Home Office

NON-TECHNICAL SUMMARY

Neuronal Mechanisms of Mammalian Reproductive and Parental Performance

Project duration

5 years 0 months

Project purpose

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

Key words

Neuroscience, Neuroendocrinology, Prolactin, Reproduction, Parental Behaviours

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

This research explores how the brain helps mammals prepare for pregnancy, breastfeeding, and caring for their young. We focus on special brain cells called TIDA neurons, which control the hormone prolactin – the main driver of these changes – and how they adapt during reproduction.

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?

TIDA neurons control prolactin release, a hormone that helps mice reproduce and care for their young by optimising resources and behaviour for parental success. To achieve this balancing act TIDA neurons are in constant conversation with lots of other brain circuits, including those that control reproduction-relevant things like eating, metabolism, and stress. Accordingly, understanding which neurons 'talk' to the TIDA system, and how these 'conversations' change across different reproductive states, is key for the promotion of reproductive health and parental performance

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

New Knowledge and Understanding:

- By focussing on how brain circuits gather reproduction relevant-information, and how their processing of this data changes according to reproductive state (*e.g.* pregnancy), we will generate important new information as to the mechanisms by which mammals adapt to meet the enormous demands of reproduction and parental behaviours (*e.g.* nursing and nest building).

Disease and Therapy:

- By understanding how brain circuits help mammals reproduce and care for their young, this project will explain how problems in these networks cause a variety of health issues and suggest new ways to treat these problems in people and animals.
- Health issues associated with dysregulation of prolactin release include infertility, miscarriage, difficulties with milk production and breastfeeding, failure to bond, post-partum depression, hormonal disruption associated with medications like antipsychotics and antidepressants, early life stress, and parental obesity, all of which can impact the health of parents and offspring.

Scientific Publications:

- We will share our results in science journals and at conferences. This lets other scientists see our methods and data, so they can check or build on our work, making it more impactful.

Collaboration and Data Sharing:

- We will team up with other scientists to share ideas and data. By adding our findings to free online databases (*e.g.* repositories for genetic, physiological, or behavioural data), we will help global brain and endocrine research and reduce unnecessary animal studies.

Public Engagement:

- We will share our findings at science fairs, talks, and in articles or podcasts. This will show why brain research matters for health and how we use animals ethically, enriching public understanding of animal dependent research.

By the end of this project (and beyond), we will have shared new ideas about how mammals reproduce and care for their young, new ways to treat related health issues, better research methods, scientific articles, new collaborations with other scientists, and public engagement, helping science and animal welfare.

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

The outputs of this project will deliver different types of benefits across multiple timescales. These benefits, realised in the short-term during the project and extending into the medium- and long-term post-completion, will advance scientific knowledge, enhance research capacity, and improve health outcomes.

Scientific Community (Short- and Long-Term):

- **Who:** Researchers in reproductive biology, endocrinology, neuroscience, and metabolism.
- **How (Short-Term):** Through publication and collaboration with other scientists we will rapidly disseminate new understanding of how the brain prepares mammals to reproduce and look after offspring. We will also share all the tools and techniques we develop.
- **How (Long-Term):** Our work will set new standards for neuroendocrine research, improving how studies are done and help shape future investigation of reproduction and health.

General Public (Short- and Long-Term):

- **Who:** Broader society, including families and individuals interested in science or health.
- **How (Short-Term):** engagement activities (*e.g.* public lectures, science festivals) during the project will raise awareness of reproductive and endocrine physiology, and hopefully inspire interest in research as a career among young people.
- **How (Long-Term):** Potential therapeutic interventions derived from our findings (*e.g.* treatments for reproductive dysfunction) could enhance reproductive outcomes and family health over decades, improving quality of life as these translate from lab to clinic.

Biological, Medical, and Health Research (Long-Term):

- **Who:** Researchers, clinicians, and institutions focused on biological mechanisms, medical advancements, and animal/human health.
- **How (Long-Term):** Though taking discoveries from the lab to the clinic is a lengthy process (10-20 years), understanding fundamental mechanisms is an essential step. Our outputs will lay the groundwork for preclinical studies and drug development pipelines, creating new ways to treat disease.

The project's outputs will benefit the scientific community with immediate knowledge and tools, the public with awareness and future health gains, and medical research with therapeutic potential. Spanning short-term gains (within 5 years) to long-term impacts (10-25 years), these benefits will influence science, education, and society, reflecting the project's broad value and responsible use of animals.

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

To maximise the outputs of this project we will implement a multifaceted strategy. These inter-related processes, detailed below, are designed to enhance dissemination, collaboration, and impact, ensuring the broadest possible benefit from our work.

Knowledge Sharing and Dissemination:

- Share all results (positive, negative, or inconclusive) in open access science journals and online databases so others can rapidly use them, avoiding repeat experiments and saving animals/limited research resources.

Effective Communication:

- Present at science meetings and talk to the public at events like science fairs or on social media, using simple summaries and infographics to explain our work.

Collaborative Efforts:

- Work with other scientists, doctors, and companies, sharing tools and ideas to make our research stronger and more useful.

Driving Translation:

- Turn discoveries into new treatments for reproductive issues by teaming up with experts, sharing new tools, and working with companies to help people and animals faster.

By integrating knowledge sharing, effective communication, collaborative efforts, and translational focus, we will maximise the outputs of this work. These processes will ensure immediate dissemination of findings, foster long-term scientific and societal impact, and accelerate the application of our research, benefiting stakeholders across academia, healthcare, and the public while optimising animal use.

Species and numbers of animals expected to be used

- Mice: 5000
- Rats: 400

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.

This project studies how the brain controls reproduction and parenting in mammals, so we need animals and ages that best fit our research goals while being kind to them. Below, we explain why we mostly use mice, sometimes rats, and mainly adult animals, with baby and young mice used for specific tests.

Choice of Animal Types:

- **Similar to Humans:** We mostly use mice because their bodies and brains work a lot like humans and other mammals, including for things like having babies and caring for them. Their brain and hormone systems are very similar to ours, which helps us learn about human health. We use rats for a few special tests because their brains sometimes give clearer signals for certain experiments, but we keep their use very low.

- **Special Tools for Mice:** Mice let us use advanced tools to study specific brain cells with great accuracy, like zooming in on just the cells we are interested in. This not only helps us ask more detailed questions but also means we can use fewer animals to get good results. Rats don’t have as many of these tools, so we only use them when their brain signals are uniquely helpful.

Choice of Life Stages:

- **Adult Animals:** Most of our experiments use grown-up mice because their brains and hormones are fully ready, which is needed to study things like pregnancy or caring for babies. These systems only work properly after the mice reach adulthood, so adults give us the most accurate and useful results for understanding reproduction and parenting in mammals.

- **Baby and Young Mice:** We use newborn or young mice in tests where we look at how parents care for their babies, like feeding or keeping them warm, since these tests need baby mice to be part of the experiment.

Welfare and Efficiency Considerations:

- **Better Methods**: Using mice means we can use precise tools to focus on just the brain cells we need, which is gentler than using less similar animals that might need bigger or more stressful tests. This helps keep the mice more comfortable and reduces the number we need to use.

- **Careful Treatment:** For all mice, whether adults or babies, we use methods like pain relief, sleep medicine for procedures, and cozy cages with toys to keep them happy and reduce any stress, as explained in other parts of this plan.

Mice and rats, are the best animals for our study because their bodies are like humans', and mice have special tools that let us study their brains accurately with fewer animals. We mainly use adult mice since their brains are fully developed for reproduction and parenting, but we use baby mice when studying how parents care for them. This balances our research needs with treating animals kindly.

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

This project uses mostly mice, and a few rats, to study how mammalian brains and hormones work together to control having babies and caring for them. We carefully plan and watch all steps to keep animals comfortable. Below, we explain the typical things we do, how often, and how long they take.

Procedures:

- **Injections:** We may give mice injections with special substances to highlight or control certain brain cells. These injections go under the skin or, sometimes, into the brain guided by a special machine to target only the neurons we are interested in. Most mice get one shot per study, but some longer studies might need 2-3 injections, spaced days or weeks apart, to check changes over time.

- **Surgical Interventions:** : For some tests, we do surgeries while mice are anesthetised to place recording devices, like tiny light fibres, in their brains. These surgeries take 30-60 minutes, and mice rest for 1-3 days afterward with pain relief and warm beds. Most mice have just one surgery, but a few might need another.

- **Post-Recovery Tests:** After healing, some mice might get special diets, like more or less food, or medicines to mimic hormones. These typically happen 1-2 times, usually through food or injections, to see how their bodies respond.

- **Behavioural Assessment:** We watch some mice to see how they act, like how much they eat, how they care for their babies (feeding or building nests), or how they move or respond. For some behavioural assessments animals may need to be housed on their own. Single housing will be kept to a minimum and avoided where possible.

- **Tissue Collection:** : At the end, animals are humanely euthanised and we take samples, like their brains, to study in the lab. This ensures they don't suffer and gives us lots of information.

Number of Procedures:

- Most mice will have just a few steps: one shot or surgery, 1-2 tests like a diet change or behaviour check, and then tissue collection. For studies that last longer, we might do a bit more, but we plan carefully to use the fewest steps and share mice between tests when possible. Mice used only for brain samples have just one terminal step, ensuring they experience the minimum amount of stress.

Duration of Experiments:

Some experiments are short, like checking brain activity, and take a few days (*e.g.* 3-7 days). Others, like watching how mice behave over time, might take weeks (*e.g.* 2-12 weeks). We make each study only as long as needed to get the answers while keeping mice comfortable.

Welfare Considerations:

- Our team is highly trained to do these steps gently and correctly, keeping stress low. We use anaesthetic and pain relief when required, and choose minimally invasive methods, like injections under the skin, when we can. Mice are checked at least once a day, or more frequently when required (*e.g.* after procedures), and staff quickly intervene if they seem uncomfortable.

Mice usually go through a few carefully planned steps—like injections, surgeries, behaviour checks, or brain sample collection—over days or weeks. These methods let us learn about the brain and hormones while treating animals with care and keeping their stress as low as possible.

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

The steps we take with mice in this project may cause mild to moderate, short-term discomfort, managed through robust refinement measures to minimise distress. Below, we explain what might happen to the mice, how long it lasts, and how we care for them.

Mild Post-Surgical Discomfort:

- **What Happens:** Mice having surgeries, like placing tiny tools in their brains, might feel pain where we made a small cut.
- **How Long:** This soreness usually goes away in 1-3 days, with pain medicine and warm resting areas to help them heal.
- **How We Help:** We check mice every day and give extra pain medicine if they’re still sore after 3 days.

Brief Weight Loss:

- **What Happens:** Some mice might lose a little weight (about 5-10% of their body weight) after surgeries, special diets, or medicines.
- **How Long:** This usually recovers itself in 3-7 days as mice get used to the changes or heal.
- **How We Help:** If a mouse loses too much weight or loses appetite for more than 5 days, we give them soft food or extra nutrients.

Slight Inflammation:

- **What Happens:** injections, like those with special substances, might cause a little redness or swelling where the needle went in, or a mild body reaction.

- **How Long:** This usually clears up in 1-3 days.
- **How We Help:** If the redness or swelling is more than mild or lasts over 3 days, we give anti-inflammatory medicine to reduce it or humanely kill the animal if it fails to resolve.

Short-Term Unusual Behaviour:

- **What Happens:** Mice might act differently for a short time, like moving less, eating less, or being less social, because of the tests we do.
- **How Long:** This usually lasts 1-5 days and goes away within a week. In longer studies (2-6 weeks), it might happen on and off.
- **How We Help:** We watch mice closely and give them cozy nests and toys so they feel safe and behave naturally.

Absence of Severe Effects:

- What Happens: We don’t expect mice to have serious problems, like ongoing pain or big health issues, because we use precise tools that only affect specific brain cells. If something serious happens, like a mouse losing too much weight or seeming very uncomfortable, we will gently euthanise them to stop any suffering..

Duration Summary:

- Most effects, like soreness, weight loss, or redness, go away in 1-7 days, and mice are fully back to normal within two weeks, even for longer tests.

The small effects we expect—like mild soreness, slight weight loss, redness, or temporary changes in behaviour—are short-lived and carefully managed to keep mice comfortable. These effects are needed to learn about the brain, but we watch closely and care for the mice to make sure any distress is kept to a minimum.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

We expect mice and rats in our project to have mild to moderate, short-term effects from our tests, which we manage carefully to keep them comfortable. Below, we explain what might happen to them, why, and how many are affected.

Mild Severity:

- **How Many:** About 70-80% of mice and all rats.
- **What Happens:** These animals get simple tests, like injections under the skin with safe medicines, special diets like more fat, or changes in their room temperature. Some mice have their brains collected after anaesthesia overdose, so they don’t feel anything.

- **Effects and How Long:** They might have a little redness where a shot was given (gone in 1-3 days), lose a bit of weight (back to normal in 3-7 days), or act a little different for a short time. Brain and tissue collection has no effect since after anaesthesia overdose.
- **Why It's Mild:** These small, short effects don't bother the animals much and go away quickly without needing a long recovery.
- **Which Animals:** This happens to some mice and all rats, with brain collection being very gentle due to the anaesthesia.

Moderate Severity:

- **How Many:** About 20-30% of mice and no rats.
- **What Happens:** These mice have small surgeries, done while they're anaesthetised, to place tiny tools, like light fibres, in their brains to study specific cells.
- **Effects and How Long:** After surgery, mice might feel a bit sore or lose a little weight (5-10%), but this goes away in 1-3 days with pain medicine and warm resting places.
- **Why It's Moderate:** Surgery is a bigger step, so it's a bit more serious, but we control the effects carefully, and mice are back to normal in a few days.
- **Which Animals:** This only happens to some mice.

About 20-30% of mice will have moderate effects from surgeries, recovering quickly in a few days, while 70-80% of mice and all rats will have mild effects, like redness or slight weight loss, that go away in 1-7 days. No animals will undergo severe suffering.

What will happen to animals used in this project?

- Killed
- Used in other projects

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 project studies how the mammalian brain controls having babies and caring for them in male and female animals, by looking at how brain signals and hormones work together. To fully understand this, we need to explore everything from tiny brain cells to how the whole body and behaviour change. We can't study this in humans because it wouldn't be safe or fair, and lab tests or computer models can't show how all these parts work together in a living animal. That's why we need to use animals, and mice are the best choice.

Rationale for Using Mice:

- **Similar to Humans:** Mice have bodies, brains, and hormones that work a lot like humans, including for things like pregnancy and parenting. This makes what we learn from mice useful for understanding human health.
- **Well-Known Brains:** Scientists know a lot about how mouse brains work to keep their bodies balanced and control behaviour, giving us a strong starting point for our research.
- **Special Tools:** Mice let us use advanced genetic tools to study specific brain cells with great accuracy, like zooming in on just the parts we need. These tools aren't as good in other animals, so mice help us get clear answers.
- **Trusted Methods:** The ways we study mice, like using special substances, watching their behaviour, or checking their health, are well-established and work best in mice because we have lots of information and resources for them.

Using a different animal would mean starting from scratch, needing more animals, taking longer, and getting less reliable results because other animals don't have the same tools or knowledge base. Mice are the smartest choice.

We need to use mice to connect what we learn about brain cells to how the whole animal behaves, giving us answers we can't get any other way. This lets us learn about reproduction and parenting while using the fewest animals possible with the minimum amount of pain and discomfort.

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

This project looks at how the brain and hormones control having babies and caring for them. We considered ways to do this research without using animals, like using brain samples, growing cells in a lab, or using computers. Below, we explain what we tried, how well they worked, and why we still need animals for some parts.

***Ex Vivo* explants (Brain Slice Preparations - Partial Replacement)**

- **Description:** We looked at using small samples of brain taken after the mouse has been humanely killed to study how brain cells work together.
- **Evaluation:** These brain samples let us use special tools to watch how certain brain cells, called TIDA cells, send signals and connect with each other in real time. This helps us learn about the brain without always using live animals.
- **Conclusion:** Studying brain samples reduces the need for live animals for some tests, but it can't show us how the whole body and behaviour, like parenting, work together, so we still need animals for those parts.

Cell Culture Systems (Growing Cells in a Lab – Partial replacement):

- **Description:** We looked at using mouse brain or hormone cells grown in a lab, either from animals or from ready-made cell lines, to see if they could effectively model the systems we are interested in.
- **Evaluation:** Growing cells is good for studying single things, like how cells make hormones or turn genes on and off, but it can't show how different parts of the brain and body work together to control things like pregnancy or caring for babies.
- **Conclusion:** Lab-grown cells aren't complex enough to fully replace animals for our research, but we might use them for smaller tests, like checking how cells react before doing bigger experiments.

In Silico Analyses (Computer Models and Data Reviews – Absolute Replacement):

- **Description:** We looked into using computer programs to mimic how the brain and hormones work or reviewing information from past studies stored in public databases to predict what might happen in our experiments.
- **Evaluation:** Computer models and data reviews rely heavily on assumptions and what's already known, so they can't give new answers about how brain cells send signals or how animals behave in real life. They also can't fully copy the complicated way the brain and body work together in a living animal.
- **Conclusion:** Computers and past data aren't ready to replace animals for our study because they can't show the full picture of how the brain controls behaviour, but they might help us plan experiments or understand results better.

Why were they not suitable?

Rationale for Non-Adoption

This project aims to learn how the brain and hormones work together in a living animal to control having babies and caring for them, looking at everything from tiny brain cells to how the whole body behaves. Other methods, like lab-grown cells or computer models, are good for studying small parts, but they can't show how all these parts work together in a real, moving animal. For example, to see how brain signals affect actions like parenting, we need a living animal, and mice are the best choice because their bodies are a lot like humans' and we have special tools to study their brains.

Commitment to Alternatives:

Even though we need mice for now, we're dedicated to following the 3Rs rules (Replacement, Reduction, Refinement) and using fewer animals and minimising harm. When possible, we'll use other methods to help, like growing cells in a lab to test ideas first or using computers to study our results. We'll keep checking for new ways to do our research without animals, and if better options come up that can show the same big picture, we'll ask for permission from the Home Office to use them instead.

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?

To figure out how many animals we'll need for this five-year project, we looked at information from earlier studies and similar projects. This helps us make a good plan while using as few animals as possible. Most animals will be mice, many specially bred to have certain traits in their genes. We'll also use a smaller number of regular mice and rats from trusted suppliers. The total number depends mostly on breeding enough mice with the right traits, but also on how we design the experiments themselves.

Breeding Estimates

We consider the following factors for breeding:

- **Breeding Groups:** We use information from past breeding to know how many parent mice we need.
- **Mice per Litter:** We base this on how many babies mice usually have each time, from past records
- **How Often Mice Breed:** We look at how often mice have babies, based on typical patterns
- **Mice Without the Right Traits:** Some baby mice won't have the traits we need, which happens when we breed for specific genes.

We reduced the number of mice needed by studying how traits pass to baby mice and by sharing special mice between researchers. Breeding exactly the mice each scientist needs helps us avoid extra breeding.

Experimental Design to Use Fewer Animals

We also keep the number low by planning the experiments carefully:

- **Group Sizes:** We work with maths specialists to calculate the smallest number of mice that will still give clear, trustworthy results for each test.
- **How Animals Are Allocated to Groups:** Mice are randomly put into different groups (for example, the "treatment" group or the "control" group) so the results are fair and not affected by chance differences between animals.
- **Managing Variability:** We reduce natural differences between animals by using mice that are the same age, sex, and genetic type wherever we can, and we often use the same mouse as its own comparison (testing it before and after a change) to keep results steady.

- **Maximising Data Output:** We collect several kinds of information from each mouse (for example, brain activity, behaviour, and hormone levels all at once) to get the most useful data possible from every animal, meaning we need fewer mice overall.

If we get new funding or our research changes, the number of mice might shift, but we'll always use past information to make careful, realistic plans. We will check our plan often and get permission from the Home Office for any big changes.

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

When planning our experiments, we found ways to use as few mice as possible while still getting trustworthy results. These steps follow the 3Rs rules to Replace animals with other methods when we can, Reduce the number we use, and Refine our work to make it gentler, ensuring our findings are solid.

- **Working with Maths Experts:** We team up with people who are great at numbers to figure out the smallest number of mice we need for each study. For example, if we expect a clear change, we pick a simpler test that needs fewer mice to give us good answers.

- **Using a Planning Tool:** We use a free online tool from the NC3Rs, called the Experimental Design Assistant (EDA), to plan our experiments carefully. It helps us use the least number of mice by building plans based on our lab's past results and information available online. The (EDA) also reminds us to randomise mice when selecting our experimental groups (so no group is accidentally stronger or weaker) and to "blind" the experiments – meaning the person doing the test or checking the results doesn't know which mice got the real treatment and which were controls. This keeps our results fair and honest.

- **Starting with Small Pilot Tests:** For new kinds of mice or untested methods, we begin with tiny experiments. These let us check if things work, improve our plans, and find the best way to do things before using more mice.

- **Getting the Most Information from Each Mouse:** We collect several kinds of data from every mouse (for example, brain activity, behaviour, and hormone levels all at once) and often use the same mouse as its own comparison (testing it before and after a change). This means we get lots of useful information from each mouse and need fewer animals overall.

- **Data Pooling and Sequential Analysis:** We mix results from similar studies when it makes sense and check our findings step-by-step. This lets us adjust or stop experiments as soon as we have answers, using fewer mice while getting the most information from each.

- **Smart Breeding:** To avoid extra mice, we keep small groups for types we don't need often. If we don't need them, we stop breeding and save their eggs or sperm for later, keeping special traits safe without raising more mice.

- **Sharing Our Resources:** We quickly share what we learn and our mouse data in free science journals and online databases. This lets other scientists use our findings instead of doing their own experiments, reducing the number of mice used around the world.

Oversight and Continuous Improvement

By including these steps from the start, we make sure we use the fewest mice possible while keeping our research strong, showing we care about treating animals kindly and wisely. We'll regularly review our plans with special team members and make updates if needed, with Home Office permission.

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

To use as few mice as possible while still getting good results for our research, we are taking extra steps beyond just planning our experiments carefully. These steps make our work smarter, avoid using animals we don't need, and get the most information from each mouse, following rules to enhance animal welfare:

- **Statistical Optimisation:** We will work with maths specialists to calculate the fewest mice needed for reliable results before each study. This ensures we use just enough animals for good science.
- **Testing the Same Mice Twice:** When it works for our research, we'll use the same mice to test both normal and special conditions. This "within subject design" means we don't need extra mice for comparison, saving animals while still getting reliable answers.
- **Using Advanced Technology:** We will use the latest tools, like special ways to focus on certain brain cells, to study mice very precisely. These tools need fewer mice than older methods because they avoid mistakes and give clear results with smaller groups.
- **Efficient Breeding Practices:** We will breed mice so most babies have the traits we need, as long as it's safe for them, to avoid having mice we can't use. For mice we don't need often, we'll save their eggs, sperm, or embryos and stop breeding until we need them again, preventing extra mice..
- **Pilot Studies:** For new kinds of mice or new methods, we'll start with tiny experiments to see if they work and make our plans better. This helps us use just the right number of mice and avoid extra tests.
- **Optimising and Sharing Ex Vivo Tissue:** We have developed optimised dissection solutions that enhance *ex vivo* tissue viability. This allows us to collect the largest and most reliable data set (*e.g.* brain slice electrophysiology) from the samples harvested. The in turn can also be shared with other scientists, giving lots more information from each mouse and reducing the total number of animals needed across research groups.
- **Sharing with Other Scientists:** We'll share our mouse samples and results with other researchers through free online platforms. This teamwork stops others from repeating our work, so fewer mice are used overall.
- **Keeping Up with New Science:** We will read new studies and go to science meetings to learn what others have found. This lets us build on their work instead of doing the same experiments, saving mice.

Implementation and Oversight

By using all these steps together, we will make sure we use the fewest mice (and rats, when needed) possible while still reaching our research goals. This shows we are serious about doing science in an ethical and efficient way, and we will keep checking our plans with our team to make them even better where possible.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This project uses carefully chosen animals and methods to study how the brain controls having babies and caring for them, while keeping animals as comfortable as possible.

Animal Models:

- **Transgenic Mouse Lines:** We mostly use mice because their brains and bodies are similar to humans', and we can use special genetic tools to study their brain cells closely. Many mice are bred with gene changes that let us control certain brain cells exactly, so we don't need harsher, less specific methods, and can use fewer mice.
- **Regular Mice and a Few Rats:** For some tests where we don't need special genes, we will use ordinary mice or rats from trusted suppliers. We'll only use a small number of rats for specific experiments to compare them to mice. Rats' brain cells send slightly different signals to the hormone system than mice do, which - even though the overall pattern is similar - account for species-specific differences in parental performance. By looking at both, we can learn more about how tiny differences in brain wiring affect parenting and reproduction – questions we couldn't answer with mice alone. This comparison gives us extra useful information without needing many more animals.

Experimental Methods:

- **Focusing on Specific Brain Cells:** We use the latest tools, like light signals or special substances, to gently turn specific brain cells on or off or watch how they work. This helps us learn how these cells affect having babies or being a parent, with minimal discomfort to the mice.
- **Terminal Anaesthesia Studies:** For experiments not requiring behavioural observation (*e.g.* tissue sampling) we will conduct procedures under terminal anaesthesia. This ensures data collection without any conscious experience of discomfort.

- **Minimally Invasive Techniques:** Where possible, when administrating substances we will use non-surgical methods (e.g. subcutaneous or intraperitoneal injections). When agents need to be delivered directly to the brain we will use minimally invasive approaches such as stereotaxic injection under anaesthesia. This anatomically targeted approach enables use of the smallest possible volume, reducing tissue trauma, recovery time and distress.

Rationale for Minimising Welfare Costs:

- **Precise Tools Keep Animals Safe:** Our special mice and methods let us focus on just the brain cells we need, so we don't harm other parts of the body. For example, we use short flashes of light to activate cells instead of making big changes, which keeps mice comfortable with little lasting effect.

- **Fewer Tests Needed:** By targeting specific brain cells, we get clear, detailed results from fewer mice, so we don't need to do as many experiments. This means fewer mice are used in tests, which is kinder for them.

How We Care for Animals:

- **Trained Team:** All our work is done by people specially trained and approved by the Home Office, so they know how to handle animals gently to keep them comfortable.

- **Good Care After Tests:** We give mice pain relief, warm places to rest, and cozy homes with toys to help them recover quickly and feel better.

- **Regular Checks:** We check animals every day to make sure they're okay. If any mouse seems too unwell, we'll gently put it to sleep right away to stop any suffering.

We chose special mice and methods in order to cause the least harm possible while getting the exact answers we need for our research about the brain and parenting.

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

This project studies how the brain and hormones control adult behaviours like having babies and caring for them in animals similar to humans. We need to use fully aware, grown-up mice to get the right answers. Below, we explain why other options, like younger mice or different animals, don't work well and how we keep mice comfortable.

Immature Life Stages:

- **What We Considered:** We thought about using young mice, like babies or those not yet grown up, because they might feel less since their brains aren't fully developed.

- **Why It Doesn't Work:** The brain and hormone systems we study, which control things like mating or taking care of babies, only work properly in adult mice. Young mice don't have these systems fully formed, so they can't show us what we need to learn.

- **Conclusion:** Young mice aren’t suitable for most of our research, but we will use a few when studying how parents care for their babies.

Less Sentient Species:

- **What We Considered:** We looked at using animals that might feel less pain, like fruit flies or small fish, because they have simpler brains.
- **Why It Doesn’t Work:** These animals don’t have the complex brains or hormones needed to study how mammals have babies or parent. Mice are much closer to humans, so their results help us understand people better.
- **Conclusion:** Animals like flies or fish can’t replace mice because they don’t have the right body systems for our research.

Terminally Anaesthetised Animals:

- **What We Considered:** We thought about using mice that are terminally anaesthetised during tests, so they don’t feel any pain or discomfort.
- **Implementation:** Where feasible, we will employ terminal anaesthesia for procedures not requiring behavioural observation (e.g. tissue collection for ex vivo analysis). These animals gently euthanised before waking up, avoiding any suffering.
- **Limitation:** Many experiments need awake mice to observe behaviours like mating or parenting over days or weeks. These tests track how brain changes affect actions, which can’t be done if mice are anaesthetised.
- **Conclusion:** We use terminally anaesthetised animals when we can, but awake mice are needed for some key studies.

Why We Need Grown-Up Mice and How We Keep Them Comfortable

We must use adult, fully aware mice because only they have the complete brain and hormone systems that control having babies and parenting, which we can’t study properly with other options. To make sure mice are undergo the minimum amount of distress, we:

- Only use awake mice when we need to watch their behaviour
- Employing terminal anaesthesia for non-behavioural procedures;
- Use special tools to target specific brain cells, making tests gentler and less uncomfortable.

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

To minimise harms while we do our science, we have built a comprehensive set of refinement measures into our experiments. These steps help reduce any harm and follow animal welfare rules.

Welfare Monitoring

- Our trained team will check on mice every day, and even more often (*e.g.* every 4-6 hours) right after surgeries or when tests require it, to make sure they are okay.
- When possible, we will use non-invasive tools, like cameras or tiny sensors, to watch how mice act and check things like their body temperature without touching them, which keeps them less stressed.
- We will use a clear scoring system to spot any signs of discomfort early, so we can help mice right away if they seem unwell.

Optimised Post-Operative Care

- After surgeries, mice will rest in warm, cozy spaces to stay comfortable and avoid feeling shocked.
- We will give them soft bedding and fun things in their homes, like toys, to make them feel safe and behave naturally while they heal.
- If mice don’t drink or eat enough after a procedure, we’ll give them extra water, soft food, or special nutrients to help them get better.

Managing Pain Well

- All surgeries will be done with general anaesthesia, carefully tailored to the procedure’s duration and the animal’s condition.
- We will give pain relief before and after surgery, with amounts set by our vet and adjusted based on how each mouse responds
- We will watch for signs of pain, like if a mouse stops cleaning itself or moves less, and give more pain relief if needed.

Animal Training and Habituation - Managing Stress

- Where tests involve repeated cage changes, handling, interventions, or use of particular equipment/environments we will get animals comfortable with the procedure in advance (e.g. gentle daily handling 5-7 days before experiments). This makes them less scared and helps our experiments work better.

Oversight and Continuous Improvement

- Every year, we will look at how we’re caring for mice and make changes if we find better ways to keep them comfortable or learn from how they are doing.
- Our team will work closely with our vet, animal care staff, and university animal welfare board to follow the law and quickly use new, improved methods, asking the Home Office for permission if we need to change our plans.

- By including these kind steps in our work, we will keep mice as happy and healthy as possible, follow the 3Rs rules (Replace, Reduce, Refine), and still get the answers we need for our research.

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

The following best practice guidance will inform our experimental design, procedural execution, and animal care protocols:

NC3Rs Guidelines and Resources:

- **NC3Rs ARRIVE Guidelines (Animal Research: Reporting of In Vivo Experiments):** These are designed to improve the reporting of animal research. They help ensure that publications include sufficient information for others to evaluate and develop the research.
- **NC3Rs PREPARE Guidelines (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence):** These provides help with planning and conducting animal research effectively. They emphasizes the importance of forming clear hypotheses, identifying non-animal alternatives, and assessing the relevance of the chosen model to the experimental question.
- **NC3Rs Refinement Resources:** We will implement specific NC3Rs guidance on refinement techniques, such as the ‘Refining Rodent Stereotactic Surgeries (Novak, NVS Edinburgh)’ and ‘Refining the Use of Head Fixation and Fluid Control in Rodents (2022)', in addition to 'Anaesthesia Guidance and E-Learning modules' from the NC3Rs Resource Library for best practice in the use of anaesthesia and analgesia, to minimise pain and distress during and after interventions like injections or surgery.
- **NC3Rs Housing and Husbandry Guidelines:** We will adopt recommendations from the NC3Rs ‘Housing and husbandry: Mouse resource (2024) and Housing and husbandry: Rat Resource (2020) to provide enriched environments, such as toys, nesting materials and social housing, promoting natural behaviour and reduce stress.

LASA (Laboratory Animal Science Association) Guidance:

- **LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2010):** For all surgical procedures (*e.g.* delivery of genetic vectors), we will follow LASA’s best practice recommendations on aseptic technique, anaesthesia, and post-operative care to minimise infection risk and optimise recovery.

Home Office and Legislative Guidance:

- **Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes (2014):** We will comply with this statutory guidance under the Animals (Scientific Procedures) Act 1986, ensuring housing conditions, cage sizes, and environmental enrichment meet or exceed minimum standards to support animal welfare.

- **Home Office Guidance on the Operation of ASPA:** We will align our refinement practices with the Home Office's advice on implementing the 3Rs, including the use of humane endpoints and welfare assessment protocols.

FELASA (Federation of European Laboratory Animal Science Associations) Recommendations:

- **FELASA Guidelines for the Refinement of Animal Husbandry and Procedures:** We will apply FELASA's recommendations on minimising stress during handling, transport, and experimental procedures, such as using tunnel handling techniques for mice to reduce anxiety (Hurst & West, 2010).

- **FELASA Health Monitoring Guidelines (Mähler et al., 2015):** We will follow these standards to ensure animals remain healthy, reducing the likelihood of confounding welfare issues that could necessitate additional animals or procedures.

Specialised Scientific Literature:

- **Wolfensohn & Lloyd's Handbook of Laboratory Animal Management and Welfare (4th Edition, 2013):** This resource will guide our welfare assessments, pain management strategies (*e.g.* use of grimace scales), and post-operative care protocols to ensure consistent refinement across all studies.

- **Data based publications on Mouse Welfare (*e.g.* Gaskill et al., 2013; Bailey et al., 2022):** We will incorporate evidence-based refinements, such as optimal thermal regulation post-surgery and enriched recovery environments, to enhance recovery and reduce stress.

By adhering to these authoritative best practice resources, we will ensure that our experimental procedures are conducted with the highest level of refinement, minimising animal welfare costs while achieving the project's scientific objectives.

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

To make sure we use the latest and least harmful ways to do our research with animals, we have made a clear plan to stay informed and work together with others. This helps us follow the 3Rs rules: Replace animals with other methods when possible, Reduce the number we use, and Refine our methods to be gentler.

Keeping Track of New Ideas

- We will regularly read scientific journals and reports from groups like the NC3Rs, who focus on better ways to do research, to learn about new tools and ideas.

- Our team will go to meetings, workshops, and online talks run by experts inside and outside our Establishment to stay updated on the newest ways to make research better.

- We will stay connected with our Establishment's animal welfare team and talk to our local AWERB to make sure we are using the best and most caring methods.

Working with Others and Sharing Knowledge

- We will team up with other scientists to share tips and use proven ways to make our research on brains, hormones, and having babies gentler for animals.
- By subscribing to 3Rs focussed newsletters, mailing lists and online platforms, we will ensure timely updates on new tools, protocols and experimental alternatives.

Using New Ideas in Our Work

- **Replacement** - If new methods, like growing cells in a lab or using computer programs, can show how the brain and body work together as well as mice do, we will test them and use them if they work for our research.
- **Reduction** - We will use better number-crunching tools and experiment plans to need fewer mice and get more answers from each one. We will also share our results with other scientists to avoid repeating tests.
- **Refinement** - We will quickly start using new, less stressful methods, like gentler ways to give medicines, improved anaesthesia protocols, or improved ways to check on mice, to keep them more comfortable. For example, if new tools let us skip surgeries, we will switch to those.

Checking and Improving Our Plan

- Every year, our team will look at how we are doing and see if we can make our experiments more benign or use fewer animals.
- Any new improvements we find will be documented, added to our plans (with Home Office permission if needed), and shared with other scientists at our university and beyond.

By embedding the proactive measures into our project framework, we will ensure that we not only stay informed about 3Rs advances but also apply them promptly and effectively to enhance animal welfare and scientific efficiency throughout this projects lifespan and beyond