Home Office

NON-TECHNICAL SUMMARY

# Bioelectronic Medicines II

**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
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

**Key words**

Neuromodulation, Physiology, Autonomic nervous system, Peripheral nerve

| Animal types | Life stages |
|---|---|
| Sheep | adult, juvenile |
| Pigs | 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 purpose of this body of work is to provide further evidence that the process of neuromodulation, alteration of an organ’s function by targeted delivery of an electrical stimulation to a specific neurological site or nerve, can be an efficacious alternative, or addition, to pharmacotherapy to treat broad ranging diseases.

**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?**

Modulating the nervous system (neuromodulation) for the treatment of locomotor, visual, auditory, and genitourinary dysfunction is a well characterised procedure in pre-clinical modelling of disease. This typically involves the implantation of electrodes encapsulated in devices, coupled with a battery power supply, to electrically excite the nervous system, to restore function that was previously lost through trauma or disease. It can also be used to record activity of the nervous system, to detect instability in the system and disease states, as well as block signals that have become pathological. This field of study is now referred to as neuroprosthetics and is now showing great promise as a potential first line therapy in the clinic, for intractable loss of function in patients after injury to the nervous system or with limb amputation.

It is now clear that similar neuromodulation techniques could be applied for the treatment of diseases associated with organs/systems such as diabetes, hypertension, rheumatoid arthritis, and inflammatory bowel disease, medically refractory epilepsy, or myocardial infarction among many others, through modulation of the autonomic nervous system. The autonomic nervous system is a component of the peripheral nervous system that regulates bodily functions, and which may be positively affected with modulation. For example, application of stimulatory devices that modulate the vagus nerve, an autonomic nerve that controls many organ functions, has shown great effect at treating animal models of disease.

The purpose of this work is to provide further evidence that neuromodulation to control organ function can be a viable alternative, or addition, to drug based therapy to treat a broad range of diseases, such as those mentioned above.

We will first ascertain whether novel targeted modulation of the autonomic nervous system can be accomplished in specific organ systems. This will be undertaken in anaesthetised animals. We shall establish an anatomical and physiological understanding, including determining the optimal interrogation point for the nerve to organ. Secondly, in a recovery setting, we will assess the surgical application of the device, the effect on the physiology over multiple applications of the therapy, and the surgical recovery profile .

We need to use large animal models, specifically sheep and pigs, because the size, anatomy, and physiology of these animals is translationally applicable to humans. Our devices are designed for human-scale anatomy, and the farm pig is the most suitable model for this when targeting nerves and organs of the viscera. Pigs are also well characterised with respect to metabolic and inflammatory pathways, and closely resemble the human physiology. Physiology and anatomy of the sheep will be relevant in cases of implants to the neck, and so these models will be justified and used in those scenarios.

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

The project aims to test the feasibility of new treatments for patients that suffer progressive diseases (including inflammatory and metabolic disorders), through modulation of the autonomic nervous system. Areas of application include immune-inflammatory, and metabolic disorders. Examples of these disorders include rheumatoid arthritis and type 1 and 2 diabetes, with around 14 and 415 million sufferers worldwide respectively. A common problem with these patients is that they become refractory to pharmacotherapy and the therapies themselves often result in debilitating side effects. The long-term benefit of this work is to provide these patients with a novel and more focussed (patient specific) treatment approach through modulation of the autonomic nervous system. Most of these diseases are life-long and thus substantial benefits accrued by this treatment (over traditional pharmacotherapy and molecular based medicines) are in reducing cumulative suffering, enhancing quality of life and reducing the cost of life-long treatment.

Overall, our efforts in advancing this new treatment modality of neuromodulation will be focused on providing a greater resolution on the mechanism of action of this treatment in the short term. Medium term benefits are to determine efficacious parameters and safety profiles for the surgical implantation of a device and the levels of electrical stimulation of the nerve. This early-stage data will allow predictions to be made on the efficacious levels (strength and duration) of neuromodulation, as well as to support refinement of the device design that could be brought to the clinical market, while refining the surgical approach for implantation. Additionally, immune-modulating and anti-inflammatory therapies through modulation of the autonomic nervous system might be effective treatments for some or all forms of epilepsy. An immediate scientific output resulting from work modulating the autonomic nervous system before and during seizures is to allow further understanding of the pathophysiology of Sudden Unexpected Death in Epilepsy (SUDEP). The longer-term aim for patients is to aid in decreasing seizure frequency and to reduce the risk of premature death in epilepsy patients.

Heart attacks (Myocardial infarctions (MI)) are another area of disease which could benefit from this type of treatment. Current treatments to reperfuse the heart can lead to further rebound injury (reperfusion injury) to the muscle due to inflammation and despite the vast number of treatments for MI there is still a large unmet need and no specific treatment directly affecting reperfusion injury. The goal,

and significant benefit to patients, is to develop a targeted therapy to reduce reperfusion injuries and prevent further damage to the heart muscle by the use of autonomic nerve modulation.

The outputs from this study will be publishable data which will be disseminated through peer review journals and meetings (we intend to do this after each set of experiments if appropriate).

Additionally, the output may be data for regulatory studies and patentable products which can be commercialised and offered clinically to patients (once regulated studies have been completed).

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

Studies in large animals will provide stronger evidence for the translation of these new therapies into humans, providing an anatomically and physiologically relevant platform to validate the implantable device, in both acute and recovery settings. Typically, industry takes about 10 years to successfully take a device from animal to man. Under our previous project licence we have reduced time by 3 years.

In the first instance the data generated from studies will aid the researchers in the selection and characterisation of new surgical implantations which will lead to their further development (e.g. in clinical trials) and could potentially lead to new therapies for immune-inflammatory, and metabolic disorders being introduced to the market.

Long term, these products have the potential to significantly enhance the quality of life for people suffering these chronic diseases or potentially cure them. This will benefit the whole society via reduction in absenteeism from work or school and reduction in demand on health services.

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

Where confidentiality agreements allow, our commercial clients will publish the information via peer-reviewed scientific journals and conference presentations in addition to patent applications.

One of the key goals of our academic clients and collaborators will be the dissemination of results in seminars, conferences, and peer-reviewed articles with open access, in order to promote the general advancement of the fields studied. Negative findings may be published to avoid duplication of work by other groups.

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

- Sheep: 75
- Pigs: 650

# 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.**

Adult sheep and pigs will be used because they have a nervous system that is sufficiently anatomically and functionally similar to that of humans and thus enable the final safety and efficacy testing of these devices. These two species are required because the specific target nerves and surrounding anatomy are slightly different between species, and we require the anatomy, physiology and surgical access to be as close to humans as possible in different scenarios. For example, sheep are anatomically & physiologically better suited for upper airway/vagal work while pigs are a better fit for studies involving the abdomen.

The anatomy of the sheep and pig nervous system is well defined, enabling robust application of surgical methods and implantation studies.

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

After arrival, all animals will be allowed to become acclimatised to the new environment.

Some animals may have blood samples taken and / or receive a pharmacological agent, for example, to modulate the immune system.

The majority of animals will be terminally anesthetised and used in a non-recovery study. Prior to surgery animals may be fasted (but no withdrawal of water) for up to 24 hours. During non-recovery surgery, animals may have implantable devices to allow neuromodulation and nerve recordings, some may undergo imaging (such as CT), samples (such as blood and organ biopsies) may be taken. Just prior to termination, under the anaesthesia, from some animals there may be collection of samples and / or biological materials, to avoid euthanising further animals for this purpose.

A proportion will be anesthetised, undergo a surgical procedure and allowed to recover. Prior to surgery animals may be fasted (but no withdrawal of water) for up to 24 hours. The surgical procedure may involve implantation of a neuromodulation device, imaging, placement of a permanent intravascular catheter. Samples may also be collected. After recovery the animals undergo a series of therapeutic subclinical neuromodulation (levels of nerve stimulation which have been determined in the terminal studies to be efficacious to produce modulation of the target organ but shown to not have off-target side effects). Imaging, such as radiology, may be performed in conscious animals. Pro-inflammatory substances may be given and blood samples may be collected. This may be repeated on a number of occasions over a 12-month period. These animals may undergo a second recovery and / or terminal surgical procedure and if recovered the subclinical neuromodulation and/or pro-inflammatory substances dosing will be repeated. Finally, the animal may undergo a non-recovery terminal procedure or be killed.

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

The larger proportion of animals used in these studies will not experience any adverse effects as the studies will be conducted under terminal anaesthesia.

With protocols and procedures that involve animals having recovery anaesthesia, some adverse effects for the animals are expected. Animals may experience discomfort and pain following surgery.

We will monitor this and provide the appropriate level of pain relief and post-operative care. Other effects include weight loss and changes to behaviour and general overall condition. We anticipate these adverse effects being transient and with good monitoring, welfare management, and nutritional supplements these effects can be alleviated. We would expect the animals to show full recovery within 7 days of surgery. If this is not the case the animals will be humanely killed.

With our specialist large animal vets, we have established clearly defined humane endpoints in all our models that minimize discomfort and pain to the animals yet allow us to address our scientific questions.

**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)?**

For the majority of animals, the studies will be conducted under terminal anaesthesia and the severity level will be non-recovery. However, as stated above, in some studies the animals will be recovered following surgery and may experience some adverse effects, but these would only cause the animal a moderate level of distress.

**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?**

Computational modelling testing has been done without using animals to give confidence that nerve stimulation may treat disease. The science cannot be advanced further without using animals. Only a whole-body system biology approach will give conclusive evidence and understanding that manipulation of the nervous system can be an effective treatment of disease.

A computer model does not yet exist to test nerve stimulation as a treatment of disease.

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

Computational modelling is relatively recent in the field of neuromodulation, it has already been successfully utilised in several neural engineering applications. Prior to starting in vivo work, we conducted simulation studies representing approximated nerve behaviour. We used multiple neurostimulation scenarios to determine stimulation current-, charge- and charge-density requirements for nerve recruitment in porcine and human splenic neurovascular bundle (SNVB). We then validated

our modelling data by measuring electrophysiological parameters in freshly explanted human splenic neurovascular bundle obtained from organ transplant donors after ethical approval and informed consent. We stimulated the explanted SNVB in an ex vivo electrophysiology preparation using a bipolar cuff electrode and recorded the stimulation-evoked compound action potentials (eCAPs) using downstream hook electrodes. These approaches have enabled the determination of clinically relevant stimulation parameters for implantable device requirements for in vivo work and ultimately to go into humans.

**Why were they not suitable?**

Computational modelling may one day replace animal modelling in this context, however there is still considerable gap in scientific knowledge around all aspects of the nervous system including anatomy and physiology. Therefore, there comes a point when the computer modelling cannot completely answer the questions and a full intact working nervous system is required. At this point the use of animals for these studies is the only option to test therapeutic efficacy of bioelectronic medicines

# 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?**

These figures have been calculated on the numbers used under the previous project licence and future plans. Up to the end of 2021 approximately 400 pigs were used under the previous PPL. Predictions for pigs for 2022 mean this number is likely to reach 500 by the end of the current PPL. The number of sheep used was lower than predicted for the previous licence. Because of this the number of sheep for this licence has been reduced.

With the ongoing projects planned for the next 5 years, (expected to increase) and additional projects intended to be started, the predicted number of animals included on this licence is a realistic estimation of the total usage over the 5 years.

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

To ensure we use minimal number of animals required to obtain meaningful and relevant data, we have extensively consulted available literature, attended experimental design and statistical courses, discussed with statisticians and NC3R staff and information provided by the NC3R. All requestors for work under a service contract will be required to justify the number of animals required and if appropriate show how they came to this number. The ARRIVE 2 guidelines have been referred to and followed where appropriate.

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

Definitive studies with sufficient animal numbers to obtain statistically significant results will be performed only after pilot studies have been performed to develop optimal methodology and assess feasibility and outcome measures. Electrophysiology methods are robust and repeatable, with minimal variation between animals. This provides clear results that give us confidence in making decisions even from small sample sizes.

In many cases, the numbers of animals required will be reduced by longitudinal measurement of responses, e.g. by serial blood sampling, multiple stimulations of nerve preps. Tissue samples at the end of studies can be collected to obtain the maximum information from a study. Also, samples taken may be used to inform other studies and provide extra data which will help advance the project or field as a whole.

# 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.**

Pigs and sheep will be used for all experiments because they are the most appropriate species to determine efficacy and safety with respect to the devices tested. Their neuroanatomy and physiology are very similar to that in humans.

We will work with manufacturers and academic experts to ensure a continued refinement approach is adopted for all implantable devices, electrodes and leads. We will work toward fully implantable devices as advancement to external wires and head caps.

Recovery studies will only be performed once surgical technique, application of neuromodulation and treatment parameters have been defined as much as possible using terminally anaesthetised animals.

All methods used for recovery animals will be refined to minimise any pain; these include appropriate provision of analgesia, use of local anaesthetics where possible for blood sampling and close monitoring of animals by large animal veterinarians and advanced trained animal technicians to recognise any adverse effects. All animals will be trained and habituated to the environment, staff and handling techniques prior to study to minimise stress.

Systemic inflammation models are well-characterised and used experimentally in clinical and non-clinical studies to determine the efficacy of medical treatments for immune / inflammatory diseases. Further development of chronic low-level inflammatory models will allow investigation of a range of diseases and the potential benefits of Bioelectronic medicines and therapies in these diseases.

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

We need to use large animal models, specifically sheep and pigs, because the size, anatomy, and physiology of these animals is translationally applicable to humans. Our devices are designed for human scale anatomy, and the pig is the most suitable model for this when targeting nerves and organs of the viscera. Pigs are also well characterised with respect to metabolic and inflammatory pathways, and closely resemble the human physiology here. Physiology and anatomy of the sheep will be relevant in cases of implants to the neck, and so these models will be used in those scenarios.

A large part of the project will be performed using terminally anaesthetised animals to define the treatment parameters of neuromodulation. Recovery animals will only be used when these parameters have been defined.

In addition, studies using rats and mice are undertaken as part of this research and help inform these large animal studies; this work is covered by a separate Home Office project licence. Studies involving pigs and sheep are one of the final steps within the project when similarity to human nerves is essential.

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

To minimise discomfort/harm to the animals, most studies are non-recovery in terminally anaesthetised animals with defined humane endpoints.

All animals will receive appropriate peri-operative care in terms of anaesthesia and pain management both during and after the procedure.

Our in-house large animal vets’ expertise further enhances animal welfare by providing close collaboration with dedicated animal care staff and ready access to highly skilled advice. Specific recovery plans have been designed to ensure the best recovery of any animal post-procedure and involve high levels of monitoring.

All animals are habituated to the environment and all recovery animals are trained prior to use for all handling procedures, such as use of a restraining crate.

Least invasive route of substance administration, appropriate needle gauge and local anaesthesia will be used where possible. Negative control groups (baseline groups) will be minimised whenever statistically feasible.

All individual study plans are reviewed including consideration of justification and implementation of refinement and reduction as part of the local protocol review process.

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

We will follow the NC3Rs guidelines on the "Responsibility in the use of animals in bioscience research" and consult all the relevant references listed therein, e.g. NC3Rs Blood sampling resource.

(Reference: NC3Rs/BBSRC/Defra/MRC/NERC/Royal Society/Wellcome Trust (2019) Responsibility in the use of animals in bioscience research: expectations of the major research councils and charitable funding bodies. London: NC3Rs.)

For substance administration the LASA substance administration guidelines will be consulted (Reference: Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider. J Am Assoc Lab Anim Sci. 2011 Sep; 50(5): 600–613.)

Animals will continually be monitored for signs of pain and distress, especially post-operative. Post-operative care will be given by specialist large animal technicians and Vets.

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

We will continuously monitor publications and the NC3Rs website for new and alternative models that could be implemented as part of this project. In addition, articles on advances in the 3Rs are regularly published on the internal Users News Forum and other relevant information is circulated by AWERB. Whenever possible we will implement these refinements into our studies.