Home Office

NON-TECHNICAL SUMMARY

Development of bioelectronic therapies for the central nervous system

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

Brain, Spine, Biohybrids, Technology, Disability

Animal types	Life stages
Mice	Adult
Rats	Adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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?

Develop new technologies to interface with the nervous system and restore function.

A retrospective assessment of these aims will be due by 22 December 2030

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

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?

Bioelectronic implants consist of implantable devices which connect with the nervous system. By reading and writing electrical information into the nervous system, bioelectronic implants can affect the function of the rest of the body. Currently, this technology has only a limited use in human patients, in the form of therapies such as stimulating deep brain regions (also known as deep brain stimulation) or specific nerves that connect the brain with internal organs, like the vagus nerve (also known as vagus nerve stimulation), which is the main parasympathetic (referring to the part of the autonomic nervous system that promotes rest and digestion) nerve connecting the brain with visceral (relating to the internal organs) organs. These are used clinically to treat conditions such as Parkinson's disease and depression. However, recent technological advances now allow us to make much smaller devices, capable of communication with small parts of the nervous system and treating many diseases. By refining the bioelectronic implants (materials and design) and stimulation techniques, we will be able to target neural (referring to the nerves or nervous system) pathways with higher specificity. Undertaking this work is important because advancing these technologies promises to expand treatment options, reduce side effects, and improve patient outcomes across a broad spectrum of conditions.

Bioelectronic implants can not only offer solutions to specific diseases, but represent a whole new class of treatments - one designed to restore health through the use of electrical communication with the body (electroceuticals), rather than making use of chemical treatments (pharmaceuticals). Overall, our approach aims to further understand and optimize recovery from conditions that affect the central

nervous system like epilepsy, Parkinson's diseases and spinal cord injury, and address the limitations of current clinical approaches.

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

This work is expected to provide novel information about the properties of the complex interaction between bioelectronics and the nervous systems. We will learn how to associate electrical signals to body functions in a more holistic approach. We will deepen our understanding on how stimulation and recording can modulate neural function and reconnect interrupted neural connections (e.g. due to lesion in spinal cord injury).

Based on our previous experience we expect by the end of the 5-year programme to have developed at least 3 bioelectronic devices ready to be used for testing in large mammals (like pigs) as the final stage in preparation for human clinical trials. Of these, we expect to have developed at least one device for every portion of the central nervous system: a brain implant, a vagus nerve implant, and a spinal cord implant for recording of physiological data with ultra-flexible device designs integrating into the neural tissue, like bendy tattoos that move and stretch with the brain and nerves.

The knowledge, expertise, and data generated from this project will significantly contribute to the continued development and optimization of bioelectronic devices, enhancing their application in other neurological conditions (referring to the process through which an individual regains lost abilities, functions, or behaviours due to brain or nerve damage) and facilitating their progression towards clinical use. The outputs from this license could play a pivotal role in accelerating the translational pathway (taking something that works in mice or rats and making it safe and effective for use in humans, adjusting it for our size, biology, and needs), ensuring the devices are robust, effective, and ready for regulatory approval in the future.

Findings will be made available to other scientists through publications in peer-reviewed journals and presentations at scientific conferences and meetings. Under our previous animal project license in the past 5 years we published over 80 papers in peer-reviewed journals. In the same period, as a lab we delivered over 110 keynote, plenary, and invited talks at international conferences and seminars.

As part of our past work, developed devices were tested acutely as a diagnostic tool in human epilepsy patients - referring to the ability to identify when abnormal brain activity (also known as seizure) is happening -, and are now beginning clinical trials for human use (referring to carefully testing a new medicine or device on people to make sure it’s safe and actually works before doctors can use it with everyone).

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

The development of a bioelectronic devices to interface with the nervous system and study their stability and performance when implanted inside the body will be of immediate benefit to the wider scientific community. In the short term, our bioelectronic devices will be used to interface with the intact/healthy brain and spinal cord to refine their design and stimulation protocols (i.e. how much and how often) to activate the neural tissue as desired. We aim to make devices that allow integration of recording and stimulation of neural tissue on a smaller device, that are simple to use implant while providing high resolution connectivity (like seeing a clear map of how different parts of the brain or

neurons are talking to each other) combined with high biocompatibility (integration with a living tissue), increasing its generality and the ability for its translation. These devices will be of great benefit to the scientific community.

By the end of the project, our refined devices will be used and validated in disease models of epilepsy, Parkinson's disease and spinal cord injury. Epilepsy is a disease that affects approximately 50 million people worldwide making it one of the most prevalent neurological disorders. Our goal is to investigate the impact of seizures on both neural activity and metabolic processes using our bioelectronic devices in chemically-induced epilepsy rodent models. This area remains largely unexplored. For Parkinson's disease (PD), we aim to reverse motor symptoms in rodent models, offering a deeper understanding of how targeted bioelectronic modulation can restore motor function. PD is a progressive neurological disorder that affects over 10 million people worldwide, making it the fastest-growing neurodegenerative condition globally. For spinal cord injury (SCI), there are an estimated 105,000 people in the UK living with a spinal cord injury and each year approximately 4,400 people are newly injured. The annual incidence of SCI in the U.S. is about 40 cases per million, totalling around 12,000 new cases yearly. SCI can occur in various ways, such as from car accidents, falls or sports injuries. Our research will focus on restoring hindlimb movement and bladder control in lumbar injury models, addressing critical functional deficits that current treatments cannot adequately resolve.

In many of these conditions, patients already have access to some treatment options involving implantable electrical devices. Our studies will enhance our understanding of how electrical stimulation can re-establish neural pathways and functional recovery in specific disorders of the nervous system (referring to the process through which an individual regains lost abilities, functions, or behaviours due to neurological damage). This makes it likely that our devices developed in this licence will be ready to follow the path of validation in large animal models leading to their ultimate use in the clinic.

There are many other diseases that our bioelectronic implants could potentially treat aside from those we work on in this licence, by just changing their design depending on the location target. This will allow other labs to apply these techniques to study a wider range of diseases, beyond those that we will work on under in this licence.

Overall, success in this program will provide practical applications to improve the quality of life for hundreds of millions of people with neurological disabilities (referring to a broad range of conditions that affect the nervous system (brain, spinal cord, and nerves) in the long term. It will also enhance understanding of the electrophysiological (referring to the study and measurement of the electrical properties) and metabolic (referring to processes related to metabolism, which is the set of life-sustaining chemical reactions that occur within living organisms to maintain life) characteristics of both normal and injured nervous systems, offering immediate benefits to the scientific and clinical communities. Additionally, new therapeutic strategies may emerge to reduce foreign body reactions (referring to the immune system's response to the presence of a material or substance that is recognized as "foreign" or "non-self") by the body to medical implants such as bioelectronic devices. When applicable, these strategies can leverage already medically-approved materials and pharmacological agents, potentially allowing for rapid application to existing clinical implants (e.g., deep brain stimulators, spinal cord stimulators, peripheral nerve stimulators, pacemakers, and joint replacement implants) following clinical trials. Together, these advancements will lay the foundation for the next generation of neural interface devices.

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

To maximize the outputs of this project, we will leverage a robust infrastructure, extensive expertise, and an unparalleled network of collaborators. The programme of work will involve over 30 researchers from our lab, including material scientists, engineers and data analysts, supported by more than £10 million in funding and access to the largest international network in bioelectronics. All fundamental methods required for designing, prototyping, manufacturing, and characterizing bioelectronic devices are well-established. These methods have been developed and refined over the past two decades.

Our previous devices are already widely adopted by leading research groups. These collaborations enable a broad dissemination of knowledge, cross-pollination of ideas, and validation of our devices in diverse contexts, significantly enhancing the impact of our work. We will continue to expand our network of collaborations as we search for new conditions which could be addressed with the treatments we are developing.

To further maximize the impact of our work, we prioritize high-quality publications and active participation in conferences, ensuring broad dissemination of the work, collaboration, and engagement with the scientific community.

Species and numbers of animals expected to be used

- Mice: 450
- Rats: 1950

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 development of implantable bioelectronic devices targeting the central nervous system requires thorough validation in animal models to ensure safety, functionality, and effectiveness. These devices must operate within the complex environment of a living organism, addressing challenges such as constant motion, temperature variation, exposure to biochemical processes, and immune responses. They must also integrate seamlessly with neural tissue (referring to the ability of a device, typically a bioelectronic or neural interface, to establish a stable, functional connection with the neural tissue without causing harm, irritation, or rejection), avoiding adverse effects like inflammation or scarring, while maintaining consistent therapeutic delivery over extended periods. These interactions and long-term outcomes cannot be fully replicated in artificial settings, making in vivo (conducted within a living organism) studies indispensable.

Rodents are an ideal choice for this research due to their anatomical (referring to the physical arrangement of tissues) and functional (referring to the natural electrical activity and functionality of the nervous system) similarities to the human central nervous system. Their neural architecture allows for

the study of motor control, sensory integration, and cognitive tasks, all of which are critical for understanding how bioelectronics may restore lost functions. Additionally, rodents can perform complex behavioural tasks, enabling researchers to test the devices in conditions that approximate human functionality. For this reason, we use adult rats, as their nervous system (brains, spinal cord, and peripheral nerves) are fully developed, and they can be trained on behavioural tasks. Importantly, studies in rodents provide a crucial translational bridge between preclinical research and human applications. The insights gained from rodent studies help refine device design, predict therapeutic outcomes, and address potential risks before advancing to clinical trials. Leveraging their widespread use in neuroscience also ensures that experiments build on existing data, minimizing unnecessary duplication and fostering an ethical approach to research.

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

Approximately 35% of animals in this work will be used for non-recovery work (terminal anaesthesia). They will undergo a surgical procedure under anaesthesia, in which one or more bioelectronic devices will be implanted in the brain, spinal cord, or vagus nerve. Animals where glucose is going to be monitored during the non-recovery procedure (less than 20% of animals in non-recovery work) may need to fast for up to 12 hours prior to the surgery to avoid unexpected fluctuations due to food intake. Cannulation (including dual-lumen intravascular catheters), intracerebral and intraperitoneal injections may cause localized tissue disruption, but these will be performed post-induction of surgical anaesthesia and prior to euthanasia.

For the remaining 65%: animals will typically be trained in a specific behavioural task for 2 weeks. Such a task may be, for example, reaching and grabbing sugar pellets. After learning the task, they will then undergo a surgical procedure under anaesthesia during which one or more bioelectronic devices will be implanted in the brain, spinal cord or vagus nerve. The wires from the implant will be run under the skin to a small port fixed to the animal's head or shoulder region. In a fraction of these animals, as part of the surgical procedure, the brain or spinal cord may also be lesioned via chemical (referring to the use of harmful substances - chemicals, toxins, or drugs- that interact with tissues or cells, causing damage) or physical injury (referring to damage caused by mechanical forces or physical trauma that physically disrupts tissue structure and function).

The animal will typically be allowed to recover and become accustomed to the implant for 1 week after the procedure. It will then be reintroduced to the behavioural task in which it was trained. Typically, this will involve 3 sessions per week for 4 weeks. During the behavioural task, a wired port will be plugged onto their head connector, and electrical signals will be recorded from their implanted neural device. As part of certain behavioural tasks (approximately 50% of animals in behavioural tasks) animals may have to be fasted. This is a commonly carried out procedure in behavioural tasks, done to motivate them to seek food rewards given during the task or to reduce the impact of feeding in the recordings. Examples of behavioural testing involve measuring motor activity on a rotating rod (called rotarod - the rodent is placed on the rod, and the device is set to rotate at a constant or gradually increasing speed), stepping tests, and other basic motor testing. The trained movements are aligned with natural rodent behaviour, and the tests will be conducted in short intervals (around 10 minutes) to ensure that they are not overly stressed.

In a fraction of the animals that have undergone behavioural training and surgery (approximately 30% of all animals), some additional procedures will be carried out. In the weeks when these animals are

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performing a behavioural task while having their device implanted, some procedures will be carried out to gather more information about the performance of the implant, such as non-invasive imaging (e.g. MRI, up to six occasions, minimum one week apart), or to make the implant more stable in the body (e.g. delivery of substances, frequency and duration based on delivery route). These additional procedures will provide little additional discomfort for the animal and may be carried out with only a brief period of anaesthesia (number of non-invasive imaging sessions will be limited to the number of anaesthesia sessions set in each protocol) or no anaesthesia at all.

A portion of animals undergoing surgery (approximately 30% of all animals) will receive a brain or spinal cord lesion to develop models of Parkinson's disease (PD) and spinal cord injury (SCI).

After ensuring full recovery from the first surgery, some animals (approximately 10%) will undergo a second surgical procedure to deliver cell therapies and assess recovery of function. Appropriate peri-operative care, defined recovery periods and refinement measures will be implemented to minimise cumulative burden. With the mitigations in place, the experience severity is not expected to increase.

Some animals (less than 10%) will also receive one or more injections subcutaneously (under the skin) or intraperitoneally (into the peritoneal cavity, the space within the abdomen that houses organs such as the intestines, liver, and stomach) as part of certain protocols for the administration of treatments. Approximately half of these will receive only one injection, with the other half receiving multiple - usually a maximum of one injection per week for the duration of the study (up to a total of 16 weeks).

Some freely-moving animals will be implanted a microdialysis probe to undergo microdialysis (a technique used to measure the concentration of small molecules, ions, or neurotransmitters in extracellular fluid in living tissues). In this case, a plastic collar will be placed around the animal’s neck to which a balance arm will be attached holding the inlet and outlet tubing connected to the microdialysis probe. While the animals are attached to the balance arm and microdialysis is being performed animals may be individually housed.

Social housing will be used by default. Animals may be housed individually if there is a risk that group housing could lead to damage to implants or connectors, potentially compromising the integrity of the experiments. The decision will be taken per case and under the guidance of the Named Animal Care & Welfare Officers (NACWO)/ Named Veterinary Surgeon (NVS). In such cases, animals will be housed separately. Animals will be reunited together in a playpen cage under supervision for at least 30 min per day to allow for supervised social interaction.

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

Approximately 35% of all animals in this work will be used for non-recovery work (terminal anaesthesia). They will not experience any notable pain or discomfort.

A significant portion of animals under this licence (approximately 65%) will undergo recovery surgery. Animals will generally be drowsy and show limited movement for approximately the first 24 hours as part of the normal recovery from the anaesthetic. Animals will also experience some degree of pain around the operated region over the first few days, which will be pre- and post-operatively treated with pain relieving medication as advised by the NVS (normally over two days post-surgery). In rare occasions (less than 5%) animals may experience postoperative complications, such as wound

dehiscence (a surgical complication that occurs when a surgical incision opens or separates) or loss of body weight, the latter generally expected to recover within the first 6 days. In case of wound dehiscence or suture opening, minor interventions such as resuturing may be allowed once, provided they are conducted under the guidance of the NACWO/NVS and with prior confirmation of the animal's welfare, with intervention taking place as early as possible within 24 hours of detecting the opening. If the wound opens and re-suturing is not possible within 24 h of detection due to the animal's welfare concerns, the animal will be killed following SCh1 methods listed in the licence.

A portion of animals undergoing surgery (approximately 25% of all animals) will receive a brain or spinal cord lesion to develop models of Parkinson's disease (PD) and spinal cord injury (SCI). Depending on the type of lesion and implant used, animals may recover full movement and feeling over the course of approximately 4 weeks, with the majority of the animals developing permanent model of motor impairment (SCI or PD) that increases the severity of the protocols to severe.

Some animals will undergo two separate surgical interventions (10%) and may therefore be at an increased risk of anaesthesia-related and surgery-related complications listed above, as well as a cumulative impact on health and welfare.

In SCI models, partial or complete hindlimb paralysis (absence of movement) or paresis (mild to moderate degree of muscular weakness) is anticipated in all animals, with effects ranging from several weeks to permanent impairment, depending on the extent and technique of the injury to the spinal cord. For example, a spinal cord hemisection (a partial injury that occurs when one side of the spinal cord is cut) at the level of the eleventh thoracic vertebra would likely cause paralysis of the hindlimb on the homolateral (same) side. If both hindlimbs are paralyzed for more than 24 hours, this outcome would be classified under a "severe" severity category. During the first one to two weeks after surgery, all animals may experience bladder and bowel dysfunction, necessitating manual bladder expression and close monitoring for constipation. In a small proportion of animals (less than one percent of all animals), a rare complication called autonomic dysreflexia could arise if the lesion is located at or above the tenth thoracic vertebra. This condition can cause an exaggerated response of the autonomic nervous system—a branch of the peripheral nervous system that regulates involuntary functions such as heart rate, blood pressure, digestion, and respiration—to painful sensory input. It can also lead to episodes of cardiovascular or autonomic instability, where the autonomic nervous system fails to regulate bodily functions properly, resulting in fluctuations or abnormalities in heart rate, blood pressure, temperature regulation, digestion, and sweating. Throughout these procedures, animals will continue to feed and drink without difficulty, although a temporary reduction in appetite and mobility might cause mild weight loss (less than 10%) and some dehydration. Most animals adapt to moving around their cages within a few days (typically two to five days) and are not visibly affected by the lesion once they have adjusted (In complete and permanent hindlimb paralysis, this will include the recruitment of the forelimbs). Brain and spinal cord lesions do not lead to additional pain beyond what is normally expected from the surgical procedure; pain relief will be managed according to standard analgesic protocols, with the option to modify the regimen as advised by the NVS.

In some cases, animals may be single-housed (for example, to protect surgical sites or to allow for accurate monitoring of fluid and food intake) . Single housing may cause stress. If signs of stress or distress appear as a result of single housing, the animals will be consolidated with other mates. If rehousing males there is an increased risk of fighting occurring.

Treatment or control drugs (placebo substances with no effect) may have a strong taste and when delivered orally in food or drinking water can make them less palatable, increasing the risk of animals losing weight or becoming dehydrated from not drinking.

Animals may experience minor crusting around the head or percutaneous connectors. This may cause some discomfort to the animals, and increase grooming around the area. In animals that wires are externalised through the head (approximately 15% of all animals), the insertion of a headcap is identified as a potential source of harm, as it may lead to transient bleeding and facial swelling due to tissue trauma during the procedure.

In some cases, the suture may reopen due to the animal scratching or natural skin retraction. This may cause minor bleeding, delayed wound healing and irritation for the animal. No other adverse effects are expected in combination with the crusting, and animals are not expected to show evidence of pain, suffering, or distress, due to the crusting.

The placement of a plastic collar in micro-dialysis experiments and the use of a balance arm could impact the animal's ability to eat or drink, particularly in the initial adaptation period, including restricted head movement, making it harder to reach food and water, stress or discomfort, and potential interference with food access, depending on cage setup and positioning of food and water.

Headcaps can fall off due to animals forcefully detaching head or percutaneous connectors (less than 5% times), typically after 1 week of implantation. Animals may exhibit over-grooming around the area prior to connector detachment. Detachment of the connector is not expected to produce a significant immediate impact in welfare, but will leave an opening in the skin that may be prone to infection.

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)?

We anticipate that most animals involved in this study will fall under either the non-recovery or moderate severity categories. Animals classified under non-recovery will undergo procedures entirely under terminal anaesthesia. All experimental work for these animals will be conducted while they are anaesthetized, and they will not regain consciousness, ensuring that they do not experience pain or discomfort.

The moderate severity category will encompass animals that will undergo surgical procedures primarily for the implantation of bioelectronic devices. These animals may experience a brief period of moderate pain following surgery, but with appropriate post-operative care, they are expected to recover fully within a few days. Although there will be mild procedures, cumulative effects make these protocols to be fully mild but moderate.

Finally, a fraction of the animals will experience severe severity. These are the animals corresponding to those protocols where two surgical procedures will be undertaken, or in which the nervous system will be lesioned (brain or spinal cord) in addition to the implantation of the bioelectronic device, and are expected to suffer major sensory-motor impairment increasing the severity to severe.

In summary:

Mice Non-recovery 45%

Moderate 50%

Severe 5%

Rat Non-recovery 25%

Moderate 60%

Severe 15%

What will happen to animals used in this project?

- Killed

A retrospective assessment of these predicted harms will be due by 22 December 2030

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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?

The use of animals is essential to achieve the aims of this project because the nervous system's complex interactions with other body systems cannot be fully replicated using in vitro (cells and tissues are grown directly in a laboratory) or in silico (computational models). Initial results will be obtained from in silico and in vitro work and our unique electronical characterization methods are among the most advanced in the world. However, animal models are necessary to study how electrical stimulation and recording influence neural activity, metabolic processes, and physiological responses in a living organism (in vivo). These interactions differ significantly between healthy and diseased states, and studying living models allows us to understand how pathological conditions such as the conditions under study (Parkinson's disease, spinal cord injury, and epilepsy) imbalances affect neural function and can be modulated for therapeutic purposes.

While alternative models can be used initially for surgical training and device placement refinement, they cannot replicate the dynamic neural and physiological responses observed in living systems. Using animals under carefully controlled and refined experimental conditions ensures that the data obtained is biologically relevant, paving the way for effective translation to clinical applications.

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

Part of the development of bioelectronic implants for interfacing with the central nervous system can be replaced or supplemented through ex vivo (organ, cells, or tissue are taken and studied outside from a living body) and in vitro work. By investigating how implants interact with brain and spinal cord tissues, we can optimize designs without initially involving animal models. However, in vivo studies will ultimately be necessary to confirm that the implants achieve the desired therapeutic effects.

The following alternative systems were considered for use in this project to minimize animal involvement:

- Ex vivo explants of spinal cord and brain tissues, connected to the implants under development. These allow detailed characterization of the features necessary to achieve a stable, functional connection with specific regions of the CNS.

- In vitro cultures of neurons, electrically stimulated using the implants being developed, to study the spread of electrical current (how much volume of tissue we are stimulating and how current is being transmitted), neuronal activation thresholds (how much stimulation is needed to activate the tissue), and the implants' ability to interface effectively with neural tissue.

- Computer simulations (or in silico work) to model electrical and mechanical properties of the implants, predict their interactions with neural tissues, and assess factors such as current spread, neural activation, and optimizing implant designs before conducting in vivo studies.

Why were they not suitable?

Although these methods provide critical preliminary data, in vivo studies remain essential to verify the long-term performance and therapeutic efficacy of the implants in living systems, where complex physiological and dynamic factors come into play.

- Ex vivo explants of spinal cord and brain tissues are valuable for studying how implants physically interact with specific regions of the brain or spinal cord tissues, allowing detailed analysis of material properties and electrical performance. However, ex vivo systems are fundamentally limited. They lack the dynamic physiological environment of a living organism, such as blood flow, immune system interactions, and tissue remodelling processes, all of which are critical for understanding how implants function in real-world conditions. Additionally, explants are only viable for short periods, making them unsuitable for studying long-term implant integration or stability. Finally, because they are disconnected from the rest of the body, explants cannot replicate the functional interactions between the central nervous system and peripheral systems, which are essential for assessing the therapeutic outcomes of bioelectronic devices.

- In vitro cultures of neurons provide a controlled environment for testing the spread of electrical current, neuronal activation thresholds, and basic material compatibility. However, these cultures represent an oversimplified version of the central nervous system. They lack the structural and functional complexity of intact neural circuits, including three-dimensional organization, synaptic connections (the junctions between neurons where they communicate with each other), and the diverse mix of cell types found in vivo. Additionally, in vitro systems do not replicate the

mechanical dynamics experienced by implants within a living body, such as tissue deformation or movement. Furthermore, in vitro experiments are limited to short-term observations and cannot capture feedback loops or other systemic processes, such as how the brain or spinal cord might adapt to long-term stimulation or respond to injury caused by the implant.

- Computer simulations allow to predict current spread, neural activation patterns, and areas of potential tissue damage, enabling iterative refinements before physical testing. However, computational models are inherently limited by the assumptions and simplifications used to create them. They cannot fully capture the complexity of biological systems, such as variability in tissue properties or dynamic biological responses like inflammation or immune activation. Additionally, while simulations can provide insights into physical and electrical interactions, they cannot test the functional outcomes of bioelectronic devices, such as restoring motor or sensory abilities, which are critical for therapeutic validation.

A retrospective assessment of replacement will be due by 22 December 2030

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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?

Estimates of animal numbers are derived from a combination of 2 factors. The first is our experience in developing neural bioelectronic devices - particularly that derived from our previous licence. The second is our engineering knowledge on the typical number of iterations (versions of devices) necessary to develop successful bioelectronic devices. Over the course of this licence, we expect to develop a progressively improved bioelectronic device. From our previous experience, we expect to have to go through approximately 15 iterations of devices before arriving to its final version. This final device will then be applied to different areas: surface and deeper structures of the brain, the spinal cord and the vagus nerve to develop bioelectronic treatments. Each of these will likely require a further 10 iterations to optimise the device for each specific application.

From our experience, each individual iteration of devices/electroceutical treatments requires approximately 25 animals to test and obtain decisive feedback on. While difficult to predict the exact numbers at this stage, this calculated estimation provides a good approximation to the number of animals we expect to use.

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

In designing our experiments, we considered the extensive groundwork required before testing devices in living animals. This includes thorough engineering tests (bench testing), computational modelling, and cell culture studies, which significantly reduce the number of iterations needed to finalize a device or treatment. We will also first optimise the surgical and behavioural procedures in pilot studies before proceeding to device implantation. This staged approach prevents unnecessary surgeries and reduces the overall number of animals required.

We incorporated best practices such as the PREPARE guidelines, the NC3R experimental design tool, ARRIVE guidelines, and comprehensive literature reviews in the bioelectronics field to accurately estimate the number of animals needed. In similar experimental setups, we have previously used statistical power calculations to demonstrate that a sample size of 10 animals per group was sufficient to achieve a statistical power of 0.8 (80%), which is the ability of our experiment to detect an effect. This would also be sufficient to achieve type I error rate (alpha) below 0.05 (5%), which is the likelihood of mistakenly concluding that an effect exists when there actually isn't one. Therefore, a sample size of 10 animals is enough for us to make conclusions from our data with good confidence. We expect these values will be similar in experiments of this licence. These tools will also guide the design of future experiments. Our estimate of 25 animals per iteration encompasses pilot studies, non-recovery procedures, and surgical implantation with behavioural testing, ensuring robust results while minimizing the use 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?

We will begin with pilot studies involving a small number of animals to assess the preliminary effectiveness of the implant being evaluated. If these initial studies show promise, additional experiments will involve larger groups of animals for more comprehensive testing. The number of animals used will be carefully determined based on the pilot results and statistical power calculations (as the one presented in the previous section to determine how many subjects are needed in a study to detect a real effect while minimizing errors), ensuring experiments are appropriately designed to detect meaningful outcomes.

To reduce reliance on animal testing, we will conduct thorough preclinical assessments, including in vitro experiments, engineering analyses, and computational modelling. These steps will help optimize device performance and increase the likelihood of success in subsequent animal studies, thereby minimizing the number of in vivo tests required.

Data acquisition and analysis will be performed using automated systems whenever possible to ensure accuracy and reduce potential biases. Animals are all expected to be sourced from reputable commercial suppliers, including specialized strains where needed, to ensure ethical and efficient use of resources.

A retrospective assessment of reduction will be due by 22 December 2030

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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.

This project will involve both rats and mice, with most experiments conducted in rats. Rats are used for most experiments because they have a nervous system that is sufficiently similar to that of humans in that the biology of brain, spinal cord axon regeneration and peripheral nerve activity is almost similar. Rats are docile and social animals, also capable of complex behaviour and skilled paw use, making it possible to achieve good behavioural outcomes. Their size is more permissive for the use of implantable neural interface technology. They will receive plenty of interaction with researchers, animal technicians and each other and provided with the best social environmental enrichments according to the latest guidelines and good practice] (https://www.nc3rs.org.uk/news/tickling-rats-social-enrichment-improve-rodent-welfare). Mice are also useful and needed when developing technology transfer to other labs or in cases requiring specific models necessary for clinical translation. For example, the cell transplantation research we draw from is exclusive to mice. This is an ex vivo approach to support the development of bioelectronic devices (see Replacement section), which involves preparing cells outside the body before transplanting them into a living organism. We use this approach to create biohybrid bioelectronic devices, which integrate living biological components, such as cells, with our otherwise pure electronic systems. Therefore, they are more compatible with biological environments and reduce the likelihood of rejection or inflammation.

Immunodeficient strains of rodents (referring to genetically modified or naturally occurring rodent models that have impaired immune systems) may be used in cases where cellular material needs to be implanted as part of the bioelectronics implant. Cellular materials are recognised by the immune system and destroyed, being therefore unable to perform their function. While local immunosuppression can sometimes be sufficient to prevent this, use of immunodeficient strains may occasionally be required to ensure integration of cellular materials into the body. Nude strains, which are strains with a genetic mutation that results in an inhibited immune response, are commonly used in xenograft studies (involving the transplantation of tissue, cells, or organs from one species to another) for this reason (i.e. they will not react and reject foreign objects).

The majority of experiments will involve implanting a bioelectronic device into the brain, the spinal cord or the vagus nerve. In some studies, additional procedures such as inducing brain or spinal cord injuries may be performed to replicate the conditions our devices are intended to treat. These surgeries will be conducted under strict aseptic conditions with appropriate pain management to ensure animal welfare.

The implants are designed to be small, flexible, and biocompatible, minimizing the likelihood of causing discomfort during their use. Extensive in vitro testing, including cell culture studies, will be conducted prior to any in vivo work to refine and optimize device performance.

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

Some aspects of this project, such as short-term evaluations of neural bioelectronic devices and electrical parameter testing, can be conducted in terminally anaesthetized animals. These non-recovery experiments account for a significant proportion of the animals used. However, for an implantable bioelectronic treatment to be viable, it must demonstrate effectiveness over extended periods. Long-term use introduces challenges such as tissue movement, inflammatory responses, and material degradation, which are critical to address because they are likely to occur in human patients. These factors can only be studied in animals where the implants remain in the body for prolonged durations.

Although less sentient species also have nerves, the human nervous system is unique to vertebrates. Rodents, particularly rats, share an anatomical nerve distribution similar to humans, with comparable nerve sizes in certain cases. This similarity enhances the translatability of the devices. Additionally, rats are widely used in surgical research due to their well-documented anatomy and are capable of learning a variety of behavioural tasks. These tasks can simulate scenarios that patients might encounter, allowing for the assessment of treatment efficacy. Together, these attributes make rodents, and especially rats, an excellent model for developing neural bioelectronic devices and advancing electroceutical treatments for human use.

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

We minimise suffering by choosing rat strains that do not nibble their toes after nervous system surgery. We will also prioritize strains such as Lewis rats, which less frequently exhibit adverse behaviours. We will use non-tail handling methods, such as cupping and tube handling, (https://www.nc3rs.org.uk/mouse-handling-tutorial) and enrichment aimed at improving their quality of life on study and ameliorating potential adverse effects. Every animal will be given plenty of time (minimum of a week) to acclimatise to the new facility before any procedure is carried out.

To minimize weight loss and enhance animal welfare, animals will be housed in enriched environments after surgical procedures. These environments will be equipped with a variety of highly palatable foods, such as seeds, dried fruit, and chocolate spread, to encourage nutritional intake and aid in recovery. Animals will be acclimatised with the highly palatable foods for a couple of days prior to surgery to be familiarised with them and further enhance their ability to maintain weight. Surgical procedures will be performed by licensed personnel skilled in the techniques, adhering to LASA guidelines for aseptic surgery. Both peri-operative and post-operative care will be provided by trained animal technician staff. After surgery, all animals will receive wet mash food or gel to support their recovery, and analgesia will be administered post-surgery as advised by the NVS. Twice-weekly weighing, instead of every two days, can improve refinement by reducing unnecessary handling and associated stress. It also provides less disturbance to husbandry routines. Discretionary post-operative fluid replacement: ensures fluids are given only when clinically indicated, avoiding unnecessary invasive procedures and associated stress for the animals.

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When animals will be required to undergo two surgeries, a minimum recovery period of three weeks will be allowed between both procedures to ensure full recovery. Progression to the second surgery will be conditional on animals demonstrating stable health and the absence of ongoing clinical signs related to the first surgery.

In non-recovery experiments, fasting will not be applied unless glucose is going to be monitored during the non-recovery procedure to avoid unexpected fluctuations due to food intake. Behavioural tasks often requires animals to be food restricted to provide an incentive for animals to learn a particular task. We will work to minimise the amount of time during which animals have to be fasting. We will limit access to food only on the day of training. Fasting will typically be done three days per week (maximum of 4 days) allowing for one day of free access to food between each session (alternating days). When intravascular blood sampling and infusion are performed simultaneously in non-recovery procedures, a dual-lumen cannula will preferably be used. This enables both fluid administration and withdrawal through a single vascular access point, reducing the number of surgical interventions and improving procedural efficiency.

Aseptic surgical techniques, including sterilising the implants prior to implantation by ethylene oxide or an equivalent method, along with the use of analgesics and post-operative care following LASA guidelines, will ensure that any discomfort is minimized, and animals will be closely observed during the healing process. Antibiotics will be used in consultation with the NVS in order to stop infections.

To minimise the impact of the bioelectronic devices, we design them to be as small as possible and to match the electromechanical properties of the neural tissue. Pilot studies and cadaveric studies are used to first test suitability of implant devices. While we often require the use of a large number of wires (to connect to the dense array of electrodes in our implants), we already make use of microfabricated wire arrays that minimise the space and damage that will occur in the subcutaneous pocket. Purposely designed and fabricated surgical tools allow to further minimise the amount of subcutaneous dead space generated as part of the implantation process.

As part of our previous work, we've optimized the design and materials of our headcaps to the point where animals can be group housed while wearing them. These headcaps are crucial for protecting the wires and providing a secure connection to the device for the experimenter. However, they can be bulky and may hinder the animal's movement around the cage and their normal grooming activities. To minimize the impact, we are designing and fabricating our own headcaps using 3D printing. This allows us to create smaller, lighter headcaps that are specifically tailored to our devices, with materials and structures that reduce their weight and bulk. Our current headcap designs weigh approximately 12 grams, which is less than 5% of the typical bodyweight of rats used in our studies. We will continue to refine our headcap designs throughout the project.

To protect the implants and minimise the risk of connector detachment, adaptations to the animals housing such as an altered lay out/removal of shelves will be implemented. These items will be substituted with other enrichment items that do not have the risk of damaging the implants such as hideouts designed to provide shelter while being large enough to avoid contact with the implants. We may also adjust food and water placement as needed to ensure animals can comfortably reach both (e.g., elevate bowls or provide long-spout water bottles).

By choosing well-established lesion models that have been extensively used in our lab throughout several previous studies, we are able to ensure a high rate of reproducibility and the least possible

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impact on the animal welfare. Furthermore we will use adequate perioperative pain relief regime and early humane endpoints. All our work is structured in a step-wise approach to only progress with interventions which are more likely to be successful. To achieve this aim we will carry out initial work under terminal anaesthesia, and use the least impactful animal model first.

Whenever possible, animals will be housed socially to promote natural behaviours and well-being. Animals may be housed individually if there is a risk that group housing could lead to damage to implants or connectors, potentially compromising the integrity of the experiments. The decision will be taken per case and under the guidance of the NACWO/NVS. In such cases, animals will be housed separately but with efforts to minimize stress, for example animals will be reunited together in a playpen cage under supervision for at least 30 min per day to allow for supervised social interaction. Additional environmental enrichment will be provided to ensure their wellbeing while minimizing the risk of damaging the implants, including but not limited to soft bedding material to promote natural nesting behaviour and comfort without interfering with the implants, and plastic tunnels or hideouts designed to provide shelter and a sense of security, while being large enough to avoid contact with implants. If signs of stress or distress appear as a result of single housing, the animals will be consolidated with other mates. For re-housing males, preventive measures will be applied to minimize the likelihood of fighting such as introducing males gradually, ensuring the environment has adequate space, and providing enrichment items to reduce territorial disputes. Regular welfare checks will be conducted to closely monitor behaviour following rehousing.

The scab formation and crusting are expected to resolve within 7-10 days with minimal topical management and cleaning the area. To further minimise this adverse effect, the sharp tips of the hind claws will be clipped as part of the pre-op procedures of the surgery to reduce potential damage from scratching. Using absorbable, finer sutures to reduce tension on the wound and improve healing will be considered. Local anaesthetics will be administered at the connection site during surgery to minimize postoperative discomfort and reduce the likelihood of scratching or irritation on the first day following the procedure. A routine inspection and cleaning programme for the implant site will be implemented to allow for early detection of any issues and timely intervention, and antiseptic and moisturising creams will be applied topically to minimise the appearance of crusting.

In a few circumstances (less than 10%), it may be appropriate to anaesthetise the animal to fully examine and clean the affected area (e.g. headcap or percutaneous connector), and potentially reclose the skin around the connector if the suture reopens (without full detachment), which will only be allowed once at any time within the first 10 days after surgery. Advice from the NVS will be sought when managing these cases. If the NVS considers that the adverse effects can be ameliorated promptly and successfully with no more than minor interventions, it will be treated accordingly.

To protect the implants and minimise the risk of connector detachment, adaptations to the animals housing such as an altered lay out/removal of shelves will be implemented. These items will be substituted with other enrichment items that do not have the risk of damaging the implants.

When administering substances to animals, the use of single needles will be mandatory to help reduce tissue damage and discomfort. If treatments or controls with a strong taste are to be delivered orally in food or drinking water making it less palatable, it will be considered the use of palatable food or sweeteners to mask the taste and reduce the risk of animals losing weight or becoming dehydrated from not drinking.

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In some animals, the placement of a plastic collar in micro-dialysis experiments and the use of a balance arm could impact the animal's ability to eat or drink, particularly in the initial adaptation period, including restricted head movement, making it harder to reach food and water, stress or discomfort, and potential interference with food access, depending on cage setup and positioning of food and water. To minimise these adverse effects, animals will go through an acclimatization period before the experiment, to ensure proper collar fit to minimize discomfort, and provide environmental enrichment to reduce stress. We will adjust food and water placement as needed to ensure they can comfortably reach both (e.g., elevate bowls or provide long-spout water bottles), offer softened food or hydration gel if needed.

Animals will also be provided with different bedding and enrichment materials in their cages to enhance comfort and promote natural behaviours. Supportive diets and food supplements will be provided post-surgery to help in their recovery. Post-op recovery will be closely reported in post-op sheets that will be filled twice a day for at least 3 days post-surgery.

Peri- and post-operative analgesia will be provided (post-operative analgesia will be provided orally with a syringe); agents will be administered in accordance with a regime agreed in advance with the NVS.

Supervisors will ensure that new personnel involved in the project receive comprehensive training on the procedures in line with LASA principles, guaranteeing that they are well-equipped to handle all aspects of animal care and experimentation.

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

All users of this licence will be required to read the published ARRIVE and PREPARE guidelines prior to start of any work and integrate these into their plan of work. They will also become familiarised with the NC3Rs experimental design website and information.

Supervisors teaching new licence holders will do so following LASA guiding principles to ensure new users are properly trained to perform any procedures necessary. This includes developing and making use of training protocols for each procedure. All work will generally be carried out in the same facility, allowing new users to have routine contact with supervisors to ensure good transfer of knowledge.

Additionally, we will encourage researchers to meticulously plan any new projects under this PPL. This planning will include outlining the structure of planned publications, detailing the experiments necessary to achieve our objectives, and calculating the number of animals required, including any pilot studies. By emphasizing adherence to ARRIVE guidelines, we will ensure that our experimental designs are robust and transparently reported. LASA guiding principles for behavioural laboratory animal science will be followed when undertaking behavioural work.

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

Team members involved in this project will stay informed about advancements in the 3Rs through various channels, including participation in animal facility user meetings, engagement with mailing lists

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and workshops hosted at our institution, regular review of the NC3Rs website, and monitoring relevant published literature. We will also maintain close communication with the Named Information Officer to ensure seamless support for our work. Additionally, we will evaluate the progress of all activities under this license and discuss the integration of any new 3Rs-based improvements into our protocols in our regular weekly group meetings.

A retrospective assessment of refinement will be due by 22 December 2030

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

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