Home Office

NON-TECHNICAL SUMMARY

Neuronal circuit mechanisms underlying cognitive memory judgements

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Cognitive memory, Decision making, Object vision, Neuronal circuit, Causality

Animal types	Life stages
Marmosets	adult
Rats	adult

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?

The aim of this project is to reveal neuronal mechanisms by which animals detect objects or events and compare them with past experiences. In other words, the project aims to reveal how brain circuits allow us to discriminate familiarity from novelty.

A retrospective assessment of these aims will be due by 4 April 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?

Identifying something from previous encountering and knowledge, or recognition, is critical for our life in many ways. For an example, loss of memory and the inability to recognise people, objects and events as familiar is a core symptom of dementia including Alzheimer’s disease in which brain degeneration slowly destroys memory and cognition. In the UK, the number of people with dementia is estimated to exceed one million in 2025 (National Health Service - Dementia guide). On the other hand, our ability to read and write also requires recognition of letters and association with sounds, and its difficulty results in a learning disorder called dyslexia, which occurs in 10% of population despite no other cognitive disabilities including verbal communication (British Dyslexia Association). Therefore, understanding how our brain recognises objects is an essential step toward preventing, treating and improving various recognition-related symptoms which significant number of people are suffering from. However, a fundamental knowledge of the neural circuits that support our recognition is lacking despite that such knowledge is the basis of effective therapy and care. Whilst whole brain imaging studies in humans identifies the brain regions that show correlated activity with recognition, it is essential to gain information about the causal roles of the identified brain regions and how these regions interact to compare visual objects with memory, provide a sense of familiarity/novelty, and retrieve their associated meanings, which eventually induces a behavioural decision that is an essential component of human behaviour. To address this issue, detailed measurements and well-controlled manipulation of neuronal activity in behaving animals are critical.

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

This project will ultimately output the knowledge that determines the key brain regions and neural circuits that, if dysregulated, may underlie the different symptoms associated with disorders of fine vision, cognition and memory of objects. As a consequence, this knowledge will provide us with greater insight into the nature of clinically relevant symptoms which can be a seed of novel treatments to improve visual cognition and memory in disorders such as dementia and dyslexia which significant number of people are suffering from. Such a knowledge base would greatly improve human life.

Specifically, I aim to identify the neurons, and their connections and communication which causally guide the perceptual identification, and memory-based discrimination of visual objects. Such neuronal circuits would be potential targets of novel medications, behavioural therapy, and stimulation therapies. For example, by selectively stimulating the neuronal circuit that signals familiarity when a dementia patient sees a family member, it would be possible to guide the patient recognise that person as familiar. Such an application possibility would be implemented by developing a wearable device in which a small computer vision camera identifies a family member, and an implanted brain manipulator stimulates the neuronal circuit to generate a safe, familiar feeling to that person. Alternatively, by stimulating the neuronal circuit that signals the shape and identity of objects, such brain manipulation technologies would also help dyslexic people discriminate letters and retrieve the sounds associated with the letters. Thus, the scientific output of this study will also have enormous socioeconomic impacts.

Equally importantly, I will gain a detailed understanding of the marmoset brain circuits and its operating principle. Marmoset is an emerging model animal to study neuronal circuit mechanisms of various cognitive functions and dysfunctions. The US, Europe, Japan, and China are making more and more investments on this small primate species because its small and smooth brain is highly suited for modern advanced technologies to investigate brain circuits underlying various cognitive functions. Therefore, the new knowledge about the marmosets' behaviour and brain circuits produced in this project will serve as a highly valuable basis for many neuroscience researchers in both the present and future and further develop the value of this primate model species. The technical developments achieved in this project will be directly applicable for studying other higher cognitive functions that are specifically evolved in primates.

Publications will be an important part of the output for all information gained throughout the duration of the project. Because the marmoset is a rising but underdeveloped model species for neurophysiology, any results and developments will be highly valuable and published in well-read journals as important advancement.

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

The intended overall benefit of this project will be directed to the society as the intended advancement in the understanding of neurophysiological basis of visual cognition and memory will have far reaching social and economic implications.

The first, immediate beneficiaries of this project output will be the basic and clinical research communities because the researchers can directly use the findings and developments produced in this project to guide their own research directions. The journal publication with Open Access, which

remains the most effective route to communicate research findings, allows the research findings to gain international impacts and prominence, relatively quickly.

The second beneficiaries would be the patients as the output of this project will help provide insight into the varied neural dysfunctions that can underlie the range of visual, cognitive and memory symptoms, guiding new treatment strategies as well as providing insight into the mechanisms by which current therapies have their efficacious actions. Those benefits will be provided to the patients through clinicians who directly advance healthcare protocols, and also through the healthcare industry which develops novel medications and devices to guide vision, cognition and memory.

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

I will communicate the output of this project as widely as possible to research communities. The outcome of this project will be published and disseminated to the scientific community in both high-impact scientific journals with Open Access and at domestic and international meetings, summer workshops, etc. My results will be targeted for publication in peer-reviewed high impact journals (e.g., Nature, Science, Cell, Nature Neuroscience, Neuron, Proceedings of the National Academy of Sciences, Journal of Neuroscience, etc.). I have a strong track record in maximising the output of the own 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 my work, thus meeting the new HEFCE policy on peer-reviewed articles and conference proceedings. I will also publish conceptual papers and reviews which are often highly cited and increase the profile of my work and the field in general.

My results will be disseminated through presentations (symposium lectures, poster presentations) at international and domestic conferences and workshops. Particularly, since I am involved in the international network of researchers, I can very effectively communicate my results to the researchers who will make the most of the project output. In addition, the techniques to study neuronal circuits that I will develop through this project will be also directly beneficial for my collaborators in the same institute who works on the same animal models, and thus our collaboration will robustly advance the understanding of various cognitive functions.

I also communicate this 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. Then, I will digest the scientific publications through Press Release as I did for my previous works which have been well discussed in media including Explica, Tech Explorist, Galileo, N+1, MedicalXpress, TechPlus, Le Scienze, New Scientist, The Scientist, Наука и Жизнь, Nauawpolsce, etc. I will also directly communicate with the society, as I previously gave lectures at a junior high school and a community centre. Thus, I will rely on publicisation by media groups, and also conversation with the non-scientific community.

Species and numbers of animals expected to be used

- Marmosets: 10
- Rats: 60

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.

In this project, I have chosen to work with adult common marmosets to study visual cognitive memory. I also work with adult rats for pilot experiments to validate and refine neurophysiological experimental techniques towards the future marmoset application.

The study of the neuronal circuit mechanisms underlying fine object vision and cognitive memory judgements 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 research techniques used in humans, such as whole-brain imaging, detect the brain activity only spatially less-finely and temporally less-precisely, and tell us only about the correlation between brain activity and behaviour which does not address the crucial issue of the causal involvement of particular brain regions and neurons in specific psychological processes. While studies of human patients with neurological damage can provide causative information, they fail to provide neuronal or circuit specificity as the damage is not controlled nor specific to particular brain structures. Since there is abundant evidence that the normal functioning of much of the brain regions is comparable across species, not only structurally but also functionally, including the behaviour it supports, this facilitates the extrapolation of findings.

The marmoset has fovea (a small region with high visual acuity) in their retina which is crucial for human-like fine object vision but is missing in rodents. 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 visual, cognitive and memory 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 memory-dependent behaviour in monkeys and humans, e.g., the prefrontal cortex (the most frontal part of the cerebral cortex) and the temporal association cortex (the side and bottom part of the cerebral 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 and temporal association cortices is not comparable. Other mammalian species such as dogs have been much less studied in neurophysiology, and comparable knowledge is not available. Pigs have one stereotaxic brain atlas, but it details only about subcortical structures, and sheep have none. Thus, the anatomical and functional organisation of their cortex is much less understood. In addition, their brain (150 g in sheep, 180 g in pigs) is far larger than the monkey brain (100 g in macaques, 20 g in marmosets) and therefore they are less compatible with modern methodologies evolved in small rodents. Other vertebrates such as reptiles and birds have brain organisation that is not directly comparable with that of mammals. Cognitive memory capacities are very much linked with the complex decision makings that are the hallmark of most primates, hence why I have chosen the marmoset.

Rats are particularly suited for refining experimental procedures for marmosets, because rats weigh 300-400 g, and the length of the skull is 4-5 cm, which are very similar to those of marmosets. Mice are

too small as they weigh only 20-30 g. This makes the rats highly beneficial as a model despite their insufficient similarity of the brain structure and functions to those of primates. Once the experimental procedures are successfully refined in rats, I will apply for amending this project licence to use those procedures in marmosets to further pursue the main objective of the study.

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

In this project, I will work with common marmoset to study visual cognitive behaviour with non-invasive brain imaging. I will also work with rats to validate and refine neurophysiological experimental techniques towards the future marmoset application.

Marmosets

Adult marmosets will be used in this study. Marmosets will be exposed to behavioural and experimental procedures with the cumulative severity which never extends beyond the moderate level. A typical study lasts between 12-24 months during which time a marmoset may receive approx. 5 anaesthetics, for restraint purposes only, e.g., magnetic resonance imaging (MRI) and positron emission tomography (PET) which allow non-invasive visualisation of brain structure and activity.

Marmosets may 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 test is important to allow animals to be directed into studies that they are most appropriate for.

Marmosets will be exposed to various behavioural procedures to aim to assess their psychological performance in tasks that measure a range of cognitive functions including perception, memory, and decision making. In most of these tests, visual stimuli are presented on the touchscreen and the monkey makes a voluntary response to the presented stimuli to receive a ‘sweet’ liquid reward through positive reinforcement. Testing in these tasks typically requires animals to be temporarily sequestered into a specialised testing apparatus. Behavioural tests typically occur daily, do not last more than 40 minutes per session, and only take place Monday to Friday. Typically, they have weekends off. When it is difficult for a monkey individual to maintain its stable test performance in intellectually demanding tasks across days, or a larger number of task trials is necessary for statistical analyses, additional motivation may also be provided by having the amount of sweet foods or the time of water access mildly restricted at home cage (the amount of water is not restricted when given) so the sweet liquid rewards in the tests are more valuable to them. The water access is restricted only gradually by analysing the behavioural performance of individual monkeys, and initially restricted only for a few hours immediately before testing. If needed, some animals may then gradually move on to longer periods of restriction 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.

For studying the behavioural effects of brain activity modulation, marmosets may receive injection of neuroactive drugs into the muscle or under the skin depending on types of drugs (typically 20-48 injections). Injection will be followed by behavioural tests, and injections of a drug and saline are interleaved to maximise the detectability of behavioural impacts.

Some marmosets may undergo brain imaging by MRI and PET (typically lasting 90 mins and 4 scans in total). For detecting brain activity by PET, an imaging agent may be administered by an intravenous route e.g. femoral or tail vein. Here, an animal receives anaesthesia for restraint purposes only. To compare the brain before and after a procedure by using the same marmoset as internal control, the scans will be performed both before and after the procedure with sufficient intervals (minimum 2 weeks).

Rats

Adult rats will be used in the study. Rats will be exposed to experimental procedures with the cumulative severity which never extends beyond the moderate level. Typical study will last up to 12 months. Rats will receive surgical implantation, and they may undergo electrophysiological experiments (to measure and manipulate electrical activity of neurons) during behavioural tests or under terminal anaesthesia. Rats may also receive brain imaging by MRI or PET.

Rats will receive surgical procedures under general anaesthesia for implanting small MRI-compatible recording chambers and miniature micromanipulators (very small movable clamps) on the skull, and for inserting microelectrodes (very thin needles with tiny electrode contacts) to the brain regions of interest through a small hole in the skull and dura mater. In electrophysiological recording sessions, the rat is gently held by hand, and the microelectrode is advanced by driving the miniature micromanipulator. Then, a small, head-mounted electrophysiological amplifier (head-stage, approx. 20x35x20 mm^3 / 15 g) will be connected to the microelectrode to record or stimulate the neuronal activity. The rats will freely explore in behavioural testing apparatus (open arena) or in their home cage, and they may collect scattered food flakes and receive neutral visual/auditory stimuli. Water and food are not restricted. Outside the recording sessions, the head-stage will be detached, while the microelectrodes, chambers and miniature micromanipulators will be kept implanted and covered by a cap to allow the animal to live normally.

Rats may undergo brain imaging by MRI or PET (typically lasting 90 mins and 4 scans in total) up to 5 times to help finely localise the brain regions of interest and their connectivity. The brain imaging may be performed before surgical procedures to plan implantation surgery, or after the surgery with the microelectrode inserted in the brain to finely determine the coordinates of electrophysiological recording. Here, an animal receives anaesthesia for restraint purposes only.

If the work with the rats is successful, I will be applying to amend the project licence to use those techniques in the marmosets.

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

In marmosets I will study visual cognitive behaviour with non-invasive brain imaging. In rats, I will validate and refine neurophysiological experimental techniques towards the future marmoset application.

Marmosets

In marmosets, 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 at the facility. 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, it is expected that animals show clinical signs of ill health. They are checked routinely in case any such signs emerge.

In marmosets, behavioural testing is based on positive reinforcement with sweet liquid rewards, and the testing apparatus is highly habituated, so they do not produce adverse effects apart from transient mild anxiety. However, in some marmoset individuals who do not maintain stable task performance in intellectually demanding tasks across days, or when a larger number of task trials is necessary for statistical analysis, the access to water in the home cage may need to be restricted to add additional motivation. This water restriction only limits the time that animals have access to water, so the amount of water is not restricted when given, 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.

Marmosets may experience transient discomfort when being handled and removed from their home cage. 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.

Animals receiving repeated injections could potentially experience soreness or bruising of the leg muscle (no incidence in the facility). After 12 injections, the animal will be examined by the NVS for signs of adverse effects. Most routes of peripheral drug administration do not result in adverse effects as a result of the injection per se. Injection sites will be alternated between legs to minimise the likelihood of this occurring and apply pressure after the injection to minimise bruising. If leg soreness or bruising is observed, injections in these animals will halt until the animal has recovered or been treated.

Given the cumulative nature of adverse effects, I do everything I 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.

Rats

In rats, 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 2 hours but can extend to approximately 24 hours. With employment of best practice treatments (e.g., full analgesic regimen) and careful 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.

Surgery including implantation and craniotomy can cause a mild pain, but the pain will be alleviated by performing the procedure under anaesthesia. Insertion of microelectrodes itself can potentially produce transient mild discomfort but no pain as there is no pain sensor in the brain. Animals are watched very closely for adverse reactions i.e., tremoring, after all craniotomy, microelectrode insertion and electrophysiological recording and stimulation, and I have robust protocols to alleviate such reactions if they do occur.

In rats, no behavioural task training is required, and therefore food/water restriction will not occur. The head-mounted devices may initially cause slight inconvenience for behaviour, but the animals will usually quickly adapt. The environmental enrichments of the home cage may also be adapted not to interfere with the implants and rats' behaviour.

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)?

Marmosets

Mild 10%

Moderate 90%

Rats

Moderate 100%

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 4 April 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?

In this project, I need common marmoset to study brain mechanisms of visual cognitive behaviour unique to primates. I will also need rats to validate and refine neurophysiological experimental techniques towards the future marmoset application.

Marmosets

The study of the neuronal circuit mechanisms underlying fine object vision and cognitive memory judgements requires a freely moving, behaving animal. Existing techniques used in humans, such as whole-brain imaging, detect the brain activity only spatially less-finely and temporally less-precisely, and tell us only about the correlation between brain activity and behaviour which does not address the crucial issue of the causal involvement of particular brain regions and neurons in specific psychological processes. While studies of patients with neurological damage can provide causative information, they fail to provide neural circuit 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 non-animal techniques will complement the animal studies presented. The brains of all animals receiving brain manipulations will be analysed post-mortem to locate the positions of microprobes and/or lesions in the brain. This will inform future surgeries and refine surgical procedures.

It is essential to use an animal model that has fine object vision and cognitive memory 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 the brain known to be involved in complex cognitive behaviour in monkeys and humans, e.g., prefrontal and temporal association cortices, 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 cortex is not comparable. Other mammalian species such as dogs have been much less studied in neurophysiology, and comparable knowledge is not available. Pigs have one stereotaxic brain atlas, but it details only about subcortical structures, and sheep have none. Thus, the anatomical and functional organisation of their cortex is much less understood. In addition, their brain (150 g in sheep, 180 g in pigs) is far larger than the monkey brain (100 g in macaques, 20 g in marmosets) and therefore they are less compatible with modern methodologies evolved in small rodents. Other vertebrates such as reptiles and birds have brain organisation that is not directly comparable with that of mammals. The complex cognitive judgements are linked to the complex environments that primates live in including marmosets, which is the main species of choice for this work.

Rats

Because the reaction of the tissue and immune system to the procedures/implants as well as the performance of neurophysiological recording/stimulation system can be assessed only in live animals, it is essential to first validate and refine those experimental techniques in a simpler animal model, before applying them to marmosets. Rats are particularly suited for this purpose because rats weigh 300-400 g, and the length of the skull is 4-5 cm, which are very similar to those of marmosets. Mice weigh only 20-30 g, so mice are too small for the implants that can weigh about 10 g. Therefore, to study marmoset neuronal circuits in the future, the use of rats for technical refinement is essential.

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

Tissue cultures including brain organoids (three-dimensional cell culture model in developmental biology) 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 nor memorise things/concepts whilst artificial computer simulations are biologically unrealistic.

A retrospective assessment of replacement will be due by 4 April 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?

In this project, I will work with marmoset to study cognitive behaviour with non-invasive brain imaging, and with rats to validate and refine neurophysiological experimental techniques towards the future marmoset application.

I am 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.

Marmosets

In deciding on group size, a number of factors are taken into account. My collaborators in the facility have extensive experience of publishing experiments on marmosets in high-impact journals with rigorous statistical peer review. I use the ‘appropriate number (n) of marmosets compatible with adequate statistical power for hypothesis testing, according to the "Reduction" principle from the 3Rs. In previous studies, the precise number of animals used in experimental 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) brain activity, both of which affect the anticipated effect size. Smaller sample sizes (3-6) have been used in the more recent years owing to the refinements in the subject-study allocation, behavioural training and surgical interventions, which have led to enhanced effect sizes. When I embark on a new study, I start off with 2 lead animals in

which I test out the hypothesis, and I look for large obvious effects e.g., behavioural changes and behaviour-related brain activity, which can be assessed 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 the effect sizes are expected to be large because I have strong hypotheses based on previous findings and by having tight within-subject control over experimental variables. Based on recently published and unpublished studies of the collaborators and myself, I have planned for group sizes of between 5-8 in this behavioural study.

Rats

In recent publications about neurophysiological experiments in rodents, majority of studies use approximately 10 animals per experiment with nonparametric statistical tests (that is more reliable but requires larger data size). In this project, rats are used to test several key technical points such as implantation of the recording chamber, semi-chronic implantation of the microelectrodes, recording performance of the microelectrodes, invasiveness of microelectrode, performance of the head-stage, etc. Accordingly, I estimate the necessary number of rats to be 10 for each test, 50 to 60 in total.

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

In this project, efforts are made to reduce the number of marmosets necessary for studying cognitive behaviour with non-invasive brain imaging. Rats will be used to sophisticate experimental techniques, which will reduce the number of marmosets required in the future studies.

Marmosets

i). Using a carefully controlled behavioural testing apparatus so that the particular cognitive abilities of interest can be studied in isolation which helps reduce variation in performance.

ii). Screening to ensure suitability of animal for particular study, thus also minimising variability.

iii). Re-using marmosets from another project held within the same facility 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 re-used, and after the completion of the previous procedure and before the intended re-use, the NVS (Named Veterinary Surgeon) 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 brain imaging, peripheral drug injections, or behavioural tests which have never exceeded the severity of the moderate level.

Rats

iv). Using MRI to target certain brain structures to ensure that the electrophysiological recording is effective in the majority of animals and reduce unnecessary repetition of the insertions of microelectrodes.

v). Using recording chambers and miniature micromanipulators to allow multiple tracks of recording in single animals.

Both species

vi). Using animals as their own internal controls (e.g., the conditions without manipulation, without drug, etc.) wherever possible to increase the power of statistical comparisons, minimise variability, and minimise the number of animals used.

vii). 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.

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 experiments in marmosets to increase the effect size in behavioural and imaging studies, and refine experimental procedures in rats towards future marmoset experiments.

When an animal is killed, organs and tissues other than brain may be shared with other research groups for various study purposes to help reduce the overall number of animals used.

A retrospective assessment of reduction will be due by 4 April 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.

In this project, I will use common marmoset to study visual cognitive behaviour with non-invasive brain imaging. I will also use rats to validate and refine neurophysiological experimental techniques towards the future marmoset application.

Marmosets

Marmosets are a particularly valuable species for our work as their relatively small primate brain makes it possible to target cortical (surface) and subcortical (inside) structures and make regionally

selective measurement and manipulation in the brain with relative ease, with minimal risk to the animal.

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

The behavioural tasks I use are designed to be able to detect neuronal activities most relevant with the cognitive memory judgment under study, maximising the ability to extrapolate findings from our marmoset studies to understand the underlying neuronal circuit basis. To avoid unnecessary stress associated with behavioural testing, my animals are (i) trained to voluntarily get into a carry box in the home cage to go to the test apparatus by appetitive training, (ii) can move freely in the test apparatus, (iii) trained in behavioural tasks by positive reinforcement, and (iv) are tested for less than 40 minutes per session in behavioural tasks.

When a marmoset individual does not maintain high task performance across days in intellectually demanding behavioural tasks, or when a larger number of task trials is necessary for statistical analysis, the access to water in the home cage may need to be restricted to add additional motivation. This water restriction only limits the time that animals have access to water, and the amount of water is not restricted when given. The restriction is introduced only gradually and only as needed through carefully analysing the task performance of individual monkeys. Initially, the water access is restricted only for a few hours immediately before testing. If needed as behavioural analysis suggests, 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.

Rats

Implantation of a recording chamber and use of a miniature micromanipulator attached to the chamber allow for insertion of microelectrodes according to the coordinate within the chamber, which increases the accuracy of targeting specific brain regions when combined with MRI than inserting the microelectrodes according to the stereotaxic coordinates (the coordinate relative to anatomical landmarks on the bone). MRI of the brain with the microelectrodes inserted will provide an accurate relationship between the chamber-based coordinate and the actual microelectrode position in the brain. This MRI-guided electrophysiological mapping of the brain increases the efficiency of localising the relevant brain regions and reduce the necessary number of microelectrode insertions in single animals, thereby reduce the total invasiveness of the experiment.

The use of semi-chronically implanted microelectrodes allows recording from as many neurons as possible in single insertions of the microelectrodes, thereby reducing the numbers of microelectrode insertions and the animals used and is much less invasive overall. Holding the rat for manipulating the microelectrodes for no more than 5-10 minutes allows for changing the depth of microelectrodes and recording from different neuronal populations in single insertions. Holding the animal avoids anaesthesia or the need to use of head fixation which offer no flexibility. All animals will be habituated to the holding procedure and thus it will cause only transient discomfort.

These are key techniques in the future marmoset application.

Both species

Terminal anaesthesia using a sedative followed by sodium pentobarbitone and trans-cardiac perfusion. 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, my collaborators in the facility and I are geared towards optimal refinement, from the choice of animals to the methods, procedures, and skills. Additionally, I make sure that my group maintains high standards and training of staff in order to ensure all our refinements are actually implemented. I review all procedures and skills of the licenced researchers working in my group, under my supervision, regularly and discuss project licence-related matters at each of my weekly group meetings.

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

In this project, I will use common marmosets to study cognitive behaviour unique to primates, and rats to validate and refine neurophysiological experimental techniques towards the future marmoset application.

Marmosets

Marmosets are the least sentient organism with fine object vision and highly evolved prefrontal and temporal association cortices that interact with each other to control the higher-order cognitive memory behaviour.

Rats

Rats are the least sentient organisms with sufficiently large body and head that can be used to validate and refine neurophysiological techniques for future marmoset experiments. Mice are too small for this purpose.

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

In this project, I will study cognitive behaviour by non-invasive brain imaging in marmosets and refine neurophysiological experimental techniques in rats towards the future marmoset application. I have 17 years of experience in neurophysiological experiments in rodents and non-human primates.

Animals will be acclimatised to their home cage, experimental apparatus, and environment before starting experiments. For acclimatisation and pain management, palatable foods such as flavoured jelly, paste or milk shake liquid will be given. Animals' health score and body weight will be regularly recorded to detect and treat any potential harms/suffering as early as possible. Animals will be kept in a pair or group in home cage to maintain their social behaviour, unless temporary isolation is required for treatment purposes e.g., recovery periods.

Marmosets

I am closely collaborating with the groups within the facility which have been performing many of the described procedures for over 30 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. To avoid unnecessary stress associated with behavioural testing, marmosets are (i) trained to voluntarily get into a carry box in the home cage to go to the test apparatus by appetitive training, (ii) can move freely in the test apparatus, (iii) trained in behavioural tasks by positive reinforcement, and (iv) are tested for less than 40 minutes per session in behavioural tasks. Analgesic, anaesthetic and antibiotic regimes have been developed in consultation with the NVS and are under continual review. I receive additional advice on anaesthesia from an experienced specialist veterinary anaesthetist with considerable expertise in primates.

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.

Rats

Rat experiments are performed in order to refine neurophysiological techniques towards the application to marmosets in the future.

The duration of surgeries is shortened where possible by technical refinements in order to minimise post-surgical complications when recovery is required. All surgeries, as well as microelectrode insertions, are performed aseptically. The home cage is enriched with various furniture and three-dimensional structure. I can gain advice from my collaborators and NVSs on best practice to reduce likelihood of post-operative complications and associated stress.

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

My 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 I follow the LASA guiding principles of aseptic surgery (http://www.lasa.co.uk/wp-content/uploads/2017/04/ Aseptic-surgery final.pdf), but I will further these principles wherever possible as part of my constant refinement strategy, especially in the case of intra-jugular catheter implantation procedures. I will receive direct updates on best practice from the N3CRs through their mailing list, and the annual Primate Welfare meeting.

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

I have several lines of information that enable us to stay informed about advances in the 3Rs in order to implement them effectively. First, I have registered to the NC3Rs newsletter. Secondly, as all the project licence holders at my establishment, I receive tremendous support from the staff at the establishment, and I receive regular critical updates from the Named Information Officer to which I pay the utmost attention and that I share with all the members of my group. Third, I regularly hold project licence-related workshops with all the members of my group to discuss the changes in procedures. I 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, I am also part of an international network of marmoset users and rodent users and I regularly have meetings and exchange best practice.

A retrospective assessment of refinement will be due by 4 April 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?