Home Office

NON-TECHNICAL SUMMARY

Neural control of puberty and fertility

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Fertility, Puberty, Neuroscience, Neuroendocrinology

Animal types	Life stages
Mice	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?

To understand how the brain is able to control the onset of puberty and how the brain controls fertility in adults

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?

The studies in this project are expected to give us a better understanding of how key groups of cells in the brain function together to control the start of puberty and adult fertility. Many humans currently suffer from infertility or disorders related to puberty and we have little knowledge of how these important events are controlled. Approximately one in five couples in the UK will suffer from being unable to get pregnant and up to 1% of children fail to go through puberty at the correct time. The ability to correct fertility disorders from conditions such as polycystic ovary syndrome (PCOS) and speed up or slow down the pace of puberty in children with too early or delayed puberty would be very advantageous. The immediate benefits of this work will be increased understanding of how the brain works to control reproductive hormone secretion. Longer-term benefits will be those of providing new information that will allow for opportunities to make new treatments for controlling puberty and fertility in humans.

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

This project will provide new information on the way in which the brain controls the activity of the gonads. It is aimed at understanding exactly how the brain controls the initiation of reproductive ability at puberty as well as how the brain controls the cyclical fertility of adult females. It is expected that these studies will provide the fundamental knowledge required to generate new treatments aimed at correcting disorders in the timing of puberty and adult human infertility conditions such as polycystic ovary syndrome and infertility due to stress.

This new information will be presented to the academic world at conferences and be available to the general public through lay presentations. It will also be provided through open-access publications in scientific journals. It is expected that this information will lead to better understanding of how the brain controls reproduction and this should enable better treatments to be developed for people with disorders of puberty and fertility.

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

In the short-term, results from this project will benefit other scientists within the UK and across the globe working on the same problem of understanding how the brain controls fertility and puberty.

We expect that understanding these mechanisms will also be very useful in developing new treatments aimed at helping people experiencing problems with puberty or fertility. For example, being able to speed up or slow down the pace of puberty in children with too early or delayed puberty would be very advantageous. Similarly, the ability to slow down the frequency of the pulse generator in women with polycystic ovary syndrome (PCOS) would be expected to help tremendously with their sub fertility.

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

The impact of this work will be maximized through -

a) presentation of findings at scientific conferences and lay user-group meetings

b) publication of all work (positive and negative observations) in open-access journals

c) providing all source (original) data alongside each publication to enable re-use and re-analysis by other scientists.

Species and numbers of animals expected to be used

- Mice: 9,120

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.

At present, the only way of investigating how specific groups of brain cells interact with one another to control processes such as puberty and fertility are through genetically-altered animal models. The great majority of these models have been developed in mice and enable the activity of specific cell populations to be modified or monitored whilst determining the effects on reproductive hormone secretion. Our work examines how fertility is initiated at puberty and then controlled in adult mice.

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

The great majority of the animals used in this project will be used for breeding and experience no adverse effects.

A typical recording experiment will involve an animal being anaesthetised for 1-2 hours and a very thin recording probe (less than 0.5 mm wide) inserted into a specific brain region with tiny screws placed into the skull and everything secured in position with dental cement. Animals may experience some discomfort and pain after surgery and will be treated with analgesics before and after surgery. After surgery, animals will be accustomed over several weeks to having their head probe connected to a very thin cable so that recordings can be made while the animals are free to behave normally in their cages. These recordings can occur for a few hours up to a maximum of 5 days so that we can record the activity of cells across the entire estrous (reproductive) cycle of a female mouse. The estrous cycles of mice are determined by looking at the cells from the vagina that are obtained by gentle water flushing. A few of these animals will, in addition, experience mild and transient discomfort from blood sampling during the recording sessions in which very small blood samples are taken from the tail of the mouse. Another small group of animals will be put on weight loss diets that are not expected to cause

distress but result in weight loss up to 15% of their normal body weight. The final procedure involving perfusion fixation (the administration of a solution to embalm the mouse) will be undertaken under non-recovery anaesthesia where the animals will only be aware of the anaesthetic being administered and may experience mild distress and no pain.

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

Animals will have surgery to implant small recording devices into the brain so that the activity of cells can be recorded. They are expected to recover quickly and will be given painkillers for two days following the surgery and post-operative care just like people recovering in hospital. Very occasionally, there can be a problem with the recording devices and an animal may need a brief further operation to correct this. In some cases, animals need to be placed back into their cages by themselves after the surgery to prevent other mice from tampering with the head devices. In these cases, mice are kept for the rest of the experiment in "open top" cages beside one another so that they can hear, see, and smell each other.

The blood sampling takes tiny amounts of blood that, together, never exceed more than 10% of an animals total blood volume. This is the same as a human giving a blood donation.

Animals will receive weight loss diets for up to four weeks that will reduce their weight by up to 15% before being placed back on normal food.

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)?

Mice: 86% mild, 14% moderate

What will happen to animals used in this project?

- Killed

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?

At present, the only way of investigating how specific groups of brain cells interact with one another to control processes such as puberty and fertility is through genetically-altered (GA) animal models. These processes are so complex that currently they can only be examined in animals. No artificial system is able to replicate this at the moment. The great majority of GA models have been developed

in mice and enable the activity of specific cell populations to be modified or monitored whilst determining the effects on reproductive hormone secretion.

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

Mathematical modelling. This is where people use mathematics to try and generate realistic models of how the brain functions.

Cell cultures including new preparations called organoids where parts of body organs are grown in laboratory dishes.

Why were they not suitable?

We are actively engaged in developing mathematical models for understanding the neural control of fertility. However, these models are severely limited by the lack of fundamental understanding of the cell groups involved in the process. As we generate more fundamental data this is being used to improve the quality of the mathematical models.

A cell culture approach for investigating fertility was developed in the 1990s (GT1 cells) but failed to provide any insight into the way individual brain cells work together to control reproductive hormone secretion.

Organoids are increasingly being used to model complex structures. However, at present no organoid preparation exists for the hypothalamus, the brain region where the cells controlling puberty and fertility reside.

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?

We have estimated animal numbers based as far as possible on projected experiments over the next 5 years and also taken into account the numbers of animals used in the current 5-year project license that uses very similar methodologies and approaches.

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

To achieve good experimental design, we have established clear experimental objectives and used randomised controls in which, as far as possible, the primary investigator is blind to the experimental

groupings. The use of inbred rodents in a consistent cage environment with skilled experimenters further minimizes variability. Our studies use “Fully Randomised” and “Repeated Measure” experimental designs as appropriate to minimize animal numbers. Fully randomised means that all variables are treated equally and treatments are given without any bias. A repeated measure design means that a series of measurements are made from the same animal so that, for example, the control treatment and test treatments are performed in the same mouse. This provides a more robust ability to detect changes to any treatment and uses very many fewer animals compared to separate groups of mice being used for each measurement or test.

The animal sample size for our work is typically 6 animals per group. This is a well-accepted sample size in our field and provides sufficient robustness for statistical analysis. However, we undertake Power Analyses for each experimental treatment and adjust the required animal number as required. Much of our work requires the placement or injection of tiny recording devices into very small brain regions. Past experience shows that even the best surgeon only has an average 75% success rate for the brain regions we target. It has been my practice that anyone in the laboratory unable to achieve over 50% success after sufficient tutoring is removed from these types of studies. Thus, for all experiments of this nature we will prepare 9 mice in the expectation of being able to use 6 mice in experiments. In circumstances where we end up with more than 6 surgically prepared mice, all will be used for experiments. Experiments will be designed so they can be published in accordance with the ARRIVE guidelines. By archiving tissue for new studies and developing recording devices that signal information without restraint, animal welfare can be improved, and data collection can be maximised while minimising the number of animals used experimentally.

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

The breeding of GA rodents is managed and coordinated across the research group by a very experienced Laboratory Manager in consultation with the principal investigator. This occurs in accord with the NC3R breeding and colony management guidelines and ensures the efficient management of the various lines and that sufficient animals are produced in a timely manner for the experiments.

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.

We use highly refined methodologies that allow the activity of brain cells to be monitored and changed in unstressed animals. We do this by allowing the animals to become very accustomed to the recording environment as well as their individual investigator over several weeks. This is very important for us as any stress stops the activity of the cells in the brain controlling fertility that we are trying to investigate.

Aside from the surgery itself, where animals are treated like humans in hospitals, the mice experience no pain or suffering over the course of the recording experiments. We use a highly refined method of taking multiple blood samples from mice in which a tiny (less than 1mm) portion of the tail tip is removed to allow frequent blood samples to be taken over 1 to 4 hours without stressing the mice. This procedure was co-developed by our group and is now used throughout the world as the best practice for examining pulses of hormone secretion in mice.

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

The brain mechanisms controlling fertility appear to be very similar across all mammals so that use of mice is appropriate for understanding function relevant to humans. The way in which and the brain of an animal like a fish controls reproduction is quite different to that of mice and humans.

We are trying to understand how the brain controls fertility in the dynamic real-life situation. Any anaesthetic stops these mechanisms from occurring.

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

We are continually looking to refine experiments through observation and the trialing of small changes to our procedures. For example, this has led to our present ability to house some animals together after brain surgery operations. Animals are housed together wherever possible in cages that have environmental enrichment such as tunnels. In the past, it was considered dangerous to house animals together after they had received head surgery as the mice would interfere with the head implants.

All surgery is undertaken using aseptic procedures and mice receive regular pain medications following surgery and are monitored for any post-surgical bleeding or weight loss. Pain relief following surgery is provided through food the animals eat. Once mice have recovered, they are accustomed to the experimental recording environment and the human investigator by gentle handling at least five days every week. This can continue for several months so that the mice become very familiar with the scientist.

The tail-tip bleeding procedure is performed after extensive habituation of mice with the same investigator handling the mouse and stroking its tail every weekday for several weeks. We have trialed applying local anaesthetic to the tail tip before cutting and bleeding but it is difficult to determine if this has any impact as the mice do not react to cutting the tip of the tail in any case.

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

We use the ARRIVE and Norwegian PREPARE guidelines, and take advice from NC3R on the management of breeding genetic mouse lines.

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

This occurs internally through information provided by the local AWERB, NACWO and NIO and externally, through local and international scientific meetings. In particular, the UK National Centre for the 3Rs (NC3Rs) provides newsletters to help keep up to date while the Laboratory Animal Science Association (LASA), Institute of Animal Technology (IAT), and the Royal Society for the Prevention of Cruelty to Animals (RSPCA) all provide additional sources.