differences between treatments without using extra animals.

-Use of shared control groups where possible.

-Use of in silico/in vitro models to generate hypotheses for in vivo work.

-Use of an experimental design where tests are repeated over time in order to account for environmental changes which might impact the experiments.

-Use of a highly reproducible epilepsy model to reduce variability, and followed published epilepsy-specific guidelines from the NC3Rs.

-Standardised husbandry procedures to minimise environmental variability.

-Closely followed ARRIVE2.0 guidelines to ensure that data generated can be reported to a high standard.

-Followed PREPARE guidelines in order to plan research experiments to a high standard.

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

-We will use pilot studies to test novel genetic interventions and generate data for power calculations.

-We will collect ex vivo brain tissue from all animals which will generate further mechanistic data about our genetic manipulations. This means that we do not need to carry out more invasive procedures in vivo in order to gather similar information.

-In instances where it is scientifically valid, we will use non-animal alternatives such as cell lines to test and/or verify particular biological effects at the level of individual cells.

-Our work will be supported by computational models and database searches which yield the most promising therapeutic targets for epilepsy, thereby prioritising interventions which have the greatest chance of success and minimising the aspect of trial and error.

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.

-Modelling epilepsy in mice using chemicals injected into the brain. The direct injection of these substances into the brain means that they act upon a very specific part of the brain and don't have any impact on the rest of the body, therefore minimising their effects. In general, the focal models used involve less frequent seizures than genetic models.

-Acute seizure models, induced by either systemic kainic acid or systemic pentylenetetrazole. These models provide a quick readout of seizure susceptibility without the need for a more prolonged chronic model. In this case, the models are more refined because they are short-lasting (maximum one hour duration). They cannot completely replace chronic models because they do not capture the epileptic brain state, but they do provide information on efficacy without the use of longer-term models.

-Induction of chronic pain using capsaicin. The direct injection into the paw is a refinement over many other chronic pain models because it does not require surgical intervention to initiate pain, and therefore negates the requirement for an extra surgical procedure. The use of capsaicin rather than other agents (e.g. complete Freund’s adjuvant) is a refinement as it triggers a more limited acute pain, whilst still capturing the necessary disease mechanisms that we aim to study and treat.

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

-It is critical to epilepsy and pain in a developed adult brain to capture the full pathophysiology of the disease.

-Embryonic life stages would not be appropriate for this work because the brain is at a very different developmental stage and treatments would not have the same effect.

-We use mice because their brain is broadly similar in structure, function and genetics to humans. Other animals would not capture human brain physiology well enough to give to able to test new epilepsy and pain therapies accurately.

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

-Daily welfare monitoring using standardised objective scoresheets with appropriate humane endpoints.

-The use of standardised and well established aseptic surgical procedures and models will minimise harms.

-Post-operative care will follow NVS and NACWO advice to minimise harms. Refinements can include use of gel food to add weight post-surgery, appropriate analgesics in consultation with NVS, and group housing post-surgery.

-Behavioural tests are not anticipated to be harmful, although mice will be acclimatised to any novel environment to minimise anxiety during tests.

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

-Opportunities for improving animal welfare in rodent models of epilepsy and seizures (J Neuroscience) - This publication arose from an expert-led working group and gives epilepsy-specific recommendations for refinements which include: Choice of animal model; Induction procedures; In vivo recordings; Perioperative care; Welfare assessment; Humane endpoints; Social housing; Environmental enrichment; Reporting and data sharing.

-PREPARE guidelines to ensure that experiments are planned to a high standard.

-The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research (PLOS Biology) - updated ARRIVE guidelines on the reporting of animal work to maximise reproducibility.

-Unilateral hippocampal CA3-predominant damage and short latency epileptogenesis after intra-amygdala microinjection of kainic acid in mice (Brain Research) - Original publication describing the animal model to be used

-The intra-amygdala kainic acid model has been refined through experience in my previous two lab groups.

-LASA guidelines for administration of substances and aseptic surgery.

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

-Working closely with the NACWO, NIO, local 3Rs manager, and AWERB throughout the project to continuously explore possible refinements to our models.

-Following any updated guidance that arrives via the NC3Rs or similar organisations.

-Engaging with NC3Rs at conferences and workshops.