



Home Office

NON-TECHNICAL SUMMARY

Neural, physiological and behavioural mechanisms underlying cognition and emotion

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

Emotion, Cognition, Mental health disorders, Brain networks, Treatments

Animal types

Marmosets

Life stages

adult, 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.

- Uses non-human primates

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 project aims to identify how different parts of our brain control our ability to learn, remember, plan, attend, display positive and negative emotions and socially interact with others. We aim to understand how these brain regions work together or individually to provide us with these abilities, how stress and genes may affect how they function, how this may lead to mental illness and how current treatments for mental illness work in the brain.

A retrospective assessment of these aims will be due by 14 October 2028

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?

Disruption of these various aspects of our behaviour (cognitive, emotional and social) are associated with a wide range of mental health disorders (such as obsessive-compulsive disorder (OCD), depression and anxiety) but also in natural aging. These disruptions combine to produce symptoms which are only successfully treated by current therapies in some people but not others. We also do not understand how they work when they are successful and so everyone gets given the same treatment in order to find out whether it will work or not. We cannot decide in advance who will be treated successfully. To move forward from this situation, it is essential we gain a basic understanding of how individual brain regions underlie the cognitive/emotional/social disruptions which create these symptoms. This issue can only be addressed in experiments with animals, in which specific, localised manipulations can be made and their effects on behaviour determined. This provides information not only on what brain regions are responsible for our cognitive, emotional and social behaviour, but also how stress and specific chemicals and genes interact with them to cause their disruption that leads to mental health disorders.

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

The results from studies performed in this project will determine the key brain regions that, if dysregulated, may underlie the different symptoms associated with disorders of cognition and emotion. As a consequence it will provide us with greater insight into the nature of clinically relevant symptoms which will help to identify groups of patients that may be better treated by particular drugs. Such a knowledge base would greatly improve clinical outcomes.

Understanding the biological (genetic) and non-biological (environmental) bases of individual differences in emotion and cognition will enable us to gain insight into both vulnerability to mental health disorders and also to differences in responsivity to drug treatments.

In addition, by characterising the development of brain circuits and determining the different time-points at which distinct circuits undergo the most change during development, and the effects of perturbation at these different time points, we will also provide further insight into the aetiology of psychiatric disorders and again help stratify patients more effectively.

We will be able to define the neural and neurochemical mechanisms by which current drugs work, which combined with better knowledge of patient symptoms, will help us to target their use in individual subjects more effectively.

Finally, we will gain a detailed understanding of the marmoset brain anatomy and connectivity that will serve as a very valuable informational framework for many neuroscience researchers in both the present and future.

Publications will be an important part of the output for all information gained throughout the duration of the project.

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

The intended overall benefit of this research will be directed to society as the intended increased understanding of the biobehavioural basis of cognitive and emotional symptoms of neuropsychiatric disorders will have far reaching social and economic implications.

The first beneficiaries of this research will be the scientific and clinical communities. The publication in journals with Open Access, which, for basic research, remains the most effective route to communicate research findings, allows the research findings to gain international impact and prominence, relatively quickly.

The second beneficiaries would be the patients themselves as the output of this research will help provide insight into the varied neural dysfunctions that can underlie the range of cognitive and emotional symptoms associated with neuropsychiatric disorders, guiding new treatment strategies as well as providing insight into the mechanisms by which current therapies have their efficacious actions.

We also intend to impact pharmaceutical company policy as they return to the development of medications for the symptoms of neuropsychiatric disorders. We recently collaborated with a pharmaceutical company to consider new drug targets for anhedonia, the loss of pleasure, a prominent symptom of several psychiatric disorders.

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

We communicate our research as widely as possible to academic and scientific communities. The outcome of our research will be published and disseminated to the scientific community in both high-impact scientific journals with Open Access and at national and international meetings and summer schools. Our results will be targeted for publication in peer-reviewed high impact journals (e.g., Science, Nature Neuroscience, Neuron, Proceedings of the National Academy of Sciences, Biological Psychiatry, Neuropsychopharmacology, Cerebral Cortex, Journal of Neuroscience). We have a strong track record in maximising the output of our work, including both positive and negative results.

Manuscripts accepted for publication will be made open-access and archived in an institutional repository to ensure the widest possible accessibility and impact of our work, thus meeting the new HEFCE policy on peer-reviewed articles and conference proceedings. We will also continue to publish conceptual papers and reviews which are often highly cited and increase the profile of our work and the field in general.

Our results will be disseminated through presentations (plenary lectures, symposium lectures, poster presentations) at international and national conferences and summer schools.

We also communicate our research to non-scientific communities. The publication in journals with Open Access will only be the first step in a wider dissemination and communication strategy aiming to immediately increase our impact on the general public. We have already published an article in The Conversation in 2021, a magazine that specialises in presenting scientific results in an easily digestible format for the public. We have also had our papers reviewed in other USA scientific outlets for the public including techexplorist, neurosciencenews and scitechdaily. Thus, we will rely on publicisation of our work by local and national media groups.

Finally, we communicate our research as widely as possible to clinically active scientists and clinicians. I am an elected fellow of the Academy of Medical Sciences which facilitates my interaction with clinicians and recently have been invite to give a presentation at the Academy of Medical Sciences in the USA. We aim to highlight how our findings impact not only our understanding of the aetiology of the symptoms found in psychiatric conditions but also insight into strategies for their treatment.

Species and numbers of animals expected to be used

- Marmosets: 300

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.

The study of the neural mechanisms underlying cognition and emotion requires a freely moving, behaving animal. It is not possible to investigate behavioural functions of the brain in a simplified experimental setting such as a tissue culture or in an artificial and biologically unrealistic computer simulation. Existing techniques used in humans, such as brain imaging are not only limited in spatial,

temporal and neurochemical resolution, but also provide purely correlative information on structure-function relationships which does not address the crucial issue of the causal involvement of particular regions in specific psychological processes. While studies of patients with neurological damage can provide causative information they fail to provide neural or neurochemical specificity as the damage is non-specific to particular brain structures. Since there is abundant evidence that the normal functioning of much of the brain is comparable across species, not only structurally but also functionally, including the behaviour it supports, this facilitates the extrapolation of findings.

We have chosen to work with a new world monkey, the common marmoset. The marmoset brain, especially the cerebral cortex has an organisation far more similar to humans than that of rodents, the latter species most commonly used in brain studies. The cognitive and emotional functions under study are poorly developed in rodents and this is reflected in their poorly developed brains compared to humans. The brain regions known to be involved in complex behaviour in monkeys and humans, e.g. the prefrontal cortex, are markedly reduced in rodents. Specifically, whilst the cortex makes up 80% of the brain in humans it makes up only 42% of the rat brain and the overall structural organisation of the rodent prefrontal cortex is not comparable. The prefrontal cortex in other species such as pigs and sheep is almost completely unknown, as are these animal's cognitive abilities and social and emotional capacities. Such higher-order capacities are very much linked with the complex social interactions that are the hallmark of most primates, hence why we have chosen the marmoset.

We are studying the brain not only in adulthood but also in development because many of the psychiatric disorders have their onset during development. Therefore, we need to understand the development of these highly developed brain regions in order to understand how they may go awry and lead to neuropsychiatric symptoms.

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

Marmosets used in this project will be exposed to several sets of behavioural and experimental procedures the cumulative severity of which never extends beyond moderate severity. A typical study lasts between 12-24 months during which time an animal may receive approx. 5 or 6 anaesthetics, only 2-3 of which will be for surgical procedures, the rest for restraint purposes only, e.g. neuroimaging. Some animals will only receive procedures as adults whilst others will receive procedures as juveniles.

All animals will first receive short, behavioural tests in the home cage that last no more than 20 mins, to determine their trait anxiety. The tests include measuring their responsivity to an unknown human that stands in front of their cage for 2 minutes and to a rubber snake placed in a box that sits on the floor of their home cage for 5 minutes. This is important to allow animals to be directed into studies that they are most appropriate for.

The various behavioural procedures marmosets will be exposed to aim to assess their individual performance in tasks that measure a range of cognitive and emotional functions including attention, behavioural flexibility, decision making, working memory, long term memory, social cognition, interest in reward and sensitivity to mild threatening stimuli (such as darkness, intermittent white noise, unknown humans, rubber snakes). In most of these tests, visual stimuli are presented on the touchscreen and the monkey makes a voluntary response to one of the presented stimuli. Testing in these tasks typically requires animals to be temporarily sequestered into a specialised testing apparatus. This testing also sometimes requires marmosets to have their water access restricted or to reduce the amount of 'sweet'

foods they get so the juice rewards in the test are more valuable to them. Behavioural tests typically occur daily, never last more than 40 minutes in a session and only take place Monday to Friday. Typically, they have weekends off.

In some cases, marmosets (adult only) will also have their heart rate and blood pressure monitored alongside the behaviour if we are studying emotional reactivity. In this case they receive a one-off surgical procedure which implants a telemetry receiver into a blood vessel (entire surgery takes 1.5 hours and return to home cage within an additional hour and a half) allowing for the remote measurement subsequently of their cardiovascular activity when they are freely moving.

In many cases adult marmosets also receive brain surgery involving: (i) infusion of viruses (e.g. AAV derived) into a specific region of the brain that enable the insertion of a designer receptor into specific neurons. These receptors are inert unless the animal subsequently receives a designer drug that specifically targets the designer receptors but has no other targets in the brain. (ii) insertion of tiny metal tubes (called cannulae) into the brain to allow subsequent infusion of substances into the targeted region whilst awake or (iii) infusion of a compound that has a permanent or longer term effect (1 week - permanent) in the brain. In some cases these procedures will be performed on juveniles at 10 months of age or older but in these cases (i) or (iii) are the preferred options as they are the least invasive.

Subsequently, having received i or ii, adult or juvenile marmosets will receive drugs peripherally (typically 20-48 injections) or through the implanted cannulae (up to 2-3 times within a week, typically 20-30 infusions in total per region) to temporarily change the activity in the specific brain region and its effects on behaviour and/or blood pressure/heart rate (adult only) measured.

Some marmosets (adult or juvenile) will undergo brain imaging (typically lasting 90 mins and 4 scans in total) to help assess the effect of the brain manipulation. Often the scans will be performed both before and after the procedure so that the brain can be compared at these distinct time periods, allowing marmosets to act as their own control. Here, an animal receives anaesthesia for restraint purposes only.

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

Overall, the incidence of adverse events is expected to be low, with the majority of procedures not anticipated to produce adverse effects. This is a result of the many steps taken to ensure best practice and to mitigate adverse impacts. Everything will be done to limit the pain, suffering, distress and lasting harm to the animals within our care at every opportunity and for every procedure. Nowhere in the project is it expected that animals show clinical signs of ill health, and they are checked routinely in case any such signs emerge.

Most adverse effects expected relate to the initial acute recovery phase following surgery, whereby complications may arise from the procedure itself (e.g. localised facial swelling) or the prolonged use of anaesthesia (e.g. protracted recovery to normal behaviour). Such effects typically resolve within 3 hours but can extend to approximately 24 hours. With employment of best practice treatments (e.g. full analgesic regimen) and extensive monitoring the overall impact of surgery to the animal is limited as much as possible. The acute phase following a surgical procedure involves the animal being actively monitored very closely for any signs of deviation from the normal recovery process. Additionally, extra

care is taken during the first week after surgery to observe any changes in normal behaviour or appearance. Long term implant sites are cleaned regularly throughout the life of the animal to prevent infection, and the cage furniture altered to minimise environmental hazards.

Behavioural testing, and the testing apparatus, are highly habituated and do not produce adverse effects apart from transient mild anxiety. However the access to water in the home cage may need to be restricted during the more intellectually demanding experiments utilising a liquid reward. This water restriction only limits the time that animals have access to water, not the volume but has the capacity to impact the animal's general well-being. This is vigilantly watched for but rarely observed. Water restriction does not affect the weight of the animals, who often ignore the water when it is first returned to their cage, suggesting that they are not very thirsty.

Animals may experience transient discomfort when being handled by an experimenter and removed from their home cage, such as is necessary for maintenance of surgical implants, or administration of substances. Discomfort associated with handling would only last a few minutes. However, they are habituated to this process over a period of time to ensure they are not stressed by the procedure and thus are not expected to experience much discomfort at all. They normally acclimate to this process quite quickly and are frequently rewarded with treats such as a small bit of marshmallow. Peripheral injections themselves can produce mild discomfort (i.e. slight bruising at leg injection site). In such a case with intramuscular injections, legs are alternated to minimise effects on the muscle and they are checked by the Vet at regular intervals (after every 12 injections) with approval required before anymore injections can be performed. Animals are watched very closely for adverse reactions i.e. tremoring, after all drug treatments, and we have robust protocols to alleviate such reactions if they do occur.

Animals may experience transient discomfort as a result of brain manipulations or peripheral physiological challenges (i.e. hormones) that alter their internal bodily and emotional states. Such effects are anticipated to produce only very mild impacts on an animal's well being with durations subject to the substance used but typically no more than 2 hours, usually much shorter.

Given the cumulative nature of adverse effects we do everything we can to limit the number of adverse effects experienced across their lifetime. This includes examining closely the transition between any procedures, especially if the animal has experienced any adverse effect or negative impact from a procedure.

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)?

Mild 10%

Moderate 90%

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

- Killed

- Kept alive

A retrospective assessment of these predicted harms will be due by 14 October 2028

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?

The study of the neural mechanisms underlying cognition and emotion requires a freely moving, behaving animal. Existing techniques used in humans, such as brain imaging are not only limited in spatial, temporal and neurochemical resolution, but also provide purely correlative information on structure-function relationships which does not address the crucial issue of the causal involvement of particular regions in specific psychological processes. While studies of patients with neurological damage can provide causative information they fail to provide neural or neurochemical specificity. Thus, this research requires the use of animals engaged in specific behavioural and cognitive tasks.

There is abundant evidence that the normal functioning of many of these neural systems is comparable across species, thus allowing a certain amount of extrapolation of findings across species. Some ex vivo techniques will be used to complement the in vivo studies presented. DNA, RNA and protein analyses will be performed on blood and brain tissue obtained pre- and post-mortem, respectively. The brains of all animals receiving brain manipulations will be analysed post-mortem to locate the positions of cannulae and/or lesions in the brain. This will inform future surgeries and refine surgical procedures.

It is essential to use an animal model with cognitive and emotional functions translatable to/similar to those found in humans. These functions are poorly developed in the rat which is reflected in the organisation of their brain. The anatomical features of those regions of the brain known to be involved in complex cognitive and emotional behaviour in monkeys and humans, e.g. prefrontal and temporal association cortex, are markedly reduced in rodents. Specifically, whilst the cortex makes up 80% of the brain in humans it is only 42% of the rat brain and the overall structural organisation of the rodent prefrontal cortex is not comparable. The prefrontal organisation of other species such as pigs and sheep is almost completely unknown, as is their cognitive abilities and their socio-emotional capacities. These complex behaviours are linked to the complex social societies that primates live in including marmosets, which is the species of choice for this work.

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

Tissue cultures (including brain organoids) and artificial computer simulations.

Why were they not suitable?

Tissue cultures (including brain organoids) are unable to contribute to a functional, behaving circuit, thus cannot perform cognitive tasks and do not express emotion whilst artificial computer simulations are biologically unrealistic.

A retrospective assessment of replacement will be due by 14 October 2028

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?

In deciding on group size a number of factors are taken into account. My research group has extensive experience of publishing experiments on marmosets in high-impact journals with rigorous statistical peer review. We use the 'appropriate number (n) of marmosets compatible with adequate statistical power for hypothesis testing, according to the "Reduction" principle from the 3Rs. The precise number used in groups has varied from study to study (from sample sizes of 3-8) depending upon prior knowledge about inter-individual variation in (i) performance of animals on the particular task and (ii) the effects of the neural manipulation, both of which affect the anticipated effect size. Smaller sample sizes (3-6) have been used in the more recent years because of our refinements to our subject study subject allocation, behavioural training and surgical interventions, which have led to enhanced effect sizes. When we embark on a new study we tend to start off with 2 lead animals in which we test out our hypothesis. We are looking for large obvious effects e.g. the abolition of anticipatory cardiovascular and behavioural arousal in an anhedonia (loss of pleasure) study, which can be seen visually in individual animals. Where possible these lead animals will be incorporated into the main study if few, if any, changes have to be made in the experimental design before the rest of the animals enter the study. It should be stressed that our effect sizes tend to be large because we have strong hypotheses based often on previous findings from our own lab and by having tight control over experimental variables. Based on our recently published and unpublished experimental manipulation studies, we have planned for group sizes of between 5-8 in the majority of our studies.

Appropriate numbers for the study of genetic polymorphisms are still being clarified. We found an n=8 group size to be sufficient to identify statistical differences in pharmacological responses and brain neurochemical and structure differences whilst molecular studies have required n=3 per group and behavioural observations needed n=15.

Similarly appropriate numbers for the study of the brain and behaviour over development are still being clarified. This work is unprecedented and so the estimation of numbers is based on findings from our own earlier developmental imaging study in marmosets as the clearest indicator of the kinds of effect sizes and background variability we might expect.

We are familiar with the PREPARE and ARRIVE guidelines (<https://proecopa.no/prepare>; <https://arriveguidelines.org>) and will ensure all our experiments are designed in adherence to these guidelines.

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

- i). using a carefully controlled behavioural testing apparatus so that the particular cognitive abilities under study can be studied in isolation which helps reduce variation in performance.
- ii). using structural MRI to target certain brain structures to ensure that the brain manipulation is effective in the majority of animals.
- iii). using animals wherever possible as their own controls to increase the power of statistical comparisons, minimise variability, and minimise the number of animals used
- iv). when separate controls are required, not necessarily matching the number of controls to the number of experimental animals but still ensuring they are sufficiently balanced to ensure statistical power
- v). screening to ensure suitability of animal for particular study, thus also minimising variability.
- vi). re-using animals when they suffered no significant adverse effects during or as a result of their previous use. Previous use will not prejudice the outcome of the study on which they are reused, and after the completion of the previous procedure and before the intended reuse the NVS has determined that they may be kept alive and that their health status and condition is compatible with proposed reuse in compliance with ASPA requirements. Re-use will not take place if an animal has received e.g. intervention surgery but an animal is re-used if all they have received is e.g. non-invasive imaging of their brain during development or peripheral drug injections/blood sampling.
- vii). repairing surgical implants whenever possible. Simple repairs occur whilst the animal is awake and minimally restrained by an experienced holder, whereas more complex repairs occur under anaesthesia. Since the repair procedure occurs to an implant rather than the animal itself, repairs are painless. However, if anaesthesia is required for more complex repairs, analgesia will also be administered. Therefore, the cost/harm of repairing a cannula on an already trained and cannulated animal outweighs the cost/harm of needing to replace the animal in the study.

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

Using pilot studies to optimise the parameters of a brain manipulation or experimental procedure and to determine the dosage of peripherally administered drugs to elicit subtle behavioural changes before

running the main study.

A retrospective assessment of reduction will be due by 14 October 2028

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.

Marmosets are a particularly valuable species to use for our work as their relatively small primate brain makes it possible to target cortical and subcortical structures and make regionally selective changes in the brain with relative ease, with minimal risk to the animal.

We use a wide array of methods in our research which have been, and continue to be, optimised to ensure least pain, suffering, distress, or lasting harm to the animals.

The behavioural tasks we use are designed to be similar to those used to test intact and brain damaged humans, maximising the ability to extrapolate findings from our marmoset studies directly into the clinical setting. To avoid unnecessary stress associated with behavioural testing, our animals are (i) trained to voluntarily get into a carry box in the home cage to go to the test apparatus (ii) can move freely once in the test apparatus and (iii) are tested for less than 40 minutes.

Water restriction for behavioural testing is only used when an animal's responses are variable in order to provide additional motivation to ensure stable and high performance across sessions. Access to water is initially only restricted for a few hours immediately before testing. If needed, some animals may then move on to longer periods of restriction, only having access to water for two hours at the end of the day to have the desired effect on their performance. This restriction only induces mild thirst, not dehydration, and to limit stress, the animals have two days of uninterrupted access to water every week, and have a break from restriction for at least a week every six months.

Systemic administration of biologically active chemicals (e.g. drugs) either through experimenter-delivered injections, or via previously implanted subcutaneous minipumps. Dosing procedures will be undertaken using a combination of volumes, routes and frequencies that themselves will result in no more than transient discomfort and no lasting harm and are the minimum number of procedures (adults and juveniles) consistent with the objectives that will minimise the already transient, mild discomfort.

For blood sampling procedures no more than 10% of total blood volume on one single occasion and 15% of total blood volume in any 28-day period will be taken following established guidelines for the marmoset (General principles NC3Rs).

Implantation of inert cannulae into the brain under anaesthesia allows for subsequent infusion of compounds through the cannulae to manipulate brain activity in localised regions and pathways (adult and juvenile) temporarily. This method replaces lesion studies which removed part of the brain, leaving the animal permanently lacking functions associated with the brain region targeted. Cannulation allows two different brain regions to be targeted temporarily and without lasting damage, and enables comparisons to be made within the same animal which is the gold standard for differentiating the functions of distinct brain regions and decreases the overall number of animals needed per study.

Infusion of viral vectors into the brain under anaesthesia to insert designer receptors into localised brain regions and pathways that can subsequently be activated by designer drugs (adult and juvenile). This is the most refined method of brain manipulation currently available and reduces the need to implant cannulae into the brain to target specific brain regions as the designer drugs used to activate the designer receptors can be injected peripherally, thereby minimising stress associated with cannulae implantation and care, and the longer periods of holding time needed for central infusions.

Being held for infusions either into the brain (through implanted cannulae), or peripheral injections (designer drugs) for no more than 5-10 minutes to induce short-acting effects on brain activity and behaviour. Holding the animal avoids the need to use primate chairs which offer no flexibility, unlike 'holders' which can adapt to an individual monkey's needs. The short-acting alterations induced by infusions and injections allow each animal to act as its own control, thereby reducing numbers, and is less invasive and more biologically relevant than inducing permanent changes in brain activity. All animals are habituated to the holding procedure and thus it causes only transient discomfort.

Monitoring of cardiovascular activity remotely in freely moving animals by implanting a telemetry (wireless) probe into the descending aorta in a single surgery under anaesthesia (adult only). Animals are back in the home cage within 2 hours of having come round from surgery, showing no ill effects. This method allows cardiovascular activity to be measured over the remaining experimental lifetime in freely moving animals thereby removing any stress induced by restraining the animal to take measurements, and allowing more relevant measurements to be made, e.g., during task performance and without the stress confounds of being held.

Subcutaneous port implantation alongside jugular vein implantation under anaesthesia to allow subsequent subcutaneous injection of radioactive drugs directly into the jugular (adult only). Animals are back in the home cage within 2 hours of having come round from surgery and show no ill effects. This removes the need for repeatedly performing a painful procedure at the time of the injection, which may even have to be performed under sedation.

MR (adult and juvenile) and/or PET (adult only) imaging under anaesthesia for restraint purposes only to measure whole brain structural, chemical or functional activity differences. This is the least invasive method for measuring whole brain activity.

Surgical procedure whereby a specialised probe is inserted into a localised brain region allowing for the measurement of a range of brain chemicals whilst under anaesthesia (adult). Animals are back in

the home cage within 3-4 hours of having come round from surgery with minimal side effects. This 'in vivo' method avoids the need to sacrifice animals to obtain the same chemical samples.

Challenge with a physiological stressor e.g. cortisol or a pro-inflammatory substance (e.g. BCG) since such stressors are a risk factor for the onset of neuropsychiatric disorders. The dose, route and number of administrations are chosen to minimise suffering.

Injection of non-harmful substances into specific brain regions in a surgical procedure, under anaesthesia in order to determine connectivity patterns in the brain. Animals are back in their home cage within 2 hours coming round from anaesthesia and exhibit no long term negative effects.

Terminal anaesthesia using a sedative followed by sodium pentobarbitone and transcardial perfusion (adult and juvenile). Complete cessation of the heartbeat is confirmed via stethoscope prior to making incisions for the perfusion for absolute certainty the animal is no longer alive and does not experience any suffering or distress during the perfusion process.

Overall, we are geared towards optimal refinement, from our choice of animals, to our methods, procedures and skills. Additionally, we make sure that we maintain our high standards and training of staff in order to ensure all our refinements are actually implemented. Thus, I review all procedures and skills of the licenced researchers working in the laboratory, under my supervision, regularly and discuss project licence-related matters at each of my weekly lab meetings.

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

Marmosets are the least sentient organism with a highly evolved prefrontal cortex that controls the expression of higher-order cognitive and emotional behaviour of relevance to our understanding of the complex behavioural symptoms of brain disorders.

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

Cortisol levels will be measured in hair and saliva samples taken from live animals via unregulated procedures, refining where possible the need for more invasive procedures (such as blood sampling).

When developing new techniques, e.g., viral infusion of designer receptors that can be activated exclusively by designer drugs (DREADDS, which activate/inactivate neurons), we collaborate with colleagues to develop the procedure in rodents, if applicable, before applying it in the marmoset.

Surgical procedures such as collecting chemical samples in a given brain region (called microdialysis) will be performed with non-recovery when appropriate, and the duration of stereotaxic surgeries have been shortened where possible by technical refinements in order to minimise post-surgical complications when recovery is required.

All surgeries, as well as intracranial infusions, are performed aseptically and we have advised numerous national and international labs on best practice to reduce likelihood of post-operative complications and associated stress.

Cannulations in marmosets at 12 months of age is at a timepoint when the size of the brain and skull are stable, and thus chronic implants are not likely to cause any more problems than are normally observed for animals above 18 months of age. Initially, we will pilot this in a few marmosets. Prior to cannulation, several adolescent marmosets will be behaviourally screened using the intended tasks to ensure that we select the most suitable candidates for cannulation.

As juveniles are still housed with their family group any adaptations required to facilitate these studies will be discussed in consultation with NACWO and NVS. For instance, we may need to consider how to adapt the diet to motivate testing adolescents without impacting the welfare of the group. We are already in the process of developing a system for assessing the weight and condition of experimental juvenile animals. We will adapt the top of the nest box doors (with flaps and/or rubber) in the home cages of the family pens as we do for our cannulated adult marmosets to avoid the cannulae getting banged accidentally as marmosets pass through the nest box door. In addition, we have performed a limited study using DREADDs to target a specific brain region allowing us to alter activity in that brain region using a systemic drug in the juveniles, reducing the need for cannulae implantation in some cases. This has been successful so will be something we will continue to use wherever possible in the future.

This laboratory has been performing many of the described procedures for over 25 years and during this time the techniques have been refined either in house, or in consultation with outside experts in their particular field to minimise pain, suffering, distress or lasting harm. Analgesic, anaesthetic and antibiotic regimes have been developed in consultation with the NVS and are under continual review. We receive additional advice on anaesthesia from an experienced specialist veterinary anaesthetist with considerable expertise in primates. Prophylactic analgesics are routinely administered prior to surgical procedures to minimise pain during the procedure and in recovery. Post-surgical or other procedure-related analgesics and antibiotics (typically oral) are given as recommended by the NVS for a given procedure, but also take into account the condition of the animal, with e.g. some individuals requiring extra pain relief early in recovery and others needing it for longer than the typical recovery window. Animals are closely monitored in the days following surgery, are always provided ad libitum water and normal diet to assist recovery, and monitoring of post-surgical recovery has recently been reviewed in conjunction with the NVS to ensure prompt identification of non-anaesthesia related symptoms, including pain.

A formalised weekly environmental enrichment programme with rotation of enrichment devices has been instituted in the colony, with new items regularly trialled and added to the rotation if successful. Items such as foraging boxes, highly palatable juice (frozen and liquid forms), swings and baskets, and textured bedding have all been utilised to enrich accommodation routinely, and/or as part of enhanced recovery after surgical procedures. Live foods (locusts and mealworms) have also been introduced to encourage more natural hunting and foraging behaviours. Animal staff and scientists interact with marmosets on a daily basis, continually monitoring the welfare and environment, ensuring that the NC3Rs guidelines on non-human primate accommodation and care are consistently met and exceeded wherever possible.

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

Our research is constantly guided by, and adheres to the Laboratory Animal Science Association (LASA), the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) and Animal Research: Reporting In Vivo Experiments (ARRIVE) Guidelines. Not only do we follow the LASA guiding principles of aseptic surgery (<http://www.lasa.co.uk/wp-content/uploads/2017/04/Aseptic-surgery-final.pdf>), but we have furthered these principles wherever possible as part of our constant refinement strategy, especially in the case of intra-jugular catheter implantation procedures. We will receive direct updates on best practice from the N3CRs as I have subscribed to their mailing list and we attend the annual Primate Welfare meeting.

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

We have several lines of information that enable us to stay informed about advances in the 3Rs in order to implement them effectively. First, we have registered to the NC3Rs newsletter. Secondly, as all the project licence holders at our establishment, we receive tremendous support from the staff at the establishment, and we receive regular critical updates from the Named Information and Compliance Officer to which we pay the utmost attention and that we share with all the members of the lab. Third, we regularly hold project licence-related workshops with all the members of the laboratory to discuss the changes in procedures. We also have an excellent working relationship with the animal care staff in our animal facility, which facilitates the implementation of advances in the 3Rs. Finally, we are also part of an international network of marmoset users and we regularly have meetings and exchange best practice.

A retrospective assessment of refinement will be due by 14 October 2028

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?