Home Office

NON-TECHNICAL SUMMARY

Nanotechnology and nanomedicine for detection and treatment of brain disorders

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

Nanotechnology, Nanomaterials, Electrophysiology, Epilepsy, Parkinson's disease

Animal types	Life stages
Rats	adult, juvenile
Mice	adult, 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?

Nanomaterials are very small materials with at least one dimension (eg. width, length, height) typically less than 100 nanometres (one billionth of a metre) in size. The overall goal of this project is to design and test novel nanomaterials or nanomaterial enabled devices for research and medical applications in brain disorders including epilepsy and Parkinson's disease.

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?

The use of novel technologies based on nanomaterials (nanotechnologies) seeks to design smarter solutions with potential to overcome key clinical problems. Metal-bases devices used to record brain activity are extremely useful, however they do have certain limitations. Novel nanomaterials, such as graphene are capable of overcoming the short comings of metals, and promise to significantly improve detection of pathological brain activity, provide advanced diagnostic clinical tools, and allow more effective neurostimulation therapy. Several neurological disorders can become drug resistant (i.e. epilepsy, Parkinson’s disease), and therefore development of novel therapies, based on either targeted neurostimulation of brain centres or circuits, novel nanomedicines, or personalised gene therapies are warranted to treat these patient populations.

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

The new information gained from this project will be shared in the form of scientific publications, conference communications and through public engagement activities throughout this project. While the timescale for these nanotechnologies to move from preclinical investigation to clinical products are more likely to be in the long term, we aim to build up rationale and justification within this project for clinical testing and validation of these technologies.

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

These communications will enable collaboration and stimulate further research into advanced biomedical nanotechnologies throughout the scientific community.

Eventually, patients will benefit from any effective new technologies for the diagnosis, monitoring or treatment of brain disorders that are based on the results obtained during this project.

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

Publication and communication of our findings will always be the primary aim of this work. The goal of every experiment conducted under this licence will be to generate valid, high quality and therefore publishable results and we will endeavour to ensure that all findings meeting these criteria will be published (majority in open access journals) to inform the wider scientific community, even if these do not support the therapeutic, diagnostic or monitoring potential of the particular nanomaterial, or nanomaterial device being investigated.

Species and numbers of animals expected to be used

- Mice: 2450
- Rats: 1650

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 are chosen for many of these studies for a variety of reasons, primarily as they are the least sentient mammalian species that will provide data applicable to humans. The disease models using these are already well characterised and validated with close similarities to the human disease such that the findings and nanotechnologies developed have the best chance of clinical translation and population benefit. Rats are used as an alternative rodent model for some of this work where either the models are more established and refined (Parkinson’s disease) or where the larger size of these rodents is necessary for the nanotechnologies being developed (e.g. recording/stimulating devices).

The majority of research will use adult animals as the diseases being investigated are those which primarily affect adults.

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

Animals will first undergo a procedure to induce the specific disease in the brain.

For Parkinson’s disease this may be surgery (injection of a neurotoxin into a discrete region of the brain) to induce a hemiparkinsonian state (affecting one side of the body). After induction of Parkinson’s disease, which will be confirmed using a combination of behavioural tests, animals may undergo an additional surgery to implant a nanomaterial enabled device for electrophysiology recording, and/or stimulation. This may be on the surface of the brain, or implanted into deep brain structures similar to deep brain stimulation probes which are already used clinically. The therapeutic effects of nanomaterial device based deep brain stimulation will be assessed by measuring electrophysiology biomarkers of Parkinson’s disease, or through behavioural tests to identify any improvement in symptoms. In some experiments, rather than stimulation, animals may be administered

nanomaterial-based therapies for Parkinson's disease and either behavioural tests, electrophysiology measurements, or a combination will be used to determine their effectiveness. At the end of the experiment, animals will be humanely killed and tissues collected for further analysis.

For epilepsy, animals will undergo a procedure to induce epilepsy which will usually be injection of chemicals to induce acute seizures or acquired epilepsy, or by implantation of cancer cells in a region of the brain that is prone to seizures (to model tumour-associated epilepsy). Animals will then be implanted with devices (usually nanomaterial enabled) for electrographic recording of seizure activity and spread. This will be used to determine the effectiveness of novel nanomedicines aiming to prevent seizures or reduce the negative impact these have on the brain, or to evaluate the impact of brain hyperexcitability and its effects on tumour growth. At the end of the experiment, animals will be humanely killed and tissues collected for further analysis.

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

From our previous work, we don't expect the nanomaterials we use to have any particularly strong adverse effects. Where unexpected or substantial reactions occur, animals will be humanely killed as these effects would likely interfere with the aims of the studies. The procedures used to administer the nanomaterials are usually the least invasive possible. Where surgical administration is needed, this will be done under anaesthesia and analgesia or additional support is provided to minimise the adverse effects associated with this.

For Parkinson's disease the 6-OHDA model of hemi-Parkinson's (our favoured model) is well tolerated. After approximately 12 weeks post-6OHDA administration, rats may show a mild rotational behaviour in the home cage, but this does not affect their wellbeing. Other mild locomotor disturbances including a shuffling gait and short strides have been reported in the literature but are infrequently observed in our studies.

For epilepsy we try to minimise suffering at all times, but in order test the efficacy of our nanotechnologies and nanomedicine it is necessary for the animals to experience the sort of seizures we wish to detect and treat in patients. Patients report that the seizures' themselves are not painful, but they do report post-seizure fatigue and they can injure themselves by falling etc. We will develop treatments to reduce seizure burden in the treated group, this does mean however that the control animals will continue to experience seizures. For this reason, we will choose a study duration of minimal length required to extract the information needed, usually less than 12 weeks. In models of acquired epilepsy some weight loss (~5-10%) is usually observed for the first couple of days after induction; but this is transient and the animals soon regain weight.

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: 100% moderate

Rats: 100% 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?

Before materials or devices are tested in animals, they will first be tested in cell cultures, or in relevant *in vitro* systems to check the safety and to help determine the doses that would be safe, or provide a particular effect *in vivo*.

For brain disorders such as Parkinson's disease and epilepsy, and tumour associated epilepsy; which are disorders of the whole brain, it is necessary to study the whole organ with intact neural networks as well as the connection with the bloodstream, lymphatics and other systems. As well as this, behavioural changes are an important measure of how effective a treatment or stimulation device is, which can only be done in animals.

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

In vitro cell cultures including 3D models and co-culture systems (up to and including organoids which are 3D *in vitro* models that contain multiple cell types and mimic organs more closely).

Why were they not suitable?

Non-animal alternatives such as those listed above can provide important information and are always used in the first instance for all new technologies and nanomaterials. This includes testing in organoid systems which is an ongoing effort by our group. However, none of these systems (including organoids) can effectively recapitulate the complex multi-system interactions of a whole organism, or the complete neural networks of the brain, as is required for our objectives.

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?

Numbers of animals have been estimated in consultation with statisticians using historical data from our own experiments with the same models and approaches with similar nanomaterials or nanomaterial devices.

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

We aim to use the minimum numbers of animals required to adequately and robustly address the research question. This has been determined with support from statisticians and use of rigorous experimental design considerations (as guided by the NC3Rs Experimental Design Assistant). Use of adequate numbers of animals will reduce variability, improve experimental consistency and confidence in outcomes. All assumptions on which sample size estimates are based will be re-evaluated once additional or new data is available from these studies and if necessary numbers of animals required will be revised for subsequent studies.

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

The main way we will reduce the number of animals we use will be to use longitudinal monitoring techniques. By implanting recording devices in animals, we will be able to measure electrophysiological biomarkers of the brain disorders overtime and in response to treatment. This will allow us to obtain data from the same animal over time instead of the more traditional method of killing a different animal at every timepoint. Where possible we will use animals as their own control (eg. comparing between stimulation and unstimulated in the same animal). Finally, at the end of each experiment we will collect as many tissues as possible in order to maximise the potential output from each experiment.

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.

Toxin-induced models of Parkinson’s disease (6-OHDA and LPS) will be used to assess the therapeutic efficacy, diagnostic or monitoring potential of novel nanotechnologies. Both these models are well characterised and reproducible model with the animals showing mild symptoms which do not interfere with daily movement or activities. The reproducibility and consistency of these moderate severity models of Parkinson’s disease will enable us to reduce the number of animals needed per study but also improve the quality of data obtained both at the biological and behavioural levels.

For epilepsy we will use a range of models in order to select the one that is most appropriate for the nanomaterial, or nanomaterial device being developed. The majority of seizures are generally mild, non-convulsive, and last less than 2 minutes. These models are reproducible and can be modulated by the use of anticonvulsant medication should the initial phenotype be more substantial than is required for the output of the experiment.

Where we are assessing the effect of brain tumour associated epilepsy, cancer cells are administered to sites of the brain known to be susceptible to seizure induction during the growth of cancer. Other than the generation of seizures, which are generally mild, animals show minimal clinical signs.

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

The animals proposed are the least sentient mammalian species. The use of non-mammalian species (eg. Xenopus, Danio) would not be appropriate for the diseases or the clinical translation of the nanotechnologies under development. Mice and rats are the most appropriate for the work being carried out as they have nervous systems very similar to humans, which allows us to effectively model the diseases we are trying to treat or diagnose. The anatomy of the brain in these animals is also more appropriate for the development of devices that may eventually be used in humans.

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

Animals undergoing surgical procedures will receive appropriate analgesia to prevent any post-operative pain, will be carefully maintained at a suitable depth of anaesthesia and may also receive additional fluid support to prevent dehydration associated with longer procedures. These animals will be provided with additional husbandry such as mash/wet food, heated housing and careful monitoring in the immediate hours following surgery until normal activity is resumed.

We will work with the animal house staff to ensure that suitable environmental enrichment is provided. Animals will be group housed if possible, however some animals with epilepsy or implanted devices will need to be housed individually.

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

All experiments will be planned and executed with reference to the PREPARE and ARRIVE 2.0 guidelines to ensure effective experimental planning and proper reporting of experiments respectively. We will follow guidance from BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinements and LASA guiding principles 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?

The researchers working under this licence will be regularly encouraged to actively stay informed on advances in the 3Rs as is required by the conditions of their PIL. We will regularly check information on NC3Rs website and newsletters and we will attend institutional and regional 3Rs symposia. Any

relevant advances, for example refinement of techniques or approaches, will be readily implemented into this project.