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NON-TECHNICAL SUMMARY

Individual differences in emotion regulation and the associated vulnerability to develop compulsion - Breeding and protocol optimisation

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

Coping, Compulsion, Individual vulnerability, Addiction, anxiety

Animal types	Life stages
Rats	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult

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 genetically engineered/altered (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?

The ability to regulate negative emotional states and cope with stress is paramount for resilience and wellbeing. Some individuals fail to maintain control over adaptive coping responses and develop compulsive displacement behaviours, while others resort to maladaptive strategies such as alcohol or drug use. These individuals are particularly vulnerable to developing Obsessive Compulsive Disorder (OCD), Alcohol or Substance Use Disorder (AUD/SUD), respectively. These disorders, which share some neurobiological mechanisms, affect ~8-10% of the world population. They destroy or even claim the lives of millions while incurring massive costs to our societies (over £ 30 billion in the UK alone), and their incidence has massively increased during the COVID-19 pandemic.

My research programme aims to uncover the biological basis of the individual vulnerability to engage in maladaptive coping behaviours and the ensuing development of compulsive disorders. Such research involves investigating the brain mechanisms of coping (both adaptive and maladaptive/compulsive) in large cohorts of rats. It is of paramount importance to optimise procedures and combinations of procedures in pilot studies to ensure they are as refined as possible while retaining scientific validity when deployed on large cohorts.

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 engineered 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 my research group using our other project licences. Therefore, data produced from the

use of these protocols will primarily be used by members of my research group. However, where appropriate, refinements will be published and shared with the scientific community in accordance with the principles of the 3Rs. We will also systematically consider publishing unsuccessful approaches on open-access publishing platforms such as F1000 Research.

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 and/or published on open-access publishing platforms such as F1000 Research.

Species and numbers of animals expected to be used

- Rats: 2100

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 and engage in coping strategies, such as, for some the use of drugs, may it be alcohol, tobacco, cocaine, heroin, fentanyl… Indeed, 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 or Alcohol/Substance use disorder (drug addiction), remains poorly understood. This prevents the development of novel, more effective therapeutic or preventive strategies for these debilitating disorders that affect millions of individuals worldwide. Our research, which relies on this optimisation & maintenance licence and a larger, experimental one, aims to understand the brain mechanisms that underlie the individual tendency to engage in maladaptive coping responses and develop Impulsive/Compulsive Spectrum Disorders (ICSDs).

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 fall short in identifying the detailed mechanisms in the brain that contribute to emotion regulation, coping (be it adaptive or not) and/or compulsion. 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 first testing them in animal models. In vitro models (e.g. rat brain slice preparations) or computer simulations

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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 that allows researchers to investigate marked individual differences in sophisticated behaviours that capture core features of these processes (emotion regulation, coping) and neuropsychiatric disorders. Additionally, the brain circuitry implicated in many neuropsychiatric disorders is highly conserved between rats and humans.

To uncover the biobehavioral basis of individual differences in coping ability and the associated vulnerability versus resilience to ICSDS across the lifespan, we will conduct investigations by deploying a large array of intracerebral measurements and manipulations on juvenile and adult wild-type and genetically engineered 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 to breed and maintain genetically modified rats as well as to optimise intracerebral techniques and pharmacological manipulations.

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 those of the other experimental licence that I hold.

Most of our rats are trained in long-lasting behavioural experiments in which they perform tasks for food or drug reward, and experience various experimental procedures such as the administration of drugs. When fully optimised, these procedures produce only transient discomfort and no lasting harm, achieved by a constant refinement of administration techniques by well-trained personnel. Thus, we constantly strive to optimise and refine our procedures.

Rats (including genetically engineered 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 intravenous or oral drug self-administration.

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

No more than 20% of the rats may be administered substances to induce dependent states. Substances that counteract these dependent states or manipulate peripheral or central systems involved in emotion regulation, coping, or adaptive vs. maladaptive motivated behaviours may also be administered.

No more than 20% of the rats with a history of drug self-administration may be exposed to escapable mild electric footshocks, which are used to assess the compulsive nature of the behaviour of these animals.

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The investigation of specific state-dependent or time-sensitive cellular mechanisms (such as intracellular phosphorylation cascades or receptor internalisation) requires decapitating the rats in a conscious state. No more than 20% of the rats will be exposed to this procedure, which our lab has refined over the years so that it is quick, non-painful and non-stressful to the animal. The rest of the animals will be decapitated under deep anaesthesia, followed or not by perfusion.

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

For the majority of our animals on this project (70%), we anticipate no more than transient discomfort and no lasting harm.

The genetically engineered rat lines that we will breed have no adverse phenotype.

The optimisation protocols are designed to enable us to minimise the impact of intracerebral procedures in subsequent experiments by optimising and refining these procedures first in a non-recovery protocol, and then in behaving rats, always using as few animals as possible (2 to 6 per sex).

When rats undergo surgical procedures, they tend to recover very rapidly and 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 a lack of interest in other sources of reinforcement and associated weight loss, and a decrease in self-care (their fur becomes dirtier). A very small number of animals given extended access to heroin self-harm in the same way that drug addicts do. 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, and/or can inflict damage to their paws.

When the compulsive nature of the drug- taking or seeking behaviour displayed by rats is assessed, rats are challenged to receive mild electric footshocks to assess if they persist in their drug-seeking/taking behaviour in the face of negative consequences. These shocks are mild and ALWAYS avoidable.

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).

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mild: 70%

Moderate: 30%

What will happen to animals used in this project?

- Killed
- Used in other projects

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 brain mechanisms underlying individual differences in coping abilities and the ensuing vulnerability to develop impulsive/compulsive spectrum disorders. This individual vulnerability is considered to stem 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 us to use a species whose brain, cognition and behaviour are similar enough to humans to offer insights into the psychobiological basis of emotion regulation, coping and neuropsychiatric conditions expressed by humans. The rat is by far the best species for establishing 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 enabling the study of large cohorts 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 compulsion.

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?

Whenever possible, we use alternative strategies, some of which involve humans, some are computer-based models, and some use Artificial Intelligence. In addition, we utilise cell culture (i.e., cells grown in a laboratory dish) and are poised to use ex vivo organoids (i.e., cells that grow in a dish and form 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 develop maladaptive/compulsive coping responses and associated ICSDs, 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 the causal mechanisms of emotion regulation and coping (be it adaptive or not) and their associated 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?

A total of 1,500 rats will be used for breeding and maintenance of genetically modified and selected strains, as well as wild-type controls. These animals will be mainly used in the experimental project licence, which, alongside the existing one, supports our research programme.

Up to 300 of these animals will be used in the present licence, with groups of 2 to 6 individuals of each sex per optimisation. 300 additional rats will be purchased to carry out the optimisation of procedures that do not require genetically engineered animals. This will enable us to carry out up to 100 optimisations over the next 5 years. The identification of these numbers is informed by our expertise and activity over the past 10 years, as well as by the systematic design of sample experiments that have been peer-reviewed through funding applications.

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 as for our larger experimental designs (enabled by our main experimental licence) we follow teh PREPARE guidelines: Planning Research and Experimental Procedures on Animals: Recommendations for Excellence. Thus, wherever possible, we implement experimental designs that minimise noise in our behavioural or biological measurements combined

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with state-of-the-art statistical analyses. The present licence will enable us to identify the ideal experimental conditions to achieve this goal on large cohorts used in our programme of research.

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 licence. 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 6 animals per sex per group) to further optimise the procedures in behaving rats.

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

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 of adaptive, maladaptive coping and their compulsive manifestations together with several experimental procedures, the combination of which will enable us to identify the environmental, psychological and biological factors contributing to the individual tendency to engage in maladaptive coping responses and the associated vulnerability to develop compulsive disorders such as Obsessive/compulsive disorder (OCD), compulsive eating or substance /alcohol use disorders (SUD/AUD).

Our methods primarily rely on awake, freely moving animals performing complex behavioural tasks for rewards (e.g., standard or high-fat/high-sugar content food, or drugs of abuse) and being subjected 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 them to minimise the distress to which our animals are subjected.

We will use models of individual differences in emotion regulation, coping responses, impulse control (high impulsivity trait), OCD and food or drug (alcohol, cocaine, heroin….) addiction.

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.

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In the case of OCD, for instance, the majority of rats tend to display compulsive behaviours for no longer than 1 hour per day, and these have no other negative consequences. To study OCD, we primarily rely on a procedure that requires rats to be food deprived to 80% of their free feeding body weight. We have over ten years of experimental evidence that 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 additional harm to the animal. To study addiction to stimulant drugs (cocaine or amphetamine) or opioids (heroin, fentanyl), rats are initially trained to self-administer drugs through a device, called a catheter, implanted into their jugular vein. We have refined the catheter implantation surgery 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.

We employ a diverse range 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, pull chains, turn wheels, or perform nose pokes) to obtain food and/or liquid (sweet solution) rewards. These tasks, which 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 is possible that rats may need 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's 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. Rats may also be trained to self-administer drugs intravenously (directly into their blood through a catheter implanted into their jugular vein). In order to establish the compulsive nature of their drug self-administration, they can be challenged with response-produced escapable mild electric foot shocks. These are always escapable, in the sense that the animals only need to refrain from responding for the drug to avoid them. Those that do not do so are deemed compulsive.

We will use systemic administration of drugs either through experimenter-delivered injections, or via previously implanted subcutaneous minipumps or slow release formulations (minipumps or pellets placed under the skin like some forms of pills in humans). In this case, each drug will be prepared in 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 are the minimum consistent with the scientific objectives. We have implemented specific methods to deliver intraperitoneal injections (injections into the abdomen, a standard administration route in rodents) to rats without restraining or stressing them. These injections are performed only by highly trained researchers.

We will use blood sampling procedures. In that case, no more than 10% of the total blood volume in 24 hours and 15% of the 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 adaptive coping, anxiety, impulsivity, OCD, and /or food or drug addiction. For this, we need to insert probes, or electrodes, inside the brain that can be connected to

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external devices to enable the measurement of brain function. We also rely on pharmacological manipulations of brain functions, and for this, cannulae are lowered into the brain, and we use injectors to infuse tiny amounts of drugs that interfere with a specific brain mechanism.

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 on fast-evolving state-of-the-art techniques, and systematically ensure that 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 at the expense of the subordinate. Similarly, when rats have received intrajugular and/or intracranial implants, they often need to be single-housed to prevent them from playing with and damaging each other’s implants.

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 engaging in maladaptive coping responses and developing compulsive disorders.

Overall, we are committed to optimal refinement, from our selection 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 ensure that we maintain our high standards to ensure that all our refinements are actually implemented. Thus, I review all procedures and skills of the licensed researchers working in my laboratory under my supervision regularly and discuss project license-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 enables the measurement of individual differences in the behaviours of interest, with some individuals displaying behavioural manifestations that have high heuristic value with regard to emotion regulation, coping responses and 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.

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We have experimental evidence that the housing conditions we use, as opposed to so-called enriched environments, do actually protect rats from developing disabling compulsive disorders such as addiction to alcohol or 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 alcohol or cocaine self-administration than rats raised in standard conditions, but instead demonstrate higher vulnerability to switch from controlled alcohol or 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 or drink alcohol for several months, those that displayed behavioural features of addiction came from the enriched environment.

For repeated intraperitoneal injections (injections into the abdonimal cavity, a standard route of systemic administration in rodents), we ensure that the smallest needles and volumes of pH-neutral injections are used and we 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 perioperative analgesia (i.e., painkillers after surgery) and use scoring sheets to monitor animal welfare for a minimum of three days post-surgery. The specific analgesics used vary between different types of experiments, and the strategies we have 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. We have progressively engineered a better catheter for self-administration experiments, accommodating the size differences related to sex. We predominantly use Sprague Dawley rats, and not the Lister-Hooded strain, which was historically used, as the former demonstrates fewer skin lesions and tolerates the effects of long-term drug exposure better than the latter. The very low incidence (actually the absence) of adverse effects around ports in 800 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 we use. 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 (https://lasa.co.uk/wp-content/uploads/2018/05/Aseptic-Surgery.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 the professional staff of our institution regarding updates on best practices from the N3CRS, and I will continue to receive 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 sources of information that enable me to stay informed about advances in the 3Rs and implement them effectively.

First, I have registered for the NC3RS newsletter and follow them on Twitter.

Secondly, like all the project licence holders at our establishment, I receive tremendous support from the staff. I also receive regular critical updates from the Named Information Officers, in addition to access to the 3Rs enquiry system and mailing list offered by my institution, which I pay close attention to and share with all members of my lab.

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

I have an excellent working relationship with the animal care staff, the Named Veterinary Surgeon and the Named Animal Care and Welfare Officer of my animal facility, which facilitates the implementation of advances in the 3Rs.

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