Home Office

NON-TECHNICAL SUMMARY

Neurobehavioural Mechanisms Underlying Mood, Anxiety and Stress-Related Mental Health Disorders

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

Behavioural neuroscience, Mental health disorders, Memory, Brain, Individual differences

Animal types	Life stages
Rats	pregnant, adult, neonate, juvenile

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

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 determine the neurobiological, neurochemical and molecular bases of psychological processes that vary across the population and can go awry in mental health disorders, particularly those associated with uncontrollable or inescapable stress.

A retrospective assessment of these aims will be due by 16 May 2029

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its 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.

Why is it important to undertake this work?

Mental health disorders are a major health burden, both in the UK and worldwide. The World Health Organisation estimates that worldwide, 301 million people live with anxiety, 280 million people live with depression, and 326 million people experience post-traumatic stress disorder. The cost of mental health disorders to the UK in terms of social, economic and health factors is extremely high (estimated in March 2022 as £117.9 billion per year).

Although treatments for mental health disorders exist, currently available treatments need substantial improvement, as they do not work for all patients. Treatment development for mental health disorders is a long-term goal for my laboratory and that of our collaborators, and we believe that treatment development will be facilitated by a better understanding of the psychological, neural and neurochemical mechanisms that underlie psychological processes that can become maladaptive or dysfunctional in mental health disorders. By understanding individual biological and environmental risk factors, it might be possible to identify those at greatest risk of developing mental health disorders, to give them access to preventative treatments (or at least access to treatments sooner). By characterising the individual psychological profiles associated with mental health disorders, it might

also be possible to identify patient 'subtypes' would benefit more from specific treatments, allowing better treatment targeting to the individual.

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

The primary output from this project will be the generation of new information about how psychological, neurobiological and neurochemical processes that are relevant to mental health disorders go awry. These will be communicated mostly through publication in scientific journals and presentation at scientific conferences, but we are also committed to communicating our findings more widely with the general public. Ultimately, the intention is that this research will lead to new treatments being developed for mood, anxiety and stress-related mental health disorders.

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

We expect our research to benefit the scientific community in the short to medium term, and in the longer term to benefit people living with mental health disorders through the development of new treatments.

In the short term, we expect to gain insight into potential drug treatments for the mental health disorders of interest (mood, anxiety and stress-related disorders). This information can be used by other researchers to test these drug treatments in small-scale and, in the longer term, large-scale studies in humans. We are also involved in the institution’s animal tissue sharing initiative, so when animals are killed at the end of the experiments, we share this information with other researchers within our institution so that they can use tissues that we do not need to analyse for our research. This helps to reduce the institution’s overall animal use.

We also expect, in the medium term, that our research will lead to the development of new behavioural treatments. The animal research allows these to be developed with an understanding of how these behavioural therapies can lead to changes in the brain, so that when they are translated to humans, we have a better understanding of how they work. We actively collaborate with researchers studying human participants and patient groups to ensure that our behavioural tasks work well and measure the same processes in humans and non-human animals.

In the longer term, understanding the risk factors that predict whether individuals are more vulnerable to developing mental health disorders may allow early interventions to be made for these people. Furthermore, it may allow for the development of personalised treatments based upon the underlying differences in psychological and neurobiological functioning.

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

We are involved in collaborations with pharmaceutical companies, which will support the impact of our research, particularly with respect to treatment development.

I have a strong track record of publication and of speaking at scientific meetings, which will publicise the work. We are also fully committed to open science, and since 2017 we have made all supporting data for our publications freely available on the university repository, for other researchers to use as

citable datasets. We also publish reliable null results, so that we contribute to a balanced interpretation of the scientific literature.

Species and numbers of animals expected to be used

- Rats: 1845

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.

We will mostly be using adult rats. For some experiments, in which we are studying the impact of early life stress on behaviour in adulthood, we will use pregnant rats and their offspring so that we can control early life experience. As we are interested in adult behaviour, the majority of our behavioural tests will be conducted in adult animals. However, as one of our objectives is to predict which individuals are at risk of developing mental health disorders, for some experiments we will take measurements (e.g. MRI images of the brain) in juvenile animals so that we can relate any differences in their adult behaviour to their previous brain development.

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

A maximum of 1845 rats will be used for this project. As the mental health disorders of interest (depression, anxiety, post-traumatic stress disorder) involve individuals being exposed to uncontrollable stressors, the majority of the animals used in our experiments (with the exception of a small number of controls, required to be naive for comparison) will undergo exposure to inescapable electric footshocks. Although trauma-inducing experiences in humans (e.g. road traffic accidents, sexual assault) are clearly different to the use of footshock in rats, these events share the fact that they are uncontrollable stressors, and the use of footshock in rats allows us to control the extent of the stress to a greater degree than using more naturalistic stressors (e.g. exposure to an aggressive male).

The typical response of rats to an electric footshock is to jump, to freeze (stay completely still other than the movements needed for breathing) for a few seconds, and then to resume normal behaviour. The number and magnitude of shocks that we use produces no physical lasting harm to the animal, but is sufficient for an aversive memory to form that subsequently affects behaviour on other sensitive behavioural tasks. We use the minimum number of shocks to achieve our scientific objectives. We typically use up to three footshocks to model adverse experiences that are within the ‘normal range’ of stressors experienced in adult life. We anticipate that ~73% of rats will experience this level of shock (and the dams required for early life stress manipulations, ~3% of the total animals, will not experience any shocks). However, to model the traumatic events that lead to post-traumatic stress disorder (PTSD), more shocks are required to model the sensitisation of the stress system found in this disorder. We have previously worked to refine this procedure, reducing the number and strength of

shocks required, and allowing animals to be housed in groups rather than experiencing social isolation stress. We have published these findings in animal welfare journals. However, ~24% of animals will need to experience more than three shocks, with the maximum number of footshocks that will be delivered in a single session being 15. The maximum number of shocks in an animal's lifetime would be 20, which would allow us to assess the effect of prior trauma on subsequent learning about adverse events. While these aversive procedures produce changes in behaviour that can be measured on sensitive tests of cognition and affect, they are not sufficient to lead to changes in home cage behaviour or normal social interaction with cage mates.

Approximately 50% of rats will also undergo manipulations of early life stress, through the administration of drugs to the pregnant mother (e.g. to model stress during pregnancy) and/or periods of separation from the mother during early life (to model early life adversity). Early life stress is a prominent risk factor for developing mental health disorders in humans, and these experiments allow us to model this in rats. We perform these manipulations to test the impact of early life stress on the behaviour of the offspring as adults. Footshocks will only be delivered to adult animals; neonatal and juvenile animals are only used for early life manipulations in our experiments, with the same animals being tested as adults to model the interaction of early life stressors with uncontrollable stressful events experienced in adulthood.

Nearly half (~49%) of the animals will undergo experiments that only involve behavioural manipulations, or behavioural manipulations with injections (e.g. intraperitoneal or subcutaneous) or oral administration (e.g. in palatable food) of drugs to modulate the function of the nervous system so that we can assess the impact on behaviour. Often rats will receive different doses of drugs or different types of drugs, so that we can compare the effects of the drugs on the individual rats' behavioural performance. The drugs administered will depend on the specific experiment, but may include drugs proposed to act as novel antidepressants, drugs that alter motivational state or attention, or that affect learning.

Just over a quarter (~27%) of rats will undergo surgical procedures in addition to behavioural testing. These procedures are necessary to allow us to directly record the activity of the brain, to deliver drugs directly to specific brain regions, or to experimentally control the activity of specific types of brain cell (either increasing or decreasing activity).

Approximately 22% of rats will undergo brain imaging in addition to behavioural testing. The rats experience brain imaging under anaesthesia, and may undergo more than one scan, including some scans as juveniles. These scans allow us to measure brain changes between rats showing different types of behaviour, and the repeated scans allow us to track this across development and to determine whether differences in brain volume reported in human patients with the disorder of interest are a cause or consequence of stressful life experiences.

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

Most of the procedures will produce (at most) transient distress and no lasting harm. Extended or excessive distress would lead us to stop the experiment and either humanely kill the animal, or seek treatment under veterinary advice.

Some of the drugs that we give to the rats may cause short-term side effects (e.g. hyperactivity) but most of the time the subtle effects of these drugs can only be seen on our sensitive behavioural measures.

Early life stress produces extended (i.e. throughout the lifespan) effects on behaviour, but these are typically only measurable on our sensitive behavioural tasks and do not involve long-term changes in home cage behaviour. Modelling of trauma through exposure to massed inescapable footshocks (up to 15 in a single session) similarly produces changes in behaviour on sensitive behavioural tasks, but does not lead to changes in home cage behaviour or social interaction with cage mates.

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)?

Moderate: 76%

Severe: 24%

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

- Killed

A retrospective assessment of these predicted harms will be due by 16 May 2029

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 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?

We need to use animals to achieve our research aims and objectives because:

- We need to manipulate early life experiences and determine individual predispositions that make individuals more likely to develop mental health disorders
- We need to understand the fundamental biological differences in the brains between individuals with and without mental health disorders
- We aim to develop new treatments for individuals with mental health disorders.

Our aims and objectives require long-term study of behaving individuals, where we have experimental control over their life experiences and can study both brain and behaviour.

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

Humans, cell culture, and computational models.

Why were they not suitable?

Human behavioural studies are useful for some of our objectives, and we aim to design experiments where the behaviours can be directly compared between humans and animals (i.e. 'translational' and 'backtranslational' research). However, we cannot fully understand the biological differences between those with and without mental health disorders in humans, as human studies are limited to correlational approaches (e.g. brain imaging) when we need to understand causal mechanisms. Furthermore, it would not be ethical (or possible) to conduct studies in humans where individuals are deliberately put at risk of developing mental health disorders (e.g. early life stress studies).

Cell culture studies can be helpful for understanding fundamental biology, but they do not link readily to the behavioural measures in which we are most interested.

Computational models do not yet fully capture the behaviours that are relevant to our research questions, because the models are not sufficiently advanced. They do not capture individual variation or early life environmental differences. However, we are beginning to use computational models to give us better insight into the behaviour of each animal, and to increase the amount of data we produce per animal in each experiment.

A retrospective assessment of replacement will be due by 16 May 2029

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 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?

The numbers of animals have been estimated by referring to the numbers used on my previous Project Licence, the numbers required for our ongoing funded research, and projections based on future

funding at similar levels. These experiments have been planned following power analyses, either using effect sizes from previously published literature or from pilot data in the lab.

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

I am well-trained in experimental design and statistics, and where additional expertise is necessary I have support from statisticians within my institution. Our statistical analyses are determined during the experimental design phase, and where possible we design experiments to allow us to measure within-subjects effects in addition to between-subject effects, to give us greater statistical power.

We refer to the PREPARE guidelines in designing experiments, with the design of each experiment checked by multiple researchers (including the Project Licence, PPL, Holder) and the Named Animal Care and Welfare Officer (NACWO), to confirm that the animal facility is capable of supporting the experiment. We report our experiments in accordance with the ARRIVE 2.0 guidelines, and make our data openly available to other researchers by publishing datasets on our institutional repository at the time of manuscript publication.

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

Where appropriate, we conduct pilot experiments to determine effect sizes, allowing us to perform power analyses where effect sizes are not available in the previous literature. We also design our experiments to collect the maximum number of behavioural measures from individual animals, including control measures to allow us to interpret our experimental effects in context. Most of our behavioural measures are collected automatically on computer, allowing us to interrogate rich behavioural datasets for the animals.

We often collect brains from the animals at the end of our experiments, to allow for further *post mortem* analyses that can be related to the individual behavioural data. We also participate in a tissue-sharing initiative run by our institution, to allow other researchers to access any unused tissues from our animals at the end of the experiments.

A retrospective assessment of reduction will be due by 16 May 2029

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

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 rats because they are the least sentient species that can model the behaviours, affect and cognitions relevant to the mental health disorders of interest. We do not attempt to model a mental health disorder 'in full' in the rats, but rather model specific psychological processes that have relevance to mental health disorders. This approach, called 'dimensional psychiatry', aims to understand the key processes that go awry in mental health disorders. These dysfunctional processes can be shared across different mental health disorders in humans, with different combinations of dysfunctional processes producing different mental health disorders. We take this approach to understanding mental health disorders, by studying these psychological processes in rats, often using behavioural tasks that can be used in both humans and rats (i.e. the tasks are both 'translational' and 'backtranslational').

As we are interested in mental health disorders where stressful environments impact the progression of the disorder, we have both scientific and ethical reasons to maintain high standards of animal welfare and to reduce extraneous sources of stress that would add noise to our experiments. We are also committed, in our task development, to refining existing behavioural procedures and have previously published this work.

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

Our research relies mostly on sophisticated behavioural testing to assess the impact of uncontrollable stress on psychological processes such as responsivity to reward, cognitive flexibility and inhibitory learning. The behavioural tasks that we use are readily acquired by rats, but would be extremely challenging for mice. The majority of our experiments test complex behaviour in adult animals, and we only use neonatal and juvenile rats where this is necessary (e.g. in early life stress procedures). We could not conduct our behavioural experiments at a more immature life stage, because neonatal animals would not be capable of performing these tasks, which often take weeks to train.

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

The bulk of our experimental work relies upon sophisticated behavioural analysis, which typically involves daily contact (at least 5 days per week) with the animals. Any illness or pain would compromise the ongoing behaviour of the rats, giving us a sensitive measure of any welfare issues, often before any clinical observations could detect changes. We also seek to refine our behavioural procedures, and have published this previously in animal welfare journals (Lab Animal).

Where animals have undergone surgery, they are provided with pre-operative, peri-operative and post-operative pain relief. Post-operative pain relief is administered for several days (typically 3) following surgery, and if signs of pain are noted while rats are undergoing increased post-operative monitoring, this is continued for up to 5 days. In the very unlikely circumstance that a rat was still showing signs of pain after 5 days, the rat is humanely killed.

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

There are no specific best practice guidelines for the specific behavioural procedures used on this project (many of which have been designed or refined for use in my laboratory). We refer to more general best practice guidelines provided by the NC3Rs, LASA, FELASA and other learned societies (e.g. the LASA/BAP/BNA/ESSWAP Guiding Principles for Behavioural Laboratory Animal Science), including for surgical procedures and other procedures such as blood sampling and administration of substances.

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

I subscribe to the N3CRs newsletter and my institution's 3Rs mailing list. I also actively participate in 3Rs sessions at conferences.

Relevant guidelines (including a link to the Norecopa databases) are made available to all researchers working on this project through an online repository, and are uploaded by the PPL Holder as they become available.

A retrospective assessment of refinement will be due by 16 May 2029

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?