Home Office

NON-TECHNICAL SUMMARY

Enteroendocrine signalling in health and disease

Project duration

5 years 0 months

Project purpose

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

Key words

Enteroendocrine hormones, gut-brain-pancreas axis, bariatric surgery

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures
- Required at inspector’s discretion

Objectives and benefits

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

What's the aim of this project?

After food consumption the gut sends signals to the rest of the body preparing it for the arrival of new nutrients. Some of these signals have already been developed for the treatment of high blood sugar with the added benefit of patients often losing weight. The same signals have also found to be strongly elevated after surgical rearrangement of the gut, a method to treat very overweight patients. However, some patients experience complications following the surgery, e.g. they feel very sick after meals, which again has been linked to signals from the gut.

We aim to understand how the gut controls these signals in health, disease and after surgery. Which cells in the gut send these signals and what do they respond to? Which cells in the body receive the signals and how do they control the whole body response?

A retrospective assessment of these aims will be due by 30 July 2024

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

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

Once we identify which signals (alone or in combination) result in weight loss and better control of blood sugar, we hope to be able to modify these signals to help patients that store too much fat and fail to control their blood sugar levels adequately. We aim to be able to modify the signals, increasing them in patients that do not have strong enough signals after a meal and thus help them to keep better blood sugar control and lose weight, whilst decreasing or blocking the signals that make some people sick after surgery, thereby improving their post-surgical quality of live.

Species and numbers of animals expected to be used

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

The cells that send the signals from the gut are very rare. We will use mice, as we have made mice in which these cells are manipulated to make a coloured label. We have made other mice that label the cells that respond to the signals. This allows us to characterise when and how the signals are sent. We will use ~15000 mice over 5 years.

Predicted harms

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

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

Most of the ~15000 mice will experience minor or mild severity. We use special mice that have been manipulated to express coloured markers either in the cells that produce gut born signals in response to a meal or in the cells that respond to these signals.
As the label is not inherited by all mice, we will use the label-negative mice as negative controls in experiments wherever possible or these mice will be culled at a young age. Even of the label-inheriting mice many will be organ donors for experiments taking place after they have been culled.
~5000 mice will undergo procedures like fattening by feeding of a fatty diet and/or will be injected with drugs to trigger signals from the gut or to alter the responses of the signal receiving cells. The mice behaviour (e.g. how much and what is a mouse eating after one of the above experiences? Is the mouse changing its energy usage?) will be investigated and mice might be bled repeatedly to measure and identify different signals from the gut. Overall this should only cause minor discomfort of short duration to the animals.
~1500 mice will undergo surgery, to either manipulate cells within their brain, with the aim to identify how the gut signals are integrated to affect feeding behaviour, or to surgically rearrange the gut in a similar way used for the treatment of very overweight people. Adverse effects will be minimised by careful post-surgical care and animals will be culled should they show signs of prolonged discomfort.

A subset of mice will be raised germ-free in special incubators – the absence of intestinal microorganism has been associated with an increased risk of caecal twisting due to enlargement of this intestinal compartment; germ-free mice will be carefully monitored by experienced staff and culled should they show signs of prolonged discomfort.

A retrospective assessment of these predicted harms will be due by 30 July 2024

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

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

We work with cultured cells wherever possible. The cross-talk between gut cells and other cells in the body and the complex circuits regulating feeding behaviour can, however, not be studied in vitro. Similarly, changes in response to surgical rearrangements of the gut can only be studied after the procedure has been performed. Whenever possible, samples from several tissues (e.g. fat tissue) will

be taken and stored from animals having undergone these surgical procedures to enable future studies.

A retrospective assessment of replacement will be due by 30 July 2024

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

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

Mice will be bred at the lowest numbers possible to keep the different strains with labelled signal producing and receiving cells alive. Harvested tissues will be shared between different group members where possible, and in vitro cultures that can be used over prolonged time frames will be established whenever possible. Pilot studies will be performed to assess variability and time courses of effects, to optimise and minimise the final group sizes for metabolic assessments of live mice. All experiments will be planned, analysed and reported in line with best practice guidelines.

A retrospective assessment of reduction will be due by by 30 July 2024

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

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

Under previously licenced work (PPL 70/7824) we have established that mouse and human signals from the gut share many similarities, justifying the use of this animal model. We constantly review the literature whilst planning our experiments and acclimatize animals to new experiences (e.g. environment changes, food changes) before the actual data is collected to minimise stress effects. Whilst we are not using a defined score sheet to assess animals after surgical manipulation, we monitor behaviour and weight progression intensively in all operated animals and for example have in the past been able to spot complications after gut-rearranging surgery early, thus minimising potential suffering. Our laboratory also has and continues to work on the improvement of the analysis of samples after they have been taken from the animal; this includes the development of better methods to measure the signals from the gut in blood, for example enabling simultaneous detection of several signals in small volumes; we adopt and develop new life tissue preparations for the characterisation of the cells that

produce or respond to the gut signals, for example brain tissue slices and gut tissue preparations kept alive for several hours for scientific characterisation, with the aim to minimise additive procedural impact on live animals.

In January 2021 we have asked for an amendment to improve the quality of brain tissue slices based on methods used in a new group members former laboratory. This should further reduce the number of mice needed to be sacrificed for this procedure.

In June 2021 we have asked for an amendment to enable the use of “germ-free” and selectively colonised mice in gnotobiotic isolators, to follow up interesting observations with “non-metabolisable” nutrient analogues - it is not possible to exclude a role of microbiota for enteroendocrine secretion stimulation by these agents in conventionally reared mice, however, should we find that microbiota are needed, this will enable us to identify which microbial metabolic pathways are essential for the stimulation.

A retrospective assessment of refinement will be due by by 30 July 2024

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?