Home Office

NON-TECHNICAL SUMMARY

Physical signals and their interaction with biochemical signalling in vertebrate tissues and cells

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

CNS development, neural disorders, mechanotransduction, mechanobiology, tissue mechanics

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult
Rats	Neonate, Juvenile, Adult, Pregnant adult
Xenopus laevis	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

Our aim is to characterise the mechanical properties* of and to investigate the role of mechanical signals*, and their interaction with biochemical signals*, on central nervous system (CNS)* tissue during development, physiology*, pathology*, and regeneration, and in other organ systems and tissues.

Glossary:

mechanical properties: the physical characteristics of something, like how stiff or stretchy it is;

mechanical signals/signalling: physical forces or movements, like pressure or stretching, that can affect how cells behave;

(bio)chemical signals/signalling: messages that cells send using chemicals to communicate and control body functions;

central nervous system (CNS): the brain and spinal cord, which control most of the body's activities;

physiology: normal healthy situation in the body;

pathology: any kind of non-healthy situation in the body.

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?

Traditionally, scientists have studied how chemical signalling affects the development, physiology, pathology, and regeneration of the nervous system. More recently, it has become increasingly clear that physical cues (i.e. physical things like pressure or stiffness that cells can sense and respond to) such as tissue stiffness (i.e. how hard or soft a tissue is) or cellular forces (i.e. pushes or pulls created by cells that affect their surroundings) are equally important for these processes. Furthermore,

chemical and physical cues are intricately interconnected and can influence one another. Cells perform the complex task of integrating, that is combining information from, both chemical and physical cues to adapt their behaviour - a process we have barely started to understand.

As both chemical and physical cues are essential for cellular and tissue development and function, it is important to address the current gap in our understanding of the mechanical properties of CNS tissue and the interplay with chemical signalling in regulating key processes during development, in adulthood and pathological processes. Clarifying these processes will help us better understand physiological and pathological processes. More research in this area could help us understand observations which are currently incomprehensible, as biochemistry alone might be insufficient to explain them. For example, cells produce chemical substances needed for communication with other cells depending on the stiffness of their environment.

We are particularly interested in how the long processes of nerve cells that send messages to other cells, called axons, find their way to their correct target location during development. This can be studied particularly well in the eye development of frog embryos. An embryo is the very early stage of a developing new animal while organs are still being built. In the eyes of frog embryos, nerve cell bodies that are located in the developing retina (the innermost layer of the eye) send their axons out of the back of the eye in a bundle, which then crosses under the brain to the opposite side of the brain, continues to grow along the surface and finds its precise target location in the visual centre of the brain. Accurate wiring of the nervous system during development is necessary for its proper functionality later in life. Wrong wiring can lead to diseases such as schizophrenia or autism spectrum disorders.

We also want to better understand what the healthy brain and spinal cord tissue "feels" like and how its mechanical properties might change during disease or after injury. To date, CNS injury often still has a poor outcome for patients. If we understand how mechanical properties change in these scenarios, we might be able to use them for diagnostic (i.e. to find out what is wrong with a patient) or even therapeutic (i.e. to treat an illness or injury) interventions in the future.

The mechanical properties of cells and tissues and mechanical signalling is highly relevant not only for CNS tissue but also in other organ systems and tissues, such as blood vessels and the lung. Therefore, we want to study cells and tissues both within and outside of the CNS.

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

- new data about mechanical properties of CNS tissue and how this may be altered during development and in pathological conditions
- new information about the role of mechanical signals in regulating CNS development and neuronal function (i.e. the function of nerve cells)
- new insights into molecular mechanisms of how tissue mechanics impact cells

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

Downloaded: 9:38am, 13 Mar 2026

In the short term, our findings will close relevant gaps in the existing knowledge which will be important for directing the future work of both us and other research groups.

In the mid-term, knowledge about changes in tissue mechanics may contribute to the development of new diagnostic approaches for CNS diseases and injuries both in human and veterinary patients.

In the long term, our work may contribute to the development of new therapeutic strategies which might improve quality of life for human patients, their relatives and friends, and would benefit society at large due to the high costs associated to the long-term nature of such CNS conditions. Such advances would likely also benefit veterinary patients suffering from similar neurological conditions, for example when they are affected by damage to their spinal cord after a road traffic accident.

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

Our group consists of scientist from a range of different subjects and has a lot of experience in biophysics, bioengineering, molecular, developmental and cell biology, as well as neuroscience. Furthermore, our work is highly collaborative (i.e. we work together with other researchers) across different departments within our university and across other universities within and outside of the UK. We regularly send members of our group to collaborating research groups and host guest researchers in our laboratory with whom we share our insights. As we have a very specific and rare expertise in measuring the mechanical properties of tissues and cells, we want to make this expertise available to other groups through collaborations, in line with the 3Rs.

Furthermore, our work is frequently presented at conferences already before publication, we upload all of our studies onto a pre-print server prior to publication, where it is freely available to everyone worldwide, and we regularly publish in highly regarded, very visible international peer-reviewed journals. We try to make sure that all relevant information, including unsuccessful attempts, are included in our publications and to give sufficient details in our 'methods' section to allow other researchers to learn from our experiences and design good future studies based on what we have done or attempted before.

We have a record of participating in public engagement activities in the past, especially with regards to our animal work in the past, and will continue to do so in the future.

Species and numbers of animals expected to be used

- Mice: 890
- Rats: 260
- Xenopus laevis: 865

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.

Downloaded: 9:38am, 13 Mar 2026

We always use the animal species of the lowest possible complexity (that is how simple or complex an animal is).

Wherever possible, we will work on *Xenopus laevis*, which are a type of frog (the African clawed frog). There are other *Xenopus* species apart from *Xenopus laevis* (for example *Xenopus tropicalis*) but whenever we say '*Xenopus*' anywhere in this document, we mean '*Xenopus laevis*' only. We work specifically with *Xenopus* embryos, which are a valuable model system for a number of reasons: Females can lay a large number of eggs several times per year. These eggs can be unproblematically fertilised, investigated and manipulated outside of the mother. Furthermore, the mother does not take care of her eggs or feel close to them, unlike mice, which look after their babies. After fertilisation, cells divide approximately every 30 minutes which means that the embryos reach the developmental stages needed for our research within a few days. The most important biochemical signalling cascades (i.e. a chain reaction) required for brain development are largely understood. Furthermore, *Xenopus* frogs are a species where we also understand the effect of mechanical signals on brain development comparatively well. It is thus a very good system to further investigate the interactions between chemical and mechanical signalling, which we currently don't yet fully understand. *Xenopus* is well-suited for such studies as embryos of the stage we are interested in do not yet have a bony skull, so that the brain can be easily accessed for microscopy and experimental interventions. Thus, we mostly use an early developmental stage of a low-complexity animal for the majority of our research.

However, differences between the *Xenopus* and human central nervous system may sometimes limit the transferability of insights we gain from the *Xenopus* experiments, particularly when investigating disorders of the CNS that are particularly relevant for humans. In order to test whether the findings which result from the *Xenopus* experiments can be reproduced (that is to do an experiment again and get the same results) in higher animals and to investigate various CNS-related diseases, we use rodents because they are mammals like humans and their neurobiology is thus more similar to the human CNS.

We are doing a lot of our rodent work *in vitro*, which means work on animal tissues or cells that takes place outside a living animal. This includes cell culture, which means growing brain cells from rats and mice in dishes in the lab. As mature nerve cells do not divide any longer, we have to use the prenatal (meaning 'before birth') brains of mouse and rat embryos. For such studies, mice are useful as they are available with various genetic alterations. 'Genetic alterations' means that these animals have changes in the instructions (genes) inside living things that decide how they grow and work. However, one can isolate many more nerve cells from rat brains, so that fewer animals are needed overall.

We also study the mechanical properties of nervous tissue. Again, a lot of these studies are conducted in *Xenopus* embryos. For other studies, we use tissue obtained from mice and rats of various ages as their CNS anatomy, that is the way their CNS is built and structured, more closely resembles the human one.

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

Female *Xenopus* frogs will be stimulated to lay eggs by injecting them with natural hormones that will make them start laying eggs. Typically, animals will be subjected to two individual subcutaneous (meaning 'under the skin') injections, spaced some days apart, and will experience brief, slight discomfort and no lasting harm. To aid the quality of eggs collected, small changes which are limited to

Downloaded: 9:38am, 13 Mar 2026

the time around egg laying, may be made to when animals are fed and to the light-dark cycle (that is when the lights are on or off) that are not expected to adversely affect frog welfare. An individual female may undergo this protocol repeatedly, with at least 3 months in between, until her egg quality gets worse.

Most of our rodents do not have any interventions carried out on them while they are alive and conscious.

Some rodents will be used to obtain tissue under terminal anaesthesia. 'Terminal anaesthesia' means that they will be anaesthetised, like for surgery, and will be killed with a humane method while they are unconscious. Some of these animals may be given analgesic drugs (that is 'painkillers') before the onset of anaesthesia, either by oral dosing (that means by giving into the mouth, which can either be by mixing it with food or by giving it directly into their mouth) or by subcutaneous injection, which may lead to brief, slight discomfort and no lasting harm.

Some rodents will have mechanical measurements conducted on their tissue under terminal anaesthesia. Some of these animals may be given analgesic drugs before the onset of anaesthesia, either by oral dosing (which can either be by mixing it with food or by giving it directly into their mouth) or by subcutaneous injection, which may lead to brief, slight discomfort and no lasting harm. It is possible that we may need to give 'neuro-muscular blocking agents' (NMBAs) to some animals. This is to prevent their muscles from twitching when their nerve cells are touched during the mechanical measurements. This might be necessary as muscle twitching could interfere with accurate readings of the mechanical measurements. We will only use NMBAs where this is really necessary, and if we do, we will put additional measures in place to be certain that animals which can no longer move are truly unconscious. The use of NMBAs is standard practice in human medicine and safe as long as monitored correctly.

Where procedures are carried out under terminal anaesthesia, the animals will only be aware of the anaesthetic being administered and may briefly experience distress and no pain. In some cases, analgesic drugs will be administered by injection after the onset of anaesthesia, which the animals will not experience as they are unconscious. They are humanely killed at the end of the experiment without regaining consciousness.

Few animals may undergo minor surgical procedures, such as embryo transfer (female mice) or vasectomy (male mice). 'Embryo transfer' means transferring embryos from their biological mother to another female (called 'embryo recipient') so that the pregnancy until the birth will happen in the embryo recipient and not the biological mother. To do that, the embryo recipient has to be mated with a male who cannot produce any offspring. Such males have previously undergone a surgery called 'vasectomy' which interrupts the biological structure through which the semen travels from the testes to the penis and renders them infertile.

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

Animals which have to be subjected to injections will suffer no more than mild transient discomfort and no lasting harm. A small number of animals may be subjected to short surgical procedures which may result in short-lived post-operative pain and discomfort.

Downloaded: 9:38am, 13 Mar 2026

Genetically altered animals which will be bred or maintained under this licence will not show any clinical signs of the genetic modification, for example because the genetic modification may simply result in some cell types of interest showing fluorescence. 'Fluorescence' is when certain substances give off light after absorbing light or energy and is often used in science to make cells light up in a specific colour so they can be identified more easily. For the purpose of obtaining tissue under terminal anaesthesia or for conducting mechanical measurements under terminal anaesthesia, we may obtain genetically altered animals from other licences if these licences are allowed to pass animals onto other licences. Such animals may show clinical signs as a result of the genetic modification, for example because this might be a disease model. Such animals would be transferred to us only if a veterinary surgeon had previously examined them and considered them fit for transport and they would be terminally anaesthetised immediately after arrival in our laboratory.

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)?

Xenopus laevis: 53% sub-threshold, 47% mild

Mice: 40% sub-threshold, 25% mild, 6% moderate, 29% non-recovery

Rats: 100% non-recovery

What will happen to animals used in this project?

- Killed
- Used in other projects
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

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?

Two different types of experiments we do require the use animals:

1) We study the mechanical properties of the central nervous system at the tissue level.

The mechanical properties of a tissue are related to the tissue's architecture as a whole. This includes the various different cell types that are present, the ways in which these cells are connected to one another, the composition and arrangement of the extracellular matrix (the non-cellular matter between cells) and the presence and orientation of larger structures, such as blood vessels. With regards to the CNS, and particularly the spinal cord, there is also a clear separation of grey and white matter with

highly aligned, parallel axon tracts in white matter. The fact that this complex architecture is required to obtain meaningful results necessitates the use of tissue obtained from an animal.

2) We study nerve cells *in vitro*, that is in cell culture.

Cell culture is generally considered a reduction method as it is done outside a living animal, using cells in a dish instead. However, we work on the central nervous system. Nerve cells typically do not divide after birth. Therefore, even when we do experiments *in vitro* we need to obtain the cells we culture from an animal in the first place as they cannot be propagated in a dish.

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

For the study of the mechanical properties of CNS tissue, we need CNS tissue. The two alternatives to fresh animal tissues would be a) surplus tissue from human neurosurgery, b) post-mortem tissue from humans and c) organoids, which are small very simplified likenesses of organs, grown in a dish from cells which are able to generate different types of tissue, used to study how real organs develop and work..

As an alternative to primary nerve cell culture, there are a few neuron-like cell lines available.

Where possible, we will use computer simulations to model certain physical interactions.

Why were they not suitable?

Previous research has shown that mechanical tissue properties are starting to change very early after death. To maintain tissue integrity, that is its correct composition, speedy and thorough sample preparation, including the use of specialised buffer solutions (meaning solutions which can keep the pH, an important measure of how acidic a solution is, stable) which are dependent on continuous bubbling with CO_2, is necessary. This fact is important for understanding why human surgical surplus or post-mortem material is not a feasible source for tissue for mechanical testing. Surplus human tissue from surgeries would become available only at a surgical centre, which would require at least 25 mins of transport to our laboratory, under ideal conditions. It would not be possible to supply CO_2 to our buffer solutions during transport and the time frame on the whole is considerably longer than would be acceptable. For this type of experiment, we are trying to keep the dissection procedure as short as anyhow possible, down to the last minute. Post-mortem tissue samples would suffer from an even greater delay due to the necessity to confirm the patient's death, move the body to a facility where a post-mortem examination may be conducted and then dissect a CNS sample from within the skull or the vertebral column, which takes a considerable amount of time due to the bone thickness. This alone renders both types of tissue unsuitable for mechanical testing.

Furthermore, there are additional problems related to both types of tissue: Surplus tissue from human neurosurgery is rare, unpredictable, normally not physiological, and small. For obvious reasons, neurosurgeons attempt to remove as little healthy tissue as possible. Often, the exact amount of tissue to be removed will be determined by the surgeon during the surgery and the removed tissue itself will usually be abnormal, for example during tumour removal. Thus, such tissue samples would be too small to represent CNS architecture as whole and predominantly unphysiological (i.e. not representing the normal healthy state) and thus not allowing us to draw conclusion about the physiological

Downloaded: 9:38am, 13 Mar 2026

mechanical properties. Post-mortem tissue would require the patient's consent prior to death, which may be inacceptable due to the psychological effects associated with it.

While the research into generating organoids has made great progress in recent years, there are still considerable limitations to how accurately these represent tissue-level architecture. The complex architecture of grey and white matter segregation with highly aligned axon bundles extending along very specific trajectories (that is a specific path which is being followed by growing axons) and the vascular system cannot yet be replicated sufficiently well to make them a viable alternative to fresh tissue samples.

Taken together, these limitations necessitate the use of fresh animal tissue for high-quality spatially resolved mechanical mapping to CNS tissue.

With regards to neuronal cell culture, cell lines available for non-primary cell culture are problematic for two reasons: There is no neuronal cell line available, there are only neuron-like cell lines available, which only resemble neurons in very specific, individual aspects, but they cannot be used to study the development of neurons over time. Furthermore, the fact that cells of such lines *can* divide in culture is per se grossly unphysiological, making it the value of findings obtained from such cells uncertain and very debated in the field.

Computer simulations used to model how objects physically interact crucially rely on information about the mechanical properties of the objects that are to be modelled. Such information is not yet fully available and our work will contribute to provide more detailed information of this sort, so that computer simulations can be used more widely in the future.

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?

A mouse 'line' or 'strain' is a specific breed of mice, similar to breed in other animals like dogs. It is possible to 'cryopreserve' a mouse line, that means freezing and storing mouse embryos at very low temperatures so they can be kept for a long time and used later to restart the line. One can also 're-derive' a mouse line, which means restarting it from frozen embryos, which is usually done to make sure the mice are free from diseases. For cryopreservation and/or re-derivation of a mouse line, we assume we might be rederiving three independent lines and have liaised with our core facility offering this service for the animal numbers required for this (approx. 270 mice, of which a large part are genetically altered mice coming from other parts of this project).

For breeding and maintenance of genetically altered mice without a harmful phenotype, we have used our annual return of procedures data from previous years to estimate the number of animals that we

will need to use for breeding and experimental procedures. From these data, we assume we need approximately 200 mice per strain. We assume we will use 3 strains of a mild 'phenotype' (that is the way an animal appears from the outside, e.g. its fur colour and any other characteristics it might have). Such strains could be for example reporter lines, in which cells of certain types are labelled, usually by being fluorescent. Such animals appear completely normal otherwise. This results in 600 mice in total. These animal numbers include building up a colony from scratch (i.e. with just a few breeder animals to start with) and generating enough animals of the experimentally required phenotype plus controls on the same genetic background (usually wild-type siblings, that is siblings which are completely normal) to carry out meaningful experiments. 'Controls' refers to animals which have been brought up and have been treated under the same conditions as the 'experimental' animals, but are genetically 'normal', in contrast to the 'experimental' animals, which will have a genetic alteration.

Some of the genetically altered mice would be used for primary neuronal cell culture. In this setting, many factors influence the cell yield (such as the number of pups per dam) and the subsequent survival of the cells in culture up to the experimentally required time-point, so that the required animal numbers have to be estimated. For mechanical data, often there are no pre-existing data as few other research groups have the expertise we do. Therefore, our experiments often have pilot character. From experience, we need approximately 8-10 animals for each the experimental and the control group (where applicable) to be able to draw sound conclusions from our data.

For experimental procedures which require tissue obtained under terminal anaesthesia, we assume we need each 16 groups of animals (including controls) with 10 animals each for both mice and rats. This results in a need for 160 mice (some of which will be genetically altered mice previously bred and maintained elsewhere on this project) and 160 rats in total.

For mechanical measurements under terminal anaesthesia, we assume we need each 10 groups of animals (including controls) with 10 animals each for both mice and rats. This results in a need for 100 mice (some of which will be genetically altered mice previously bred and maintained elsewhere on this project) and 100 rats in total.

Xenopus numbers are based on the our annual return of procedures data from previous years to estimate the number of animals that we will need for breeding required for experimental procedures (approx. 400; some of these are genetically altered frogs bred and maintained elsewhere on this project) plus animals required for breeding and maintenance of genetically altered lines (approx. 540 in total for 3 different lines).

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

We always aim to use as few animals as strictly required for our experiments.

Due to the novel nature of our experiments and the considerable amount of variation due to environmental factors outside our control (e.g. number of embryos per pregnant female used for primary cell culture, seasonal effects on *Xenopus* embryo viability *in vitro*), we cannot calculate the exact number of animals needed for each experiment. However, we have a lot of experience with the experiments in question and can base our results on the numbers previously needed for comparable, successfully completed, experiments from the past. We optimise external conditions as much as

Downloaded: 9:38am, 13 Mar 2026

possible (e.g. strict temperature control for rearing of *Xenopus* embryos, rigorous sterile preparation and working procedures when handling material for primary cell culture to avoid contamination and infection) to minimise the number of animals needed.

Where possible, we design experiments in such a way that control groups can relate to more than one experimental group, so that no additional control groups are needed for related experiments.

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

We believe that the greatest impact on the reduction of animal numbers is careful colony management. Therefore, we take great care when setting up animals for breeding. We aim to breed slowly, rather than quickly expand a colony, to make use of all possibly available animals and reduce the numbers of animals of unwanted genotypes, that is the specific combinations of genetic instructions (genes) an animal has. Whenever possible, we use animals of unwanted genotype for tissue or for optimisation of experimental procedures or pass them on to other researcher for such purposes. We have a long record of sharing tissues with other research groups (both requesting and offering tissues) via the 3R mailing list of our institution and have contributed suggestions for how to make communication through this channel even better.

Concerning *Xenopus* frogs, we share eggs with other research groups to reduce the number of females needed as often there are enough eggs from one laying for several researchers. We also use 'double ovulation' which means that the same female lays eggs twice in a row, similar to what happens in nature, which reduces the number of females we need overall. Where this is possible and practical, we may buy frozen sperm from genetically altered male frogs from resources such as the [redacted] rather than breeding and keeping the genetically altered frogs themselves. As sperm from one frog can be stored for a long time and used for a number of fertilisations, this would reduce the number of animals needed.

When we conduct a new type of experiments, we start with a small number of experiments, called a 'pilot study', to find out how big the effect is that we are investigating. This initial small study allows us to better calculate the numbers needed for a larger study. Usually, the animals used in the pilot study can also be included in the data of the larger study, which reduces the numbers of animals needed.

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.

- *Xenopus laevis*: Predominantly controlled egg laying for *in vitro* fertilisation. To a small degree production and maintenance of genetically altered animals without a harmful phenotype.

    - Least possible impact as low complexity animal and minimally invasive procedure (subcutaneous injections of physiological agents only).

    - *In vitro* fertilisation is required for experimental procedures which require fertilised eggs of defined developmental stages. Natural mating, which means putting a male and a female animal together to allow them to mate naturally, is unsuitable for this as it constitutes a prolonged process which results in fertilised eggs of various developmental stages. Furthermore, *in vitro* fertilisation can be conducted in a cleaner environment, reducing the contamination with pathogens (substances which can cause disease), and thus increasing the relative numbers of healthy embryos at experimental stages. This in turn leads to a reduction of animal numbers required.

- Mice: Breeding and maintenance of genetically altered animals without a harmful phenotype.

    - Such mouse lines are required where *Xenopus* models are not available (e.g. due to their more complex tetraploid genome, see next question) or useful (due to differences in CNS structure or function). This may include reporter lines for cell type-specific mammalian structures or lines that allow *in vitro* 'knock-out' of specific genes. *In vitro* 'knock-out' means that we will use cells in a dish (outside a living animal) and apply techniques to them which hinder the production of specific products encoded by a certain bit of genetic information (gene), usually to study what this specific gene is needed for. Such animals themselves appear completely normal on the outside and do not suffer any pain, suffering, distress or lasting harm.

- Mice: (Re-)derivation of mouse lines.

    - Poor overall health status or contamination with certain pathogens within a mouse line might require rederivation. To establish a new mouse line, 'founders' may be bought in as embryos. 'Founders' mean male and female animals used to start breeding a mouse line.

    - This constitutes a refinement in itself as the disease burden on the line may be lowered or transport of postnatal ('after birth') animals can be avoided.

    - The latest and most refined methodology will be used, so that only minimal pain and very short-term distress will be caused to the animals who will not experience suffering or lasting harm.

Rats and mice: Obtaining tissue or conducting mechanical measurements under terminal anaesthesia.

- As animals are unconscious and do not regain consciousness after the procedure, this is the maximally refined procedure possible to obtain the required data. Animals will only experience induction of anaesthesia and no pain, suffering, distress or lasting harm.

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

We will always use the animal species of the lowest possibly complexity. Wherever possible, we will work on *Xenopus laevis*, and specifically, on *Xenopus* embryos at an age where they are not yet

protected under ASPA, as they do not yet feed. Thus, we are already using a non-protected form of a low-complexity species. The procedures applied for here are required to obtain such animals.

Where the role of certain genes is to be investigated, using *Xenopus* can be problematic due to the fact that it has four of each chromosome (tetraploid) rather than two (diploid) as mammals do. A chromosome is a structure inside cells that carries genetic information (genes). Therefore, genetic modification of *Xenopus* can be difficult and we often use genetically modified mouse models instead.

In rodents, where procedures are not breeding and maintenance of genetically altered animals or procedures related to the (re-)derivation of such lines, our protocols are entirely conducted under terminal anaesthesia.

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

Xenopus: We are constantly reassessing refinement strategies for *Xenopus* and implementing changes, e.g. gentle massaging of females, which imitates natural mating. If frogs are being massaged for egg release, then they are able to be housed with another frog from their home tank and placed in system water. When handling *Xenopus*, their eyes are always covered to prevent stress. Water in holding tubs is changed regularly. All frogs are housed in enriched tanks and when out for laying, attention is given to placement of their holding tank (i.e. never underneath a light, dark lids are used etc.).

Mice: We consider good communication between animal facility staff and researchers key to refinement and regularly exchange observations and ideas. Therefore, we will also communicate with other researchers who have used the relevant mouse lines before to learn about the refinement measures they may have developed or considered, or the suppliers breeding these animals. We use handling methods that do not scare the mice, such as handling in a cardboard tunnel or transferring on a cupped hand rather than lifting by the tail. When injecting, we use every needle only once to ensure the tips is as sharp as possible which will minimise pain during injection. When we were using genetically altered strains with a harmful phenotype in the past, we used carefully composed scoring charts which we modified and updated as appropriate where we found this would improve welfare. This included minimal handling of sensitive animals and keeping them in particularly quiet environments. We have also successfully used other refinement measures such as extra soft bedding, long-nozzled water bottles and feeding soaked pellets from the easily accessible trays on the cage floor, where this was useful for a specific mouse line.

Rats: Whenever possible, rats will be given access to a play pen at regular intervals. Such enrichment is already available in our facility. In close collaboration with our animal technicians, we have successfully trialled and implemented refinement strategies for our animals in the past. This included stress reduction during transport by letting the rats get to know the transport boxes we use in a play pen on several occasions before transport. Where rats are used to physical contact with humans, they will receive a cuddle before procedures to calm them down. Experience shows that this reduces the time for animals to go to sleep under volatile (i.e. gas) anaesthesia and results in animals going to sleep more calmly.

Downloaded: 9:38am, 13 Mar 2026

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

We will use the information provided by the PREPARE guidelines and the LASA surgical guidance and stay in touch with our NIO about new guidelines that may be published in the future.

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

We regularly obtain information about 3R advances by the institution's named information officer (NIO). We will regularly check information on the NC3Rs website. We are in regular contact with our named animal care and welfare officer (NACWO) and the named veterinary surgeons (NVS) team and discuss possible refinement strategies. We are in contact with other researchers using *Xenopus* within our own institution and across other institutions and exchange information about refinement strategies with them via an international email list.

Downloaded: 9:38am, 13 Mar 2026