Home Office

NON-TECHNICAL SUMMARY

Biobehavioural basis of vulnerability to impulsive/compulsive spectrum disorders - Optimisation and service protocols

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

No answer provided

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

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

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

What's the aim of this project?

To optimise procedures and produce GA rats for use on other project licences held by the group.

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?

To use the optimisation protocols in pilot studies to optimise procedures and combinations of procedures to ensure they are as refined as possible while retaining scientific validity.

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

The expected outputs will be refinements to procedures and experimental designs and demand matched production of genetically altered rats.

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

The data obtained from the optimisation protocols will be used to inform experiments conducted by members of the research group using the other project licences held by this group. Therefore data produced from the use of these protocols will primarily be used by members of our research group. However where appropriate refinements will be published and shared with the scientific community in accordance with the principles of the 3Rs.

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

Refinements to procedures and experimental design will be published and discussed at national and international meetings.

Species and numbers of animals expected to be used

- Rats: 1300

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 majority of individuals in the world are exposed to stress, or use drugs recreationally, may it be alcohol, tobacco…, sometimes itself as a means to deal with stress, a so-called coping strategy, we are not equally capable of developing adaptive coping strategies or to maintain a recreational, controlled use of drugs. The individual vulnerability to lose control over coping strategies or drug use, which results in Compulsive Disorders such as Obsessive Compulsive Disorder o drug addiction, remains poorly understood. This prevents the development of novel, more effective therapeutic or preventive strategies for these debilitating disorders that affects millions of individuals worldwide. Our research, which relies on two complementary experimental project licences alongside this one, interests itself with the environmental, psychological, neural and cellular factors that contribute to this individual vulnerability to develop Impulsive/compulsive Spectrum Disorders.

This research is only possible with the use of animals because human studies (e.g. brain imaging studies) are useful but can only provide correlative data that do not address causation and short fall of identifying the detailed mechanisms in the brain that support the vulnerability to develop Impulsive/Compulsive Spectrum Disorders. Furthermore, it is not ethically possible to study the genetic and/or environmental factors that underlie predisposition to, and the development of, neuropsychiatric disorders in humans.

Similarly, it would not be possible to develop new treatments for brain disorders without testing them in animal models first. In vitro models (e.g. rat brain slice preparations) or computer simulations cannot be used because the modelling of behaviour in these systems is not sufficiently advanced.

Thus, we use rats because they are the least sentient species which allows researchers to investigate marked inter-individual differences in sophisticated behaviours that capture core features of these neuropsychiatric disorders. Additionally, the brain circuitry implicated in many neuropsychiatric disorders is highly conserved between rats and humans.

In order to uncover the biobehavioural basis of the vulnerability vs resilience to ICSDs across the lifespan, we will carry out investigations, deploying large array of intracerebral measurements and manipulations on juvenile and adult wild-type and genetically modified rats. It is therefore important that such procedures, which are invasive, are optimised prior to being deployed on large experimental cohorts of rats. The purpose of the present project is therefore the breeding and maintenance of genetically modified rats as well as the optimisation of intracerebral techniques and pharmacological manipulations.

The identified need for optimising our procedures stems from our commitment to animal welfare. Indeed, most of our animals run in long-lasting behavioural experiments in which they perform tasks for food or drug reward, and experience procedures, when fully optimised, that produce only transient discomfort and no lasting harm, achieved by a constant refinement of administration techniques by well-trained personnel.

Any adverse effects are discussed with the Named Veterinary Surgeon. If these cannot be quickly ameliorated then animals are killed to prevent suffering.

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

Rats in this project will either be bred and maintained in order to be used in the two other protocols on this licence or on our other licences.

Rats (including GA animals with no adverse phenotype) used for optimisation procedures will be subjected to intracerebral manipulations under terminal anaesthesia, with no recovery or will receive 1 intracranial procedure, and up to three surgeries overall (including the implantation of an indwelling catheter), in order to optimise manipulations or measurements of brain mechanisms while the animal is performing behavioural tasks, including drug self-administration.

Rats (no more than 60%) may be exposed to behavioural procedures aiming at measuring their motivation, impulse control or other cognitive functions, for which they sometimes need to be single housed and/or food deprived.

No more than 20% of the rats may receive administration of substances to induce dependent states. These may also be administered with substances that couteract these dependent states.

No more than 15 rats overall may experience experimentally-induced Parkinson's disease.

The investigation of specific cellular mechanisms requires the decapitation of the rats in a conscious state.

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

For the majority of our animals on this project (75%), we anticipate no more than transient discomfort and no lasting harm.

The genetically modified rats lines that we will breed have no adverse phenotype.

The two optimisation protocols are designed to enable us to minimise the impact of intracerebral procedures in subsequent experiments, by optimising and refining the said procedures first in a non-recovery protocol, and then in behaving rats, always using as few animals as possible (2 to 5).

When rats undergo surgical procedures, then tend to recover very rapidly and they are provided with post-operative care, including the use of analgesics.

When rats are trained to self-administer drugs, some (up to 25%) may develop several behavioural characteristics similar to those presented by human beings suffering from a drug addiction, including lack of interest in other sources of reinforcement and associated weight loss, decrease in self-care (their fur becomes dirtier). A very small number of animals we see them self-harm in the same way that drug addicts do when they are when given extended access to heroin. This is because high levels of heroin intake can cause changes to the way nerves in the face and mouth behave: heroin is an analgesic (i.e. it affects feeling and pain perception). When rats can no longer feel the pain some start to nibble their toe nails and toes can inflict damage to their paws.

When rats have become dependent on a drug, such as heroin, upon induction of withdrawal they display typical signs of physical withdrawal, including wet dog shakes, or piloerection, but as in humans, these signs wear off rapidly (within 24 to 48 hours).

In the case of the induction of Parkinson's Disease, rats tend to present a transient lack of motivation that is associated with the neurodegenerative process of the dopamine neurons, hence they need to be supplemented by highly palatable food or feed (including by oral gavage) until they recover (between 3 and 12 days overall).

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)?

Non-recovery rat protocol – 100%

Mild rat protocol – 100%

Moderate rat protocol – 100%

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

- Used in other projects
- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

It is not yet possible to achieve our objectives without using animals, and, especially, rats. Our research, which investigates the inter-individual differences in the vulnerability to develop impulsive/compulsive spectrum disorders, which result from an alteration of many complex, intricate, brain mechanisms and associated cognitive (understanding and perception) and emotional processes which we do not yet understand, even remotely, enough to be able to contemplate modelling them using algorithms or Artificial Intelligence.

The nature of our research therefore requires we use a species whose brain, cognition and behaviour are similar enough to humans to offer insights into the psychobiological basis of human neuropsychiatric conditions. The rat is by far the best species to establish the neural and neurochemical mechanisms underlying inter-individual differences in behaviour, cognition and neuropsychiatric disorders which cannot be investigated in humans.

The rat is so far the only species in which individuals have been shown to have representation of the relationship between their actions and their consequences, to establish coping strategies, to differ in impulse control and in their propensity to take drugs, even to develop maladaptive habits and compulsivity.

Most of our animals undergo long duration behavioural experiments (which last up to 14 months) in which they perform tasks for food or drug reward, and experience procedures that, provided they are fully optimised, produce only transient discomfort and no lasting harm. It is therefore fundamental to optimise these invasive procedures, and that is precisely the goal of the present project licence.

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

We use alternative strategies some of which involve humans, some are computer-based models and some use Artificial Intelligence. In addition, we use cell culture (i.e. cells grown in a dish in the laboratory) and ex vivo organoids (i.e. cells that grow in a dish and form into organ like structures).

Why were they not suitable?

As our objectives, which will be achieved through the use of other complementary project licences, require complex behavioural tasks allowing for the identification of individual vulnerability to compulsive disorders, we cannot rely on in vitro (non-animal) models because these cannot reproduce the integrative function of the brain that is the focus of this research.

Since these experimental procedures are designed to optimise the intracerebral and pharmacological procedures we need to use in order to achieve our research goals in rats, the purpose of the present project licence, must be carried out in the same species.

Human studies cannot allow for a lifetime longitudinal study addressing causal mechanisms of neuropsychiatric disorders, they yield only correlative data and rely very often (if not exclusively) on retrospective analyses which prevent the identification of factors of vulnerability measured prior to the development of the disease.

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?

1000 rats will be used for breeding and maintenance of genetically modified/selected strains and wild-type controls. This will enable us to use up to 2500 such transgenic animals in the two experimental project licences which, alongside the present one, support our programme of research.

150 rats will be used in each optimisation protocol, with groups of 2 to 5 per optimisation, that will enable us to carry out up to 20-25 optimisations over the next 5 years.

The identification of these numbers is informed by our expertise, activity over the past 10 years, as well as by the systematic design of sample experiments that have been peer-reviewed through applications

for funding.

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

This project licence is designed to enable us to reduce the number of animals being used in subsequent experiments under our other project licences.

Here, for each optimisation strategy, wherever possible, we implement experimental designs that minimise noise in our behavioural or biological measurements and that are always combined with state of the art statistical analyses.

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

We will use efficient breeding and use both male and female offspring in subsequent experiments carried out under the authority of our other project licences. We will rely on computer modelling or in vitro assays prior to optimising procedures in a non-recovery setting. We will eventually rely on very small samples (approx. 2 to 5 animals per group) to further optimise the procedures in behaving rats.

Together the incremental strategy designed for this breeding and optimisation licence will enable us substantially to optimise the number of animals we plan to use in our overall programme of research.

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 we will use several animal models and methods, the combination of which will enable us to identify the factors of vulnerability to develop compulsive disorders across a wide range of conditions and their biological basis in the brain.

Our methods mostly rely on awake, freely moving animals performing complex behavioural tasks for rewards (e.g. food, or drugs of abuse) and subject to invasive recordings or manipulations of the brain while performing these tasks.

We are fully committed to minimise the cumulative severity of all our procedures as much as possible and have been constantly refining all our procedures in order to minimise the distress to which our animals are subjected.

We will use models of inter-differences in impulse control (high impulsivity trait), Obsessive Compulsive Disorder, drug (alcohol, cocaine, heroin….) addiction and Parkinson's Disease.

We have, over the years, refined our models so that we can study the psychological and biological basis of profoundly debilitating and distressing disorders in humans such as OCD or addiction, with minimal adverse consequences in our rats.

In the case of OCD for instance, rats tend to display compulsive behaviours for no longer than 1 hour per day, and these have overall no other negative consequences. To study Obsessive Compulsive Disorder, we primarily rely on a procedure that requires rats be food deprived to 80% of their free feeding body weight. These rats are absolutely fine and display no behavioural or physiological signs of distress.

For drug addiction, we implemented procedures that enable us to measure the compulsive nature of drug seeking and taking that characterises addiction (in other words the drug is used despite disastrous negative consequences for the user and their relatives/carers) without overall harm to the animal. To study drug addiction, rats initially trained to self-administer drugs through an indwelling catheter implanted into their jugular vein. We have refined this procedure so that it lasts no more than 10 minutes and rats recover very rapidly with no signs of distress. All our surgeries are performed under aseptic conditions and rats are given appropriate analgesia prior to, during, and after the surgery.

For Parkinson's Disease, the procedures we use enable the rats quickly to recover from the initial motivational effects of the sudden development of the condition and ensure they do not develop too profound a motor deficit.

We use a wide array of methods in our research. One is the testing of rats in behavioural tasks in which they are given the opportunity to solve puzzles, work (press levers) to obtain food rewards. These tasks, that are designed to investigate specific psychological or behavioural mechanisms, are not regulated, meaning they do not cause any distress or harm to the animal. However, it can be the case that rats have to be slightly food restricted to engage with the task. In that case food is delivered every day following the behavioural session. Food is given in quantities large enough to maintain the animal body weight between 90 and 100% of their free-feeding weight. It is also better for the animal's health as there is strong evidence that as in humans, free-feeding in rats results in decreased longevity.

We will use systemic administration of drugs either through experimenter-delivered injections, or via previously implanted subcutaneous minipumps or slow release formulations (like some forms of pills in humans). In this case, each drug will be prepared with double distilled water or sterile vehicle, in autoclaved glassware and subsequently filtered prior to use. Dosing procedures will be undertaken using a combination of volumes, routes and frequencies that themselves will results in no more than transient discomfort and no lasting harm and is the minimum consistent with the scientific objectives. We have implemented specific methods to deliver intraperitoneal injections to rats without restraining and stressing them and only highly trained researchers perform these injections.

We will use blood sampling procedures. In that case no more than 10% of total blood volume in 24hrs and 15% of total blood volume in any 28-day period will be taken.

We will use methods designed to measure or manipulate the activity of specific brain areas while rats are behaving, and/or expressing OCD, drug addiction, impulsivity. For this, we need to insert probes,

electrodes inside the brain that can be connected to external devices that enable the measurement of brain function. We also rely on pharmacological manipulations of brain functions and for this cannula are lowered into the brain and we use injectors to infuse tiny amounts of drugs that interfere with a specific brain mechanism inside the brain. We will also use the technology of virus-mediated expression of genes in the brain to measure of control brain function. For all these methods, the surgical procedures are all carried out under aseptic techniques and the rats are habituated to be connected to external devices and are always free to move when connected. We use the least invasive methods drawing in fast evolving state of the art techniques and systematically ensure to procedures are optimised and refined (which is the very purpose of this project licence) before using them on larger cohorts.

When rats are food restricted, they often need to be single housed as the dominant would otherwise experience no food restriction and the expense of the subordinate. Similarly, when rats have received intrajugular and/or intracranial implants, they often need to the single housed in order to prevent them from playing with, and damaging each other's implant.

Isolated animals tend to gain more weight than controls; they are more responsive to stimuli predicting reward (in specific behavioural tests) and are hyperactive as measured by specific behavioural tasks. Additionally, we have shown that rats raised in an enriched environment are more vulnerable to develop compulsive disorders.

Overall, we are geared towards optimal refinement, from our choice of animals, to our methods, procedures and skills. First, and foremost, the present licence will enable us to optimise and refine on small groups of rats the intracerebral and pharmacological procedures that will subsequently be used on large experimental groups. The present project licence is therefore testament to our commitment to refinement.

Additionally, I make sure that we maintain our high standards in order to ensure all our refinements are actually implemented. Thus, I review all procedures and skills of the licenced researchers working in my 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?

Rats are the least sentient organism that enable the measure of inter-individual differences in the behaviours of interest with some individual displaying behavioural manifestations that have high heuristic value with regards to the Impulsive/Compulsive Spectrum disorders we are investigating.

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

When rats have to be singly housed for the purpose of protracted self-administration or behavioural training, not only are they handled at least twice a day, during behavioural training, at least 5 days a week (in average), but they are also brought to test boxes several hours per day, at least five days a week.

We have experimental evidence that these housing conditions, as opposed to so-called enriched environments, do actually protect rats from developing disabling compulsive disorders such as addiction to cocaine. Thus, our recent work on environmental enrichment has actually shed new light on the rather anthropomorphic view of the benefit of enriched environments for rodents, as it is likely more difficult to define what is an enriched environment for rats than it is for humans, for whom the definition is very much specific to each individual. Indeed, we have, in a very challenging environment, demonstrated that rats raised in a highly enriched environment are less likely to engage in high levels of cocaine self-administration than rats raised in standard conditions, but instead demonstrated higher vulnerability to switch from controlled cocaine intake to addiction. Thus, of all the 48 rats (24 from a standard condition, 24 from an enriched environment) trained to self-administer cocaine for over 80 days in this experiment, 6 became addicted to cocaine, and they were all from the enriched environment.

For repeated intraperitoneal injections we ensure that the smallest needles and volumes of pH-neutral injections are used and use procedures we have developed and refined, and validated by our Named Veterinary Surgeon, whereby stress is minimised delivering these injections without restraining the animal.

Each of the rats on the optimisation protocols on this licence will undergo neural manipulations (brain surgery) and/or implantation of intravenous catheters for long-term self-administration of drugs of abuse. We take great care to minimise suffering following surgical procedures and minimise the risk of infection and/or catheter damage by using the most elaborate techniques (the present licence is actually designed further to optimise each bespoke intracranial manipulation to further decrease its potential impact on the animals) that have been developed and constantly refined in collaboration with the Named Veterinary Surgeon.

We routinely administer peri-operative analgesia (i.e. pain killers after surgery) and use scoring sheets to monitor animal welfare for a minimum of three days post-surgery. The specific analgesics used varies between different types of experiment and the strategies we have now in place to ensure continuous analgesia through the perioperative period have been designed with the Named Veterinary Surgeon.

We have been investing a lot of time and efforts in refining our skills, techniques, procedures and equipment, and we will continue to do so over the next five years. Over the past years, we have developed and refined, together with our Named Veterinary Surgeon, a scale to enable the daily monitoring (using an electronic reporting system we have also implanted and refined) of the status of the back mounted cannulae ports in rats trained to self-administer drugs. We have also substantially improved our aseptic techniques adopted from the principles of the Laboratory Animal Science Association (LASA) guidelines, to comply with our high throughput requirements.

We have progressively engineered a better catheter for self-administration experiments. Relying on a stepwise, iterative empirical strategy, we have decreased the size of the mesh that is inserted under the skin to secure the port, in order to decrease the surface it occupies under the skin, hence the risk of irritation and physical damage to the surrounding tissue. Having verified, in collaboration with the Named Veterinary Surgeon that this change yielded positive outcomes in terms of damage to the skin around the port, we then increased the rigidity of that mesh to stop it being grabbed and stretched by the rats, which on some occasions resulted in local irritation. Having observed another improvement, we then decreased the diameter of the port cannula (tube) which reduced the overall size of the device.

We predominantly use Sprague Dawley rats, and not the Lister-Hooded strain which were historically used, as the former demonstrate fewer skin lesions, and tolerate the effects of long-term drug exposure better than the latter. The very low incidence (actually the absence) of adverse effects around ports in 200 rats that had the new monitoring procedure illustrates the improvements made by my laboratory and we will endeavour to further the refinement of this, as well as all the other procedures used in my laboratory. All improvements will be disseminated as widely as 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 PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) and the ARRIVE (Animal Research: Reporting In Vivo Experiments) 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 also benefit from the tremendous support provided by UBS to the Cambridge community re updates on best practice from the N3CRs, and I will continue receiving direct updates from the NC3Rs as I have subscribed to their mailing list.

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 me to stay informed about advances in the 3Rs in order to implement them effectively.

First, I have registered to the NC3Rs newsletter and follow them on Twitter.

Secondly, as all the project licence holders at our establishment, I receive tremendous support from the staff at the establishment, and I receive regular critical updates from the Named Information and Compliance Officer to which I pay the utmost attention and that I share with all the members of my lab.

I hold project licence-related workshops at least twice a year with all the members of my laboratory to discuss the changes in procedures.

I have an excellent working relationship with the animal care staff in my animal facility, which facilitates the implementation of advances in the 3Rs.