Home Office

NON-TECHNICAL SUMMARY

Mechanisms underpinning neuronal excitability in health and disease

Project duration

5 years 0 months

Project purpose

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

Key words

Pain, Inflammation, Neurodegeneration, Cancer, Therapy

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant, aged
Naked mole-rat	adult, juvenile, neonate

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?

To extend knowledge of mechanisms governing how nerves are switched on and off in the context of pain, control of breathing and neurodegeneration, conditions where levels of oxygen and interactions between the immune and nervous systems are important. Anticipated benefits are understanding potential new drug targets for pain relief and the treatment of neurodegenerative conditions associated with ageing, such as Alzheimer’s and Parkinson’s 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?

Chronic pain is a hallmark of conditions like osteoarthritis, irritable bowel syndrome, cancer and endometriosis, affecting approximately 40% of the adult population in the United Kingdom. Unfortunately, current medications are deemed inadequate at controlling pain by two thirds of chronic pain patients, either because the drugs do not work, or the side effects experienced are too unpleasant.

Neurodegenerative diseases like Alzheimer's disease which affects approximately 1 million individuals in the United Kingdom, and disordered breathing syndromes such as central sleep apnoea, are similarly poorly managed, a few treatments improving symptoms, but no therapies providing a cure.

A major risk factor for both chronic pain and neurodegenerative diseases is ageing and in the United Kingdom the proportion of the population aged over 65 years of age will increase from 16% to 30% by 2030, thus the prevalence of conditions for which age is a risk factor is set to increase.

In both chronic pain and neurodegenerative conditions, changes in neuronal excitability and function lead to the symptoms observed. In recent years, it has become clear that changes in how the immune system interacts with the nervous system play pivotal roles in disease progression.

This work will focus on increasing our understanding of how the properties of nerves change from health to disease in the context of chronic pain, disordered breathing and neurodegeneration, and the role of non-neuronal cells in these processes. Results will open avenues to developing new therapies to treat pain and neurodegeneration.

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

The main output of this project will be new information about the mechanisms by which chronic pain and neurodegeneration (neuronal death) are caused.

For example, the way our experiments are designed, by comparing health to disease, we will be able to identify key molecules involved in specific disease processes, e.g. what molecules are released into the knee joint during arthritis and how do they alter neuronal excitability to cause pain? Similarly, by comparing the neurobiology of the long-lived naked mole-rat, which is highly resistant to cancer and neurodegeneration, to the biology of the mouse, we aim to identify molecules the support the healthy ageing of the naked mole-rat, which could be targeted to prevent and / or treat ageing-related conditions, such as neurodegeneration in humans.

All new information generated will be published, firstly online through the open access pre-print bioRxiv server, a process that ensures rapid dissemination of our outputs, and secondly in peer reviewed journals. In addition, we will communicate our findings at scientific conferences through poster and oral presentations.

We will also make sure that our work is communicated to the public in an appropriate way, for example, by providing lay summaries of our work and through the production of short films and animations, all of which we have a strong track record of delivering.

In the long-term, we would hope that our work would lead to the generation of new therapies for the treatment of chronic pain, disordered breathing conditions, and neurodegeneration, but this is beyond the scope of the current project.

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

In the short-term, the main benefits from our work will be to other researchers, both in academia and the pharmaceutical industry, as well as to the general public through our public engagement work. Communicating with the public to increase understanding of what scientists do is not only crucial to educate and inspire, but also as a point of accountability considering how most research within the United Kingdom is funded. We currently collaborate with multiple pharmaceutical companies making us well placed to provide immediate benefit to their research programmes through our findings.

In the long-term, beyond the time of this project, we anticipate that findings from our research could lead to the development of new therapies to prevent and / or treat chronic pain conditions and neurodegenerative diseases.

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

To maximise our outputs, we will continue our strong track record of collaborative work. For example, I run an animal sharing platform, which provides an online presence through which researchers can contact me to arrange collaborations and maximise the sharing of tissue to gain the greatest amount of information from any individual animal.

To maximise dissemination, we post all our findings, both positive and negative, on the open access pre-print bioRxiv server, which enables results to be posted online prior to peer review. In addition, we

will continue to post "Tweetorials" of our published work through Twitter to reach the maximum possible audience; these are often written in an accessible way for non-experts to also understand.

Species and numbers of animals expected to be used

- Mice: 13960
- Other rodents: No answer provided

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.

Chronic pain is whole body experience, involving activation of a peripheral nerve that generates responses in the central nervous system, and the experience of pain is modulated by how the nervous system interacts with other parts of the body, for example the immune system. Similarly, in neurodegenerative diseases, such as Alzheimer's disease, the disease occurs in a complex organ, the brain, and involves multiple different cell types. Consequently, to study how nerve function changes between health and disease it is necessary for us to study whole organisms.

This project will use mice at all life stages, which are the most appropriate species for studying pain. Firstly, for studying pain affecting joints (e.g. osteoarthritis) and internal organs (e.g. endometriosis), it is necessary to use a whole organism that has the appropriate body parts, i.e. a purely in vitro (i.e. cell-based) study or one involving invertebrates like Caenorhabditis elegans that lack joints would not provide the same degree of insight that an in vivo (i.e. whole animal) study in a mammal can. In addition, technologies developed for genetically altering mice enable greater mechanistic insight to be generated than in other rodents. Mainly adult mice will be used, but in some experiments young mice will be used as well to study the developmental aspects of both pain and neurodegeneration.

We will also use naked mole-rats because they are long-lived rodents that display a variety of exceptional biology. For example, naked mole-rats are highly resistant to cancer, do not develop neurodegenerative conditions throughout their approximately 40-year life-span, can cope with fluctuations in atmospheric oxygen and carbon dioxide that regulate breathing, and display highly unusual pain biology. As a mammal, the naked mole-rat provides a unique opportunity to study biology at its extreme, for example, if we understand what is special about nerves within the naked mole-rat brain that make it resistant to neurodegeneration, this could be translated to be of benefit to humans. We will use naked mole-rats of all ages, from soon after their birth through to the end of their naturally occurring lifespan because this will enable us to see what changes do, or do not occur, within their nervous system that enable them to age healthily.

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

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 inflammation, such as arthritis and colitis, skin cancer, or experience fluctuating oxygen and carbon dioxide levels, procedures which will involve a moderate level of pain and distress; an animal would only experience one such experimental condition.

In arthritis models, this is likely to involve swelling of the joints; 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; synovial fluid will also likely reduce the severity of joint inflammation compared to synthetic agents, although these latter agents are also very useful due to their reproducibility of effect and known mechanism of action, how the pain they produce is evoked is less understood. Most of these inflammatory arthritis models will last a maximum of 7-days, but one that mirrors aspects of osteoarthritis last 28-days due to the progressive degradation of cartilage and bone that thus better recapitulates the human condition. A further arthritis model involves making a small injury to the knee joint that leads to gradual onset of osteoarthritis over several weeks, involving similar processes to the human condition, thus making it a good model to aid translation from bench to bedside.

In colitis models, animals are likely to show low activity and experience diarrhoea as occurs in human inflammatory bowel disease (IBD), but periods of colitis will generally last days, rather than weeks. Some animals may experience 2-3 bouts of colitis to simulate the relapse and remission that humans with IBD also experience, remission from inflammation often not meaning a remission from pain, and thus our approach enables us to study pain mechanisms at all stages of disease.

In cancer models, cancer is induced in the skin on the back of the animal over a period of weeks and is not expected to induce any significant symptoms beyond tumour growth, metastasis to another body location being exceptionally rare.

In bladder pain and endometriosis models, animals will likely urinate more often, but produce a smaller volume, as occurs in humans with similar conditions. Bladder pain models are generally short lasting (e.g. up to 7-days) using an anti-cancer agent that causes bladder pain in humans (i.e. we accurately modelling a human condition). Endometriosis models are longer lasting (up 2-months) due to the gradual onset of the condition, i.e. animals do not experience symptoms until long after induction of endometriosis.

In some experiments, animals will be injected with substances that enable the tracking and modulation of sensory neurones in 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, bladder or vagina. 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, bladder pain or endometriosis. The distension procedure is required to simulate the type of natural stimulus that evokes pain in humans with such conditions, e.g. through passage of matter through the gut, a filling bladder and uterine contractions during menstruation.

In all animal models of pain-related diseases, animals will undergo behavioural tests to measure how disease and disease intervention affects their behaviour. Many of these behavioural tests will monitor the impact of pain on spontaneous behaviours, e.g. how much less burrowing does an animal display when a joint is inflamed, whereas other tests will measure the time taken to respond to a stimulus, such as a fibre being pushed against the hind paw.

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 reduced animal use.

In some experiments, animals will be transported between two facilities (a 15-minute journey by an appropriate vehicle) 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 breathing is regulated and how 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 and the minimum number of procedures will be performed on each animal to minimise the overall impact on an animal's well-being. 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.

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

In all of our pain models, we anticipate that the animals will experience some degree of discomfort and heightened sensitivity in behavioural tests, due to accurate simulation of the human condition being modelled; the discomfort is expected to be lessened in our studies that aim to interfere with the pain mechanisms that we identify. Most arthritis, bladder pain and colitis models only last a number of days, but one arthritis model lasts up to 16-weeks because it is a slowly progressive condition with animals not experiencing measurable pain for the first 12-weeks, and similarly, our endometriosis model develops slowly and so although it lasts approximately 2-months, symptoms do not occur in the initial weeks. Weight loss is not generally expected apart from in the bladder pain and colitis models. Behavioural tests used will sometimes evoke a transient sensation of pain, but that is the method required to measure how the disease affects pain and set a standard against which we can investigate how interventions affect the pain experienced.

In our cancer pain models, whereas mice develop tumours, naked mole-rats do not, moreover, because we are investigating skin tumours on the back of the animals, these are generally deemed to be low impact to the animal's well-being and no particular distress or weight loss is observed.

All surgeries conducted will be under anaesthesia, thus minimising the pain and discomfort experienced, although transient weight loss in the few days following surgery is anticipated; pre- and post-surgery pain relief will be administered to lessen the discomfort experienced where it does not interfere with the scientific objectives.

Exposure to low oxygen or high carbon dioxide will be relatively brief (maximum of 1-hour) and will cause mice to show altered breathing rate during the period of altered oxygen / carbon dioxide exposure, but this is not the case for naked mole-rats who are resistant to such changes in oxygen and carbon dioxide.

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)?

There are no severe protocols associated with this project.

Most animals will experience surgery and / or a form of pain syndrome (e.g. arthritis) or cancer, which would be of moderate severity.

Mice exposed to low oxygen or raised carbon dioxide would be considered as moderate severity, but this would be mild for naked mole-rats that are resistant to low oxygen or raised carbon dioxide and continue to breathe normally.

Breeding of genetically altered animals will be of a mild severity, as will animals acting as control or sham conditions in our arthritis, colitis and other pain models.

Overall severity proportions:

Mice: 9.2% mild, 12.7% moderate

Naked mole-rat: 100% mild

What will happen to animals at the end of this project?

- Killed
- Kept alive

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?

This research will investigate complex, whole organism pathologies, such as inflammatory bowel disease. To study such pathologies, it is necessary to study whole, intact organisms because of the interactions that take place between different body systems to drive disease pathology, e.g. the nervous system and immune cells in the blood.

Moreover, to correlate our work with humans experiencing similar conditions and to thus aid translation, it is necessary for us to study how an underlying pathology affects an animal's behaviour, which can only be done if whole animals are used. For example, humans with joint pain are less active due to activity-induced pain and we use similar assays to measure how arthritis and other conditions in mice affect their natural digging behaviours. Similarly, to study how changes in atmospheric oxygen or carbon dioxide affect breathing and the function of nerves in the brain, it is necessary to study an animal that has similar physiology to a human.

Studying less complex organisms, such as worms, would be inappropriate because they do not have the necessary physiology that would enable us to simulate human pathologies, i.e. arthritis is a disease of the joints and worms do not have joints, nor do they have brains which precludes studying neurodegenerative conditions of the brain, or the brain regions that control breathing.

Lastly, using purely cell-based or organoid models prevents would be scientifically inappropriate as they do not permit the study of interactions between different body systems that are critical in the conditions that we will be studying, nor do they enable us to study how neuronal activity, and modifying that activity (e.g. via novel painkillers developed through our work) impacts animal behaviour.

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

We do not only conduct whole animal work, but rather structure our computational, cellular and whole animal work to form coherent research projects, with the additional benefit of using human tissue when appropriate.

To meet the objectives of the proposed research, the following hon-animal alternatives that we have considered are as follows:

• Computational approaches to identify drug targets for treating disease

• Human induced pluripotent stell cell derived sensory neurones (human cells, which can be transformed to be like neurones)

• Other cell lines / organoids

• Non-protected animal species (e.g. worms and leeches)

Why were they not suitable?

• Computational approaches are ideal for screening through 1000s of compounds to identify top "hits" that might interact with a target of interest and this a method that we have used and published.

However, such computational approaches themselves cannot alone provide information on mechanisms of disease pathology, be it how endometriosis-mediated pain progresses over time, or how a change in oxygen level changes brain activity to result in altered breathing.

• Human induced pluripotent stell cell derived sensory neurones (hiPSC-SN) are neuronal cells of human origin that are helping for screening purposes (e.g. does my compound of interest modulate activity of a certain molecule in a human cellular background), which is how we use them in our lab to help validate results from rodents. However, such hiPSC-SNs are homogenous in nature, unlike the true diversity of sensory neurones in a whole animal where we and others have shown that neurones supplying different targets have different properties. In addition, a purely cellular system does not permit us to examine the complex pathologies of conditions such as osteoarthritis or inflammatory bowel disease where interactions between the nervous system and cells of the immune system and other non-neuronal cells are important for disease pathogenesis.

• Other cell lines/organoids, as with hiPSC-SNs, provide a good background for studying the structure - function aspects of how specific molecules function, an approach we regularly use in the lab. However, such a cell-based or organoid-oriented approach does not provide insight into the complex, whole organism physiology and pathology that we are studying in this project where multiple body systems interact with each other to modulate behaviour.

• Non-protected animal species (e.g. worms and leeches), every organism with a nervous system has nerves dedicated to detecting potentially harmful stimuli and enabling safe interactactions with its environment. However, the physiology and pathology that we need to study in this research are specific to what occurs in mammals, e.g. one cannot study a condition affecting joints in a species without joints (e.g. worms/leeches), nor can one study regions of the brain involved in sensing oxygen or that undergo neurodegeneration in an organism that lacks a brain/a brain similar to that of humans (e.g. worms and leeches / fruit flies).

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?

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. Such analyses will be driven by prior experience and data from the wider scientific literature.

In addition, where appropriate, pilot tests will be carried out in a small number of animals to assist with experimental design and refine group size estimates. For example, to determine the efficiency with which neurones can be labelled with a certain agent, this would be conducted in small number of animals and refinements made where necessary.

Overall numbers have been estimated based upon two key factors:

• The number of mice required to breed the correct number of genetically altered animals of the required genotype for the different types of genetically altered animals to be used, which is based on our lengthy experience of working with such animals.

• Consideration of the number of different test and control groups that will we analyse for each scientific protocol involving mice and naked mole-rats. The group size for each experiment being estimated using statistical analysis for each specific protocol to ensure that the minimum number of animals will be used to produce statistical useful results.

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

We keep up with the latest literature to make sure that we are only conducting experiments that are scientifically appropriate and to prevent the meaningless repetition of studies conducted by others elsewhere.

Where possible, we conduct computational and cell line work before proceeding to using animals. For example, computational modelling can help to identify compounds most likely to provide the desired effect on a molecule that regulates one aspect of neuronal excitability, which can then often (but not always) be tested in a cell line to validate computational results before taking the lead compound into whole animal work.

We have also conducted pilot tests to assist with experimental design and refine group size estimates, alongside use of online reference tools, such as the NC3R's Experimental Design Assistant.

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

The 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. For example, subsequent to behavioural studies looking at inflammatory bowel disease, we would isolate the colon, as well as sensory neurone tissue to perform experiments on both tissue types, and from behavioural studies analysing joint pain we would isolate sensory neurones and knee tissue to support different forms of analysis; we often also take the spleen for harvesting immune cells. Where possible, we always conduct studies where the animal acts as its own control, for example, in many inflammatory arthritis models, we only induce inflammation in one joint of an animal, which means that the other joint acts as a healthy joint (pathology in the untreated joint, due to, for example, offloading from the treated joint has not been observed), thus reducing overall animal use.

Specifically, regarding the naked mole-rat, it is a highly unusual animal and therefore whenever an animal used experimentally, we ensure that tissues are shared widely using an online sharing platform.

Use of this platform means that it is very common that tissue from one animal supports multiple projects, for example different brain regions can be taken for studies looking at neurodegeneration and the regulation of neuronal activity by oxygen, the skin is taken as a source for developing cell lines, heart, liver and skeletal muscle support studies examining energy regulation with ageing, and further tissues are often shipped internationally (e.g. blood to country X to examine properties of the naked mole-rat immune system and skin to the country Y to examine properties supporting the mole-rat's unusually strong and supple skin that shows limited scarring after injury).

All members of the research team also subscribe to an internal tissue sharing scheme, which supports sharing of animals, techniques and resources.

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.

Mice are used because they represent good choice of animal model for the study of the specific pain related conditions in this project e.g. colitis and arthritis, being an organism in which disease models, pathology and behavioural paradigms are well developed and understood. Furthermore, we have generated significant preliminary data using mice that will be used as a basis upon which to build in the experiments proposed in this project, and such preliminary data has helped us refine the experimental approaches used, the number of animals required and the severity of the procedures conducted.

In addition, mice are the mammalian species best developed in terms of our ability to manipulate their underlying genetic make-up and produce so called ‘genetically altered animals’, which enables us to make mechanistic insights into disease pathologies that were previously impossible, i.e. we can use such genetically altered animals in our studies to analyse the roles of specific of genes of interest in disease pathology and behaviour.

Many of the models we use simulate very closely the disease in humans. For example, the bladder pain model results from administration of an anti-cancer drug that is frequently used in humans with cancer and causes bladder pain as a side effect, the main colitis model is induced through a change in diet and shows relapse/remission like inflammatory bowel disease in humans, one of the key arthritis models involves mechanically destabilising the knee joint leading to gradual onset of osteoarthritis and injury initiated / slow onset pathology regularly underpins osteoarthritis in humans, and the endometriosis model to be used reproduces the types of internal injury that occur in the human condition. Because we are studying pain mechanisms, providing standard pain relief throughout a study would compromise our scientific objective of understanding the key molecules that drive pain. To provide as much comfort for animals as possible and thus to minimuse suffering, we will enrich their environment as appropriate, e.g. providing food in the form of mash (available on the cage floor to

prevent the need for animals to rear up to obtain food) and placing heat pads under individual cages as an extra supply of warmth.

When tamoxifen is used as a gene inducing/deleting agent, animals can experience weight loss. To minimise any potential harm to an animal, no experimental procedure will be conducted until any weight lost has been regained.

We will regularly carry out behavioural tests, many of which are non-interventional behavioural tests to measure the impact of a condition on an animal's behaviour, for example, how does the condition an animal experiences affect their digging behaviour, a natural exploratory behaviour exhibited by mice?

Naked mole-rats are being used because they display an insensitivity to carbon dioxide/tissue acidity and low levels of oxygen, alongside extreme cancer resistance and absence of neurodegeneration over a long lifespan, combined features that are unique among mammals. By comparing mice to naked mole-rats we can identify the molecular basis for the differences in naked mole-rats and thus learn more about oxygen/carbon dioxide sensing, cancer and neurodegeneration in other mammals, including humans. Because naked mole-rats are resistant to the conditions that we will be exploring, they will experience minimal pain and distress under experimental conditions, e.g. agents that induce skin cancer in mice do not in naked mole-rats and they continue to behave normally in conditions of lowered oxygen. Some naked mole-rats will receive hormonal injections to help us generate cell lines, which would lead to reduced animal use in the future, but the hormonal injections themselves do not causing any suffering of lasting harm.

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

In this project we will study pain, how oxygen regulates breathing and how nerves in the brain die with ageing. For all of these conditions, an organism with a nervous system and brain is required, i.e. use of worms or flies would be inappropriate as they do not have the parts of the mammalian body that we need to study to stand the best chance of translating our findings to aid human health and that of companion animals. Moreover, many of the human conditions that we wish to study, e.g. osteoarthritis and endometriosis, require the use of adult mammals as they are conditions that impact humans in adulthood. In addition, to study mechanisms of joint pain, it is necessary to study animals that have the same musculoskeletal apparatus as humans, and thus worms and fish that lack joints would be inappropriate species to study.

Because we wish to study how disease progression affects an animal's behaviour over time, use of terminally anaesthetised animals is not possible. For example, the main model of osteoarthritis that we use lasts 16-weeks with behavioural symptoms only occurring from approximately week 12, and therefore to study how the progressive nature of the condition affects behaviour (and how our interventions modulate the behaviours), it is necessary to monitor animals over time, not under anaesthesia.

Naked mole-rats are the only mammal exhibiting their highly unusual biology and hence it is not possible to replace them with another species. For example, certain reptiles also exhibit low prevalence of cancer, but translating our findings from a mammal are going to be far easier than from a non-mammalian species like a gecko.

Lastly, for all our work using mice and naked mole-rats, it is important that we can compare our results to others in the field and benefit from insights that they make. In all areas we are working, pain, cancer, neurodegeneration and regulation of breathing, mammals (primarily mice) are the species of choice because disease models are well-established and transgenic tools are available.

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

In general terms, the welfare impact on the animals will be minimized through use of: sterile surgical technique, pain relief when required that does not impair the scientific objectives of the study, 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 pain conditions on an animal’s behaviour.

For animals experiencing conditions lasting several days or weeks, they are regularly monitored (e.g. using a disease activity index scoring system for mice with colitis and measuring joint width for animals experiencing experimentally-induced inflammation) and weighed.

On days where animals experience surgery, they will be regularly checked with peri-operative pain relief and easily accessible energy rich food provided.

We have constantly refined the environmental conditions that our animals live in, for example by providing ramped/bendy tubing and enlarged tunnel/chamber habitats 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. Moreover, for experiments where we wish to measure how changes in inhaled oxygen and carbon dioxide affect the amount of oxygen and carbon dioxide in the blood, we will use a non-invasive method where the animal is under anaesthesia and placed in a machine that scans the animal with light to produce a form of ultrasound imaging, rather than taking multiple blood samples and measuring in an external machine.

Where genetically altered mice are used, inducible constructs will be used wherever possible to limit any impact of gene expression/deletion on behaviour. Moreover, where possible we will limit gene alteration to the cells of interest, which in the case of this project will usually be peripheral sensory neurones.

Lastly, we will use non-aversive handling techniques where possible, and always allow acclimatisation of the animals when brought into the facility before starting a study, as well as acclimatisation to any behavioural apparatus before initiating the study.

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

We will conduct our research according to the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines and make sure that we report our findings in line with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

We will also use online resources, such as the humane endpoints webinar (https://www.humane-endpoints.info/en), NC3R's resources for breeding and colony management in genetically altered mouse colonies (https://www.nc3rs.org.uk/3rs-resources/breeding-and-colony-management), and NC3R's experimental design assistant (https://www.nc3rs.org.uk/our-portfolio/experimental-design-assistant-eda).

In addition, for all aspects of our surgical work we will use the Laboratory Animal Science Association (LASA) Guiding Principles for Preparing for and Undertaking Aseptic Surgery and for our cancer research following the advice of Workman et al (2010) Guidelines for the welfare and use of animals in cancer research, British Journal of Cancer, 102, 1555-1577.

Lastly, we will keep up with the latest scientific literature to monitor advancements in the field about how to look after our animals and conduct experiments in the most refined way.

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

We will make the following steps:

• Routinely check the NC3R's resource library and keep abreast with, and implement where appropriate, any new advances in refinement, reduction and replacement that are relevant to our project.

• Have regular discussions with the Named Persons and animal technicians within the facility to review current approaches and whether there are any new 3Rs opportunities.

• Continue our subscription to an internal tissue sharing scheme, which also provides an opportunity to keep up to date with 3Rs news and events, as well as take advantage of opportunities to share tissues and knowledge.

• Regularly check attend NC3R's workshops where appropriate and monitor publications in journals, such as Laboratory Animals and Lab Animal to stay abreast of the latest developments in all aspects of the care and use of animals in biomedical research.