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NON-TECHNICAL SUMMARY

# Molecular Mechanisms of Neuronal Excitation and Neuroinflammation

**Project duration**

5 years 0 months

**Project purpose**

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

**Key words**

Pain, Inflammation, Neurodegeneration, Hypoxia, Ageing

## Retrospective assessment

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

## Objectives and benefits

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

**What's the aim of this project?**

The nervous system is important for normal bodily function. Malfunction of the nervous system presents in a variety of ways: chronic pain, i.e. regular pain over a period of months, disrupted bowel function (e.g. chronic constipation or inability to control bowel movements), cancer pain and dying off of neurones leading to impaired brain function (e.g. as occurs in Alzheimer's disease and Parkinson's disease) – many of these conditions are related to ageing. Many people suffering from chronic pain are poorly treated by current medications, either due to a lack of efficacy (the drugs do not work), or due to intolerable side effects. Similarly, in conditions where brain neurones die off (e.g. Alzheimer's disease and Parkinson's disease), no cure is available just treatments to ease a patient's suffering. To develop new drugs, it is first necessary to better understand how the nervous system works and what goes wrong in disease: enhanced understanding of how neurones work in health and disease underlies future drug discovery and clinical breakthroughs.

**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.**

**What are the potential benefits that will derive from this project?**

By understanding how the sensory nervous system works, with a focus on inflammatory and cancer pain sensation and diseases associated with neuronal death in the brain, our work will lead to fundamental advances in our knowledge of neuronal function. With enhanced knowledge, our work will aid the development of new therapies to treat chronic pain (especially that associated with inflammation, e.g. arthritis and inflammatory bowel disease), as well as treatments for disordered breathing syndromes (e.g. central sleep apnoea) and conditions associated with neuronal death in the brain (e.g. Alzheimer's disease and Parkinson's disease).

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

**What types and approximate numbers of animals will you use over the course of this project?**

6888 mice and 328 naked mole-rats are estimated to be used over a period of 5 years.

# Predicted harms

**Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.**

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?**

Many of the procedures detailed in this proposal will be conducted using general anaesthesia to ensure that pain and/or distress are limited during a procedure before animals are allowed to recover with appropriate post-operative care (e.g. soft food, pain relief post-surgery and heated recovery cabinets, as

well as use of heat mats during surgery). Small groups of animals will develop different forms of arthritis/colitis (both acute and chronic), skin cancer, or experience fluctuating oxygen and carbon dioxide levels, procedures which will involve a moderate level of pain and distress. In arthritis models, this is likely to involve swelling of the joints and grooming of the affected area; one arthritis model uses the joint lubricating fluid from human individuals (or dogs) with arthritis to induce arthritis-like conditions in mice and use of this substance will aid translation of our findings back to humans, it will also likely reduce the severity of joint inflammation compared compared to synthetic agents. In colitis models animals are likely to show low activity and experience diarrhoea as occurs in human inflammatory bowel syndrome, in cancer models, cancer is induced in the back skin of the animals and so is not expected to induce any significant symptoms beyond tumour growth. In some experiments, animals will be injected with substances that enable the tracking of sensory neurones around the body so that we can determine, for example, how the properties of neurones supplying the knee change during arthritis – these substances are inert and no adverse effects are expected beyond that experienced by the procedure itself; injection of substances to track neurones in the gastrointestinal tract (gut) and other internal organs requires surgery, from which animals are expected to make a rapid recovery. From such work, we aim to identify new drug targets and these will be investigated by administering substances by the most appropriate route to determine how they ameliorate disease/pain progression, i.e. drugs or other substances administered to modulate pain pathways in disease are expected to reduce the adverse effects of the, for example, arthritis being experienced. A small number of animals will undergo surgery to insert wires that will enable us to measure the responses of muscles to distension of the gastrointestinal tract. Animals are expected to make a rapid recovery from surgery and the distension process itself evokes a short-lasting pain response that may be heightened in animals experiencing colitis. A small number of animals will undergo spinal surgery to enable optical control, e.g. different coloured lights can be used to excite different proteins and switch nerves on and off, and thus manipulate how neurones communicate with each other. Neither the surgical procedure, nor the optical stimulation is not expected to produce any long-term pronounced harm. In some studies, female animals will undergo drug-induced superovulation – a procedure to induce multiple eggs that is used in humans for in vitro fertilisation – harvesting of eggs will enable us to establish cell lines that in the long-term will lead to greatly reduced animal use. In some experiments, animals will be transported between two facilities (a 15-minute journey by an appropriatevehicle) to enable access to equipment that measures in a non-invasive way how cells in the blood respond to changes in the amount of inhaled oxygen and carbon dioxide. Results from these experiments will tell us how much oxygen and carbon dioxide get to the brain, which will help us understand the mechanisms by which nerve damage can occur because long-term changes in brain oxygen and carbon dioxide are associated with nerves dying. We do not expect animals to die as a result of the procedures performed. All animals will be humanely killed at the end point of each study using approved methods and tissues used for further analysis, including sharing with other research groups to maximise the use of available tissues and thus minimise further animal use as far as possible.

# Replacement

**State why you need to use animals and why you cannot use non-animal alternatives.**

This work will examine complex behaviours such as pain and breathing control and to assess complex behaviours it is necessary to study whole organisms because interactions take place between different

body systems, e.g. the nervous system and immune cells in the blood, rather than each system acting alone. Indeed, although cell lines and computational methods are used to complement our *in vivo* research, they themselves do not provide in depth information on complex behaviours that are the result of complex neuronal circuits functioning at the whole organism level. Similarly, non-protected animal alternatives, such as the nematode worm or fruit fly, do not have the same level of neuronal development as mammals, which would limit the direct relevance or 'translatability' of our work to the field of human medicine.

We will also develop cell lines from naked mole-rats that will lead to replacement of animal tissue with cells for future experiments and thus in the long-term act to reduce total animal number.

# Reduction

**Explain how you will assure the use of minimum numbers of animals.**

Statistical analyses will be employed ahead of starting experiments to ensure that the minimum number of animals will be used, as is necessary to produce statistical useful results. Experimental blinding (i.e. the experimenter is unaware of what the genetic make up of an animal is, or whether they have received a drug or control substance etc.) will be used as part of appropriate study design and animals used are inbred to produce more reliable results (inbreeding reduces the genetic variation and thus limits one possible source of variation in experimental measurement) resulting in a lower overall number being used. We regularly design experiments so that multiple tissues can be taken from each animal after its death to decrease the overall number of animals used. We regularly use cell lines in place of freshly isolated neurones to examine the structure and function of any proteins of interest that we identify in this study, which we combine with computational methods to try and develop new therapeutics/drug treatments. Lastly, when inducing joint inflammation, this only occurs in one knee/ankle joint of an animal, which means that the other joint acts as a healthy joint and thus each animal acts as its own control, thus reducing overall animal use.

# Refinement

**Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.**

Mice are used because they represent good choice of animal model for the study of the specific pain related conditions under study e.g. colitis and arthritis. Moreover, we have generated a large amount of preliminary data using mice that will be used as a basis upon which to build during the proposed experiments in this Project Licence. Mice are also the mammalian species best developed in terms of our ability to manipulate their underlying genetic make up and produce so called 'genetically altered animals'. Our studies will enable analysis of genes of interest in animals specifically bred to be lacking that gene, which is essential for many of our aims. Naked mole-rats are being used because they display an insensitivity to carbon dioxide/tissue acidity and low levels of oxygen that is unique among mammals. By comparing mice to naked mole-rats we can identify the molecular basis for the difference

in naked mole-rats and thus learn more about how chemicals are ‘sensed’ in other mammals, including humans. Welfare impact on the animals will be minimized through use of: sterile surgical technique, pain relief when required and humane killing at end of study, or at predetermined humane endpoints to prevent unnecessary suffering, we also use non-interventional behavioural tests to measure the impact of arthritis/colitis on an animal’s behaviour. We have constantly refined the environmental conditions that our animals live in, for example by providing ramped tubing and enlarged tunnel/chamber habitats, group housing for our naked mole-rats to more closely mimic the living conditions and hierarchical colony structure that they would have in their natural wild setting. Using a non-invasive method for measuring how changes in inhaled oxygen and carbon dioxide affect the amount of oxygen and carbon dioxide in the blood is a refinement on taking multiple blood samples and measuring in an external machine.