Home Office

NON-TECHNICAL SUMMARY

Biobehavioural basis of the individual vulnerability to impulsive/compulsive spectrum disorders

Project duration

5 years 0 months

Project purpose

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

Key words

emotion, motivaiton, impulse control, addiction, individual vulnerability

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

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?

The aim of this project is to understand the environmental, psychological and biological factors that contribute to individual vulnerability to develop the compulsive behaviours that represent the hallmark features of Impulsive/Compulsive Spectrum Disorders (ICSDs) such as Obsessive-Compulsive Disorder or drug addiction.

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?

The cost incurred by ICSDs to our society and the burden they put on our health care system reflect mainly the limitations of our current knowledge of both their pathophysiology and aetiology. This limited understanding thereby prevents the development of novel, more effective preventive and therapeutic strategies.

It is therefore of paramount importance to better understand the biobehavioural basis of the individual vulnerability to develop compulsivity.

Thus, why only some individuals exposed to stress, experience distress or take addictive drugs recreationally lose control over their behaviour and go on to develop compulsivity remains a key question to be addressed that goes beyond the understanding of the neurobiological adaptations to distress, chronic stress or drug exposure. Indeed, since we are all subjected to these challenges it is pivotal to dissociate the psychological, neurobiological and environmental mechanisms that support adaptive coping strategies and impulse control from those which, in vulnerable individuals, aberrantly support the switch to compulsivity.

Additionally, this work will shed new light on our understanding of adaptive coping strategies which current and future generations will need to deploy in the face of new challenges, such as social media or globalisation.

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

This research will deliver new knowledge that will enhance our understanding of the biobehavioural basis of the vulnerability to develop Impulsive/Compulsive Spectrum Disorders (ICSDs) and identify new drug targets.

Outputs for all objectives are primarily behavioural and data collected through performance on specific procedures aiming to operationalise in the rat impulsive or compulsive behaviours or associated behavioural traits of vulnerability that have heuristic value with regards to the human situation.

Our understanding of the neural and cellular substrates of these behaviours will come from associated neurophysiological outputs such as neuronal activity patterns and circuit mapping in behaving or anaesthetised rats. Our understanding of the molecular mechanisms of these behaviours, within the identified neural circuits, will stem from investigations of the expression of specific molecules (such as metabolic products, mRNAs, proteins, small molecules acting as neuromodulators) within brain tissue assessed post mortem. Usually, molecular data are generated using the same procedures as for behavioural data, but animals are killed at a specific experimental time point whereas the investigation of neurophysiological and neuropharmacological processes in non-behaving animals is performed under terminal anaesthesia. Thus, for some of our objectives, the brains of the rats will be harvested for subsequent post-mortem analyses (using a wide array of contemporary neuroscience techniques, such as in situ hybridisation, RNAscope, immunohistochemistry, western blot, qPCR, next-generation omics, single-cell transcriptomics...).

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 ICSDs 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 and rapidly to gain international impact and prominence, will only be the first step in a wider dissemination and communication strategy aiming to immediately increase our impact on the general public. Our goal is to help the general public realise that compulsive disorders, and especially addiction, are indeed psychiatric conditions that are not simply the behavioural manifestation of poor willpower, a view that has long contributed to a damaging prejudice towards those who suffer from these conditions.

The second beneficiaries would be the patients themselves as the output of this research will help improve the awareness of ICSDs as brain disorders, and facilitate their prevention and treatment by personalising medicines and other interventions, including at school, in those individuals deemed most at risk from developing maladaptive coping mechanism and/or harmful levels of drug use in the future. Such long-term benefits will depend on the efforts we continually make to provide to inform the general public and clinical policymakers.

We also intend to impact pharmaceutical company policy as they return to medications development for disorders such as addiction. We have therefore recently developed a jointed research project on drug addiction with a pharmaceutical company.

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

We are fully committed to communicating our research as widely as possible to academic and scientific communities. The outcome of our research (may the results be positive or negative) 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, Current Biology, Nature Neuroscience, Neuron, Biological Psychiatry, Neuropsychopharmacology, Journal of Neuroscience, Molecular Psychiatry). We have a strong track record in maximising the output of our work, with the systematic publication of 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 commentaries which are often highly cited and increase the profile of our work and the field in general. This activity greatly increases the research profile of my group and enables the rapid communication of new findings to a wide scientific community.

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

We are fully committed to communicating our research as widely as possible 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. Our goal is to help the general public appreciate that compulsive disorders, and addiction especially, are psychiatric conditions and not simply the behavioural manifestation of a poor willpower, a view that has long contributed to a damaging prejudice towards those who suffer from the condition. Thus, we will rely on publicisation of our work by local and national media groups.

We will continue actively to participate in local and national public engagement events to educate the public about our work, including teachers and students at schools and other educational institutions.

We will also use other means to increase our impact, especially through the powerful vehicle that represents arts.

We are fully committed to communicating our research as widely as possible to clinically active scientists and clinicians. We intend also to have an impact on approaches to treatment. Addiction theory and computational models have also evolved to incorporate notions of inhibitory control, actions and habits rather than earlier assumptions that the key to addiction is to be found exclusively in the dopamine reward pathway, which is instead the point of entry of addictive drugs to exert their rewarding effects.

We will investigate the translational potential of our findings in human subjects as a way to validate our discoveries in rats. The successful demonstration of cross-species convergence would enable targeted interventions to be developed based on candidate neural markers.

Species and numbers of animals expected to be used

- Rats: 9000

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. However, around 10% of those who take drugs eventually lose control over their intake and develop the compulsive relapsing pattern of drug seeking and taking that characterises addiction. In other words, we are not equally vulnerable to developing maladaptive coping strategies and/or drug addiction.

The environmental, psychological and biological factors contributing to this individual vulnerability to lose control over coping strategies or drug use, which results in Compulsive Disorders such as Obsessive Compulsive Disorder or drug addiction, remain poorly understood. This prevents the development of novel, more effective therapeutic or preventive strategies for these debilitating disorders that affect millions of individuals worldwide.

Thus, our research interests itself with the identification of the environmental, psychological, neural and cellular factors that contribute to the individual vulnerability to develop Impulsive/compulsive Spectrum Disorders (ICSDs) across several nosological boundaries.

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 the investigation of 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. The study of the environmental, psychological and biological basis of the vulnerability to develop as debilitating a psychiatric condition as OCD or drug addiction requires the establishment of these conditions in the animal model. However, our experimental approach has been refined so that the overall suffering induced by the development of these compulsive behaviours

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.

For this, we have a dedicated optimisation and maintenance licence the purpose of which is to optimise and refine each of the procedures that will be used on relatively large cohorts of animals under the present one. 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.

We take the welfare of the animals very seriously. Most of our animals are exposed to long-lasting behavioural experiments in which they perform tasks for food or drug reward, and experience procedures, e.g., injections that produce only transient discomfort and no lasting harm, achieved by a constant refinement of administration techniques by well-trained personnel.

Animals are monitored and handled frequently (often undergoing daily testing). In the case of drug self-administration procedures, the daily monitoring of the status of the port area is facilitated by a severity scale developed in collaboration with previous Named Veterinary Surgeons that is used as a visual reference by all members of staff to monitor the animals.

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

We are fully committed to using the minimum number of animals required to obtain data that are statistically and biologically meaningful. We carefully design our experiments to maximise the behavioural data collected from each animal, and to minimise distress. Each of our experiments is designed on the back of a robust statistical approach that relies on state-of-the-art statistical tests and models.

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

Rats used in this project (including GA animals with no adverse phenotype) will be exposed to several sets of behavioural and experimental procedures the cumulative severity of which never extends beyond moderate severity.

The various procedures rats will be exposed to aim at measuring their individual performance in tasks that operationalise, for instance, attention, behavioural flexibility, impulse control, anxiety trait, decision making, boredom susceptibility, interest in appetitive outcomes and sensitivity to negative ones (such as mild electric foot shocks) as well as goal-directed or habitual instrumental responding. Testing in these tasks, as well as coping-related tasks sometimes requires rats to be exposed to food restriction and single housing conditions for the entire duration of an experiment (up to 12 months).

Rats (no more than 30%) will be exposed to procedures that enable the assessment of adaptive, or maladaptive (compulsive) coping strategies in the face of distress; these procedures require that rats are food-restricted (between 80% and 85% of their theoretical free-feeding weight) and singly-housed for a period of at least 15 days and up to 12 months overall.

Our research also focuses on the contribution of interoceptive mechanisms to the vulnerability to develop ICSDs. For this we use a drug discrimination task that requires systemic administrations of a drug over multiple daily sessions. Thus, no more than 40% of the rats may receive multiple systemic

administrations of substances that trigger specific interoceptive states or dependence. These may also be administered with substances that counteract these dependent states.

In the case of drug self-administration, rats (no more than 50% overall) are given the opportunity freely instrumentally to respond for a bolus of cocaine, heroin or other addictive drugs delivered directly into their bloodstream through an indwelling catheter connected to a syringe. Most rats maintain control over their drug intake, as humans do, but some, those which underlying psychology and neurobiology we seek to understand, eventually develop drug seeking habits that persist despite adverse consequences (such as exposure to response-produced, hence escapable, mild electric foot shocks), the hallmark of the compulsive nature of addiction in humans. These shocks are always avoidable and their maximum intensity kept very low. There is no alternative to such procedure to measure the persistence of a behaviour despite negative consequences that characterises compulsive disorders.

Rats (no more than 40% overall) may be exposed to several stressors such as maternal separation, social defeat and/or mild unpredictable stress to measure the influence of stressful events on their subsequent resilience or vulnerability to develop impulsive/compulsive spectrum disorders (ICSDs).

Alone, or in conjunction with any combination of the previously described procedures rats (up to 50%) may be subjected to intracerebral manipulations either while behaving or under terminal anaesthesia. Thus, they will receive up to 2 intracranial procedures, prior to any additional procedure performed under terminal anaesthesia.

Rats lay also be implanted with subcutaneous minipumps or slow-release formulations in order chronically to influence a central or peripheral system and/or trigger dependence. Half the animals that will have developed dependence will then experience precipitated withdrawal.

The investigation of endophenotypes of vulnerability requires blood samples to be collected from peripheral vessels from the animals (no more than 30% overall). No more than 10% of total blood volume will be collected in any 24hr period and not more than 15% in any 28-day period.

Rats (no more than 10% overall) may experience experimentally-induced Parkinson's disease because the study of the individual vulnerability to develop Impulse control disorders in Parkinson’s Disease requires a Parkinson’s Disease state is induced in behaving rats.

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.

When rats undergo surgical procedures, they 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 toenails and toes can inflict damage to their paws. While there is no alternative to these side effects of chronic exposure to heroin, these adverse effects are minimized by early end points.

The study of the biobehavioural basis of drug addiction that intends to have a real impact on the life of the millions of individuals who suffer from this debilitating psychiatric disorder requires the identification of compulsive, maladaptive behaviours in a subset of animals exposed to the drug for long periods of time. As a matter of fact, over the past two decades, all the behavioural, psychological and biological mechanisms that have been identified in the rat to underlie this vulnerability to develop compulsive drug seeking and taking using our methodology have been subsequently demonstrated I humans, thereby demonstrating the clear translational value of our multidimensional and heuristic approach.

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). There is no alternative to the induction of dependence and withdrawal to study the cellular and molecular basis of these phenomena and their contribution to compulsive behaviour.

When rats are encouraged to develop coping strategies in the face of distress, some (no more than 30%) lose control over their coping strategy and progressively develop behavioural manifestations that are similar to those shown by individuals suffering from Obsessive/Compulsive Disorder. Thus, these rats show excessive ritualised behaviours such as grooming and adjunctive drinking behaviour. These behavioural are not harmful to the animal.

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)?

Protocol 1: Moderate, 100%

Protocol 2: Moderate, 100%

Protocol 3: Moderate, 100%

Protocol 4: Moderate, 100%

Protocol 5: Moderate, 100%

Protocol 6: Moderate, 100%

Protocol 7: Moderate, 100%

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

- 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 investigates the individual vulnerability to develop impulsive/compulsive spectrum disorders, which results 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 procedures carried out under this PPL will have systematically be optimised, including using our optimisation 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. For instance, having shown in rats that a high impulsivity trait predicts and exacerbated vulnerability to develop early-onset Parkinson's Disease, we have established sibling studies combined with brain imaging analyses in human populations to further investigate the biological and environmental basis of such vulnerability. We have shown that the siblings of early-onset PD patients were more impulsive than those of late-onset PD patients, thereby confirming in humans the striking observation originally made in rats. Similarly, having shown in rats that the development of maladaptive habits results in an exacerbated vulnerability to relapse mediated by negative urgency, we have designed longitudinal studies in humans combined with EEGs to further the neural correlates of the influence of negative urgency on craving and relapse. 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) in order to identify responses to drug exposure at the cellular level that do not require a behaving organism.

Why were they not suitable?

As our objectives, which will be achieved through the use of a complementary project licence, 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 across the lifespan that is the focus of this research.

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?

The number of animals required for these experiments is based upon the historical usage of animals in our laboratory. Overall, with a requirement of 48 to 96 rats per experiment, we have used ~6000 rats in the last five years in spite of the COVID pandemic. In the next five years we will therefore use a maximum number of 6400 rats. These numbers reflect the absolute maximum range of the estimated numbers for the next five years.

Provided our research is mostly based on inter-individual differences, when the distribution of the population allows for an objective criterion to select specific groups such as a bimodal distribution, the

group size is dependent upon the distribution. In the other cases, the groups used to perform between-subject analyses will be selected in the upper and lower quartiles of the population.

In order to maximise the differences between the experimental groups, we will, therefore, carry out experiments with cohorts of 24 n animals, with 1<n<4 for each experiment. We do not foresee the use of cohorts of more than 48 rats for any of our experiments.

Our group sizes (n= 5-12) are based both on power statistics and reference to the established scientific literature that are necessary to obtain statistically and biologically meaningful data with an initial target of alpha = 0.01. By applying this strategy, we are very confident that results will be statistically relevant even if, especially with protracted self-administration experiments, we have to kill animals during the experiment.

For experiments for which we can make no predictions about the size of the behavioural effect a manipulation can cause or how a behavioural difference relates in terms of neurophysiological or cellular processes, these experiments are conducted over several runs, and the early runs are used to calculate effect sizes and to perform power analysis to inform the final group sizes.

If we typically anticipate n = 5-12 animals per group, our between-subject design analyses will always be computed along with dimensional (correlation, regressions, factorial regressions, PCA, cluster analyses) analyses in order to make the maximum use of each individual within a design aiming at emphasising inter-individual differences.

We have recently implemented a new strategy whereby each study that requires a final cohort of 48 rats is often (wherever appropriate) ran in two independent experiments. Thus, not only do we ensure internal reproducibility of the effects observed between the two runs, but provided we reach a statistically and biologically meaningful result at the end fo the first experiment we are in a position not to run the second experiment and spare half the number of animals initially planned.

Wherever possible, we use within-subjects experimental designs (e.g., testing the effects of different drug doses on a specific ongoing behaviour in a Latin-square design) as this increases statistical power and reduces the numbers of animals required.

Where between-subjects comparisons have to be made (e.g., where each animal’s behaviour can only be manipulated once without compromising the interpretation of subsequent data) there are a minimum of two experimental groups (one control group and one experimental group), and sometimes additional controls (e.g., if we need to demonstrate that a drug given systemically is acting in the brain to influence behaviour, then a similar drug that does not cross the blood-brain barrier may be given to another control group). If the vehicle for the peripherally-acting drug and the centrally-acting drug differ, then another vehicle group may be included for the peripherally-acting drug).

For all experiments, we use the minimum number of control groups that we can in order for the experimental data to be interpretable. Nevertheless, we will make sure our group size is big enough to start with in order to avoid having to replicate an experiment in which only specific individuals (i.e., upper and lower quartile) are taken into considerations for between-subject analyses, which would run counter to the principles of the 3R’s. We are indeed 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?

We are committed to, and have succeeded in, using the fewest animals required for statistically and biologically meaningful results. Recent publications in high impact journals, the data of which were acquired under the current PPL, report highly novel and impactful results with relatively low sample, but big effect (e.g., partial eta squared values) sizes.

Our approach, based on inter-individual differences, inherently makes uses, and capitalizes on individual variability, rather than trying to control for it, alongside a highly standardized behavioural neuroscience methodology that dramatically minimizes external sources of variability that contribute to decreasing the power of our experiments.

We will continue to design our experiments using the minimum number of animals deploying a range of strategies that we have implemented over the years and were previously validated by a biostatistician. The key principles on which our statistical design relies are:

1. Aiming for an overall statistical level of significance of $p < 0.01$ and large effect size (partial eta squared values (>0.14) which we report systematically in our publications) between individual groups, i.e., animals showing vulnerability, and those showing resilience to, neuropsychiatric disorders, generally selected in the upper and lower quartiles of the population or between experimental conditions, i.e., chemogenetic inhibition of a specific neuronal ensemble vs non inhibited control.

2. Combining between (or within when possible) subject design with dimensional analyses in order to strengthen the power of the overall conclusions. We have been very successful in applying various descriptive analyses including cluster analyses, factorial analysis or Principal Component Analysis with either simple, multiple or factorial regression analyses to draw firm conclusions from relatively small samples.

3. Optimising animal use through conducting ‘pilot’ experiments under our maintenance and optimisation licence to verify that brain manipulations or biological measurement are producing the intended effects at a neural level, and to calculate effect sizes (and statistical power) to identify how many animals are required for analysis.

4. Using within-subjects designs whenever possible, as these have greater statistical power than between-subjects designs and require fewer animals.

5. Blinding the experimenter to the behavioural data for the post-mortem histological verifications or interrogations of the neural, cellular and molecular substrates or correlates of the psychological and behavioural mechanisms under investigation.

Over the past year, we have developed new machine-learning-assisted classifiers to characterise rats that are resilient to, or at risk of developing addiction irrespective of the cohort to which they belong. This new tool (publication under preparation) will be deployed in all our experiments to further reduce, wherever possible, the size of the cohorts we initially train to study identify those individuals that eventually develop ICSDs.

We have much expertise in experimental design and statistics, ranging from parametric to non-parametric analyses, sophisticated analyses of variance and covariance, and associated post-hoc tests, dimensional analyses, such as multiple and factorial regressions, as well as data mining such as k-means clustering, principal component analysis and distribution fitting. However, if further advice is needed, we will consult statisticians.

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

We have implemented a standardized approach of training cohorts of 48 rats at the same time, allowing us to obtain at least 5 to 10 rats (depending on the nature of the compulsive behaviour under investigation) with extreme behaviours from a cohort with a very similar history, may it be related to handling, behavioural training, housing conditions or surgery and peri-operative care.

To this aim, we have developed highly standardized procedures and high throughput surgical techniques under aseptic conditions. The latter is also very important for ensuring that catheter/cannulae/probes life expectancy of the animals undergoing surgery first is not reduced by the number of days of surgery spent on subsequent animals.

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 are subject to invasive recordings or manipulations of the brain while performing these tasks.

We are fully committed to minimising 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 to 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. Alternatives, such as genetically engineered SAPAP mouse, display profound adverse phenotypes such as excessive self-grooming-induced skin lesions.

When rats are food restricted, they often need to be singly 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. Indeed, we have 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, they 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. In addition, we demonstrated that the exacerbation of the vulnerability to develop compulsive drug use in rats by environmental enrichment generalised to alcohol since rats raised in an enriched environment are more likely than rats raised in a standard environment to relapse to alcohol drinking after abstinence and to compulsively do so, in that they would persist in drinking alcohol at relapse even if it is adulterated by the bitter tastant quinine.

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. There are no alternatives to the use of these procedures, but our constant refinement has helped us achieve an attrition rate of almost 0% by 80 days of daily training while the average worldwide is over 5% by two weeks, with only two other laboratories in the world reporting chronic self-administration experiments lasting more than 6 weeks, but with an attrition rate of over 15%.

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 result 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.

In order to determine the role of stress during neurodevelopment and or adolescence/adulthood, rats will be exposed to maternal separation stress and/or one of two types of stress during adolescence and/or adulthood. Our stress procedures have all been refined and are far superior to alternatives that, for most of them, require exposure to inescapable shocks, restraint and/or forced swimming.

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 a 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?

Any new procedure, or combination of procedures, used under this project licence will systematically be previously optimised using my dedicated optimisation licence.

All our current procedures are constantly being refined.

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 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. Similarly, we have shown that environmental enrichment exacerbates the vulnerability to relapse to alcohol drinking and to persist in doing so despite the adulteration of acohol by a bitter tastant such as quinine.

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 unique procedures undertaken under this licence will have previously been optimised and refined, either as part of our ongoing refinement strategy or using my optimisation licence. Thus, rats on this licence undergo neural manipulations (brain surgery) and/or implantation of intravenous catheters for long-term self-administration of drugs of abuse that will have been systematically optimised. Indeed, 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 effort 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 was 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 receive direct updates on best practice from the N3CRs 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.