Home Office

NON-TECHNICAL SUMMARY

Brain network changes in a rodent model of schizophrenia

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

Memory, Animal behaviour, Electrophysiology, Schizophrenia, Psychedelics

Animal types	Life stages
Mice	adult
Rats	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 identify how the activity of brain networks and cognition are altered in a well-established rodent model of schizophrenia. The work will determine the ability of both standard and novel drugs to restore altered brain activity and behaviour to normal levels.

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?

Schizophrenia is a devastating brain disease that affects approximately 24 million people worldwide. It is frequently associated with significant personal distress and impairments in personal, family, social, educational, occupational and other important areas of life. People with schizophrenia are 2 to 3 times more likely to die early than others in the general population (10 years on average) due to suicide and/or physical illnesses that include cardiovascular, metabolic and infectious diseases. Despite the acknowledged devastating effect of schizophrenia on so many sufferers, their families and other care-givers, the current range of drug treatments cannot improve all aspects of the disease and the severe side-effects of available drugs can lead to patients giving up their treatment. **In particular, current therapies for schizophrenia fail to improve patient symptoms such as memory, attention and decision-making deficits.** These cognitive problems (symptoms that impact how we think, feel and act) are reported by patients as having the biggest negative impact on their quality of life. Indeed, these problems make it hard for sufferers to firstly find and then secondly hold down a job, further impacting their ability to re-join and make a positive contribution to society. Thus, there is a pressing need for research efforts to find new treatments for schizophrenia that target these devastating cognitive symptoms.

Our knowledge regarding changes in the brain that give rise to the symptoms of schizophrenia is still extremely limited, both from studies in patients and animal models. This project will address this gap by using a well-established rodent model of schizophrenia to reveal for the first time the activity changes in the brain that produce cognitive deficits. We will then use this knowledge to determine again for the first time the extent to which we can restore normal brain function with both commonly prescribed medications for schizophrenia and novel therapeutic strategies.

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

Outputs from this project will primarily be additions to academic knowledge. These outputs will be communicated as new information via: (a) poster and oral presentations at national and international

scientific conferences; (b) publication of results in academic scientific Journals that are, wherever possible, open access to the public. Overall, our hope is that the project will greatly expand our knowledge regarding disease-relevant changes in brain activity that underpin schizophrenia and how new interventions may normalise such activity.

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

Short-term benefits will be the ongoing publication of new insights into how changes in brain activity underlie cognitive impairments in our model of schizophrenia. By the end of the project, we hope to have quantified how these deficits can be rectified by novel drug treatments such as psychedelics. Longer-term benefits will be to validate further our disease model of schizophrenia such that it can play an even more important role in new drug development. This will be underpinned by the project's novel identification of abnormal brain activity patterns as a new target that can be added to our existing disease-relevant anatomical and behavioural markers of schizophrenia. These we believe will be extremely attractive to pharmaceutical companies with an interest in developing new treatments for schizophrenia.

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

Our work fits within the wider research effort into treating schizophrenia. This will naturally encourage collaboration between our lab and others; for example, we can take tissue from our functional experiments and run further complementary analyses to correlate our findings with, for example, established changes in brain anatomy and neuronal markers of disease. It is important to disseminate the knowledge gained from our work irrespective of whether our experimental outputs support or refute our initial experimental hypotheses. So, we will seek to publish all completed experiments, whether the approaches were successful or not, as reporting 'negative' findings to the field is as important as communicating 'positive' outcomes. To maximise the relevance of our laboratory animal work to patients in the clinic we will perform studies in both female and male animals. Our chosen disease model can be created in both male and female animals; however, it has been investigated and defined most thoroughly in female animals. Our strategy will, therefore, be to obtain new information first in females and then repeat those studies in males.

Species and numbers of animals expected to be used

- Mice: 2000
- Rats: 1000

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.

We choose to use rodents as many aspects of their behaviour and brain anatomy are fundamentally similar to humans. Our pre-existing knowledge of these aspects in rats and mice are also far greater than for any other species, including primates. Thus, rodents provide useful models of normal and, with suitable alteration, abnormal human brain and behaviour. We induce our rodent model of schizophrenia in adult animals and this reflects very well the specific symptoms and biological markers for the human condition. Whilst it is possible to induce similar changes in pre-term rodents, this can result in a less specific model for schizophrenia, rather, producing a more general model of abnormal brain development that reflects symptoms across many different diseases. Therefore, we feel our adult model provides a more specific representation of schizophrenia.

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

An animal will typically experience the following:

1. Schizophrenia model induction will be accomplished by injection of a drug (phencyclidine), typically by dosing every day for up to 10 days; control animals will be dosed similarly but receive only saline. We will then allow 7 days for phencyclidine to clear out from the animals' circulation. Behavioural testing will then test for the presence of a characteristic memory deficit in model animals.

2. Either:

(a) behavioural testing to probe multiple types of memory and decision making. Drugs may be used (typically by injection or oral dosing) to determine their capacity to modify or restore behavioural deficits; or

(b) implantation of recording and stimulating devices into target areas of the brain under anaesthesia These will measure functional brain activity/connectivity during the performance of behavioural tasks. Drugs may be used (typically by injection or oral dosing) to determine their capacity to modify or restore behaviour and functional brain activity; or

(c) we will test for the benefits of non-pharmacological ‘treatment’ by allowing animals to exercise, typically by running in wheels. Similar aerobic exercise is beneficial in human patients, so has the potential to reduce or replace their need for drugs, but we do not know how aerobic exercise exerts these improvements in brain function and behaviour.

Experiments will typically take up to 3 months but some will take up to 10 months in order to determine how long behavioural and brain activity changes persist. Some of our behavioural tasks also take weeks to months for animals to learn. We will in parallel also test for positive response to drugs over this extended period.

Psychedelic drugs. A particular treatment focus in this project will be on psychedelic drugs. These compounds have been shown in recent human clinical studies to be highly effective for a range of cognitive and neuropsychological disorders (such as major depressive disorder) that are difficult or impossible to treat in many people with currently prescribed drugs. Whilst psychedelic drugs produce positive changes in the behaviour of patients, often after only a single treatment, we have little understanding of how they change brain activity to produce this behavioural improvement. Thus, we will test psychedelics as a priority in our project to expand our knowledge of their mechanism of action in the brain and their ability to treat cognitive symptoms in our animal model for schizophrenia. To

determine whether psychedelics out-perform current antipsychotics we will compare their effectiveness against existing 'best available' human therapies. Permission to use restricted substances: Psychedelics and phencyclidine are controlled drugs in the UK and their use falls within the Misuse of Drugs Act (1971). In order to use these drugs in this project we have obtained all appropriate licensing and permission from the UK Home Office for that use in our research.

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

Our model induction process using phencyclidine does impact on the behaviour of the animal for a short time after every dose. Thus, animals can appear slightly confused and unaware of their surroundings. However, this passes quickly (less than 1 hour) and the duration and intensity of these drug effects reduces quickly after each dose as the animal develops tolerance. Once established, model animals are virtually identical to controls in terms of their general behaviour - it is only when given cognitive tests or drugs that differences can be seen in model animals.

Some behavioural tests will require food restriction of the animal. This is because in these tests we use food to encourage behaviour during training and testing - so animals receive food for correct performance during the task. Animals are expected to lose some weight during food restriction periods and this will be monitored very closely. If their weight loss approaches 15% compared to free-feeding animals, we will reinstate free feeding to shift their weight towards the optimal (around 10%) reduction figure compared to controls; we expect such weight gain to occur within 24-48hrs of return to free-feeding. We will only restrict food immediately prior to and on behavioural testing days; on all other days, animals will have free access to food and will always have free access to water.

Recovery following surgery will result in some pain for the animal as it recovers from the anaesthetic but this will be controlled by pain-reducing drugs and animals will be monitored closely during their recovery. During recording after the recovery period, the animals will have to carry small recording and stimulating devices on their head, but these are very light so only have a minimal impact on the natural behaviour of the animal. The latter will be aided by prioritising recordings where measurements are transmitted wirelessly from these head-mounted devices so that there is also no requirement to connect the animal via wires to the equipment in these cases.

Drugs used to modify behaviour and brain activity are of course likely to affect cognition, but we will minimise the chance of any serious adverse effects by selecting doses from the literature that are known to be safe. In cases where that evidence is not available for selected compounds, or we have not used them before, we will perform a pilot experiment in a small group of animals where we test intended experimental doses of that drug incrementally (one dose per animal) and monitor adverse effects over 1 hour. We will monitor the lowest dose first for adverse effects before progressing to the next highest dose. We do not expect adverse effects from dosing but if these do arise and last for 1 hour the animal will be killed humanely immediately.

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)?

Moderate severity:

(i) Animals that experience model induction via injections over several days of drug/vehicle (or are drug naive) followed by behaviour with/without further drug challenge - 70% of animals

(ii) Animals that experience model induction via injections over several days of drug/vehicle (or are drug naive) followed by brain activity recording under anaesthesia - 15% of animals

(ii) Animals that experience model induction via injections over several days (or are drug naive), then experience electrode implantation followed by brain activity recording during behavioural tasks with/without further drug challenge - 10% of animals.

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 impossible to mimic brain and behaviour interactions in cell systems, so studies using live animals are vital to obtain a greater understanding of normal and abnormal mental states and to test the effectiveness of new drugs. Some *in vitro* approaches, however, may provide valuable tools to screen potential treatment compounds prior to their use in animals.

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

Computational models, in vitro preparations.

Why were they not suitable?

This work must use whole animals, as understanding behaviour and the required brain activity to produce that behaviour is a central feature of the project. This cannot be studied effectively by using reduced in vitro preparations, and computational approaches lack the required complexity due to insufficient biological data. To date, there is no suitable alternative to the use of rodents for behavioural studies that do not involve human subjects and we are still extremely limited in our ability to measure neural activity directly from the human brain.

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?

Behaviour/behavioural pharmacology studies. Our general expectation for animal numbers is that we will require 10 per group for behaviour. This is based on data acquired by us previously where we have seen robust outcomes using this cohort size. We will monitor closely for any changes in our data that may support an increase or decrease in required sample sizes, for example, to show a significant effect of drug treatment. Where there is limited or no previous relevant information for a specific experimental design, we will compute and predict sample sizes that are likely to be sufficient to provide a robust experimental result based on previous most relevant experiments from ourselves or other groups. Data analyses will be carried out according to a pre-specified statistical analysis plan drawn up in conjunction with professional statistician experts.

Studies recording brain activity. Numbers required for these depend on the question and technique at hand and, most importantly, the size of the effect in our experimental animals. Similar studies in the literature typically require a sample size of approximately 8-10/group and this matches well with our estimated group size of 10 to see drug treatment effects. Studies recording brain activity are usually carried out by accumulating recordings from one or two animals at a time. This sequential use of animals will allow us in many cases to make ‘waypoint’ checks well before we reach our predicted required group sizes. This will ensure that we are not using too many animals, or that we need to add more animals to achieve our scientific goals. For example, if we predicted that a group size of 10 animals would be required to effectively test our hypothesis, we can double check ‘halfway’ through an experiment, for example, once we have 5 animals in each group, whether we will still need that total of 10 animals per group.

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

Experimental design. We will use experiments with several variables, where we predict that those variables will have some effect on our experimental measures (e.g., improvement in memory). These variables include the type of animal (e.g., control or disease model groups) and treatment (e.g., high or low doses of a particular drug or periods of exercise with short or long duration). Such designs allow us to test whether each variable has an influence on its own and whether together they have a combined effect (e.g., changing the dose of a drug may produce different behavioural changes in model versus control animals). By combining several variables in this way, we increase our knowledge about effects in our model animals whilst keeping the number of animals and experiments required to a minimum.

The quality of our measurements will be maximised by good training of researchers. To reduce the chances of experimenter handling affecting results, we will use tasks wherever possible in which animals can learn and perform at their own pace, that is, without repeated handling by the experimenter. To make our testing as fair as possible we will: randomly allocate animals to treatment groups; minimise the chances that we get a particular result simply due to the order in which animals experience experimental stages; and where possible ensure that experimenters are unaware of whether animals are in a particular treatment group until all data capture and analysis stages have

been completed. To maximise the quality of our results we will adhere to national standards ("ARRIVE" and “PREPARE” guidelines) when planning and carrying out our research and publishing our findings.

Whilst most animals will experience either no drug or a single drug exposure, we require some animals to experience more than one drug/compound to produce a change in behaviour. In cases of repeated challenge this will be for cases where we could not substitute a naive animal. This approach will also reduce the number of animals required to meet our Objectives.

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

When testing a drug that is new to the laboratory, we will first conduct a pilot experiment using a small group of naïve animals. Experimental doses will be given incrementally (one dose per animal) and we will monitor for adverse effects over 1 hour. We will test the lowest dose first and if there is no adverse effect progress to the next highest dose in the next animal. This procedure will provide a safe dose range for our experimental groups.

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.

We will use mouse and rat species as these are of low neurophysiological sensitivity, yet their brains show good anatomical and functional similarities to those in the human brain. All procedures in this license range from non-recovery to moderate severity.

Our chosen animal model for schizophrenia develops both behavioural/cognitive deficits and abnormal changes in the brain that mimic very well those seen in schizophrenic patients. Importantly, for model animals their overall general behaviour is unaffected and deficits are only observable when you test for them. Thus, our model induction produces no observable long-term pain, suffering or distress.

Our subsequent behavioural measures will often require animals to simply behave spontaneously; for example, we will measure whether they can detect a change in their environment that requires prior memory of, for example, the type of objects encountered previously. Some animals will be trained to perform a task, which will require food restriction on training and testing days as we use food to reward learning and correct task performance. We do not expect significant adverse effects from this food restriction but will monitor the weights of these animals very closely. If we detect substantial weight loss than cannot be reversed by full food provision the animal will be killed humanely immediately.

In experiments where we record brain activity from animals during behaviour, we will use well-established techniques developed over the last 60 years. This requires animals to undergo recovery

surgery under general anaesthesia where we will minimise the chances of infection and peri-operative analgesia to reduce pain. Animals will be monitored closely post-operatively for several days for signs of pain and further analgesia will be provided if required. These procedures are now well established in our labs.

In experiments where we dose animals with drugs we will use doses, routes and volumes that are suitable for the species. We will make sure that any novel compounds are tested in a small group of animals first to ensure safety and tolerance before dosing our experimental groups. All drugs are intended to improve or normalise cognitive performance of animals.

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

Our experiments require measurements of behaviour and cognition in adult rodents to model disease-relevant changes in the human adult population. Rats and mice are relatively simple species when compared to human but they exhibit similar cognition and brain anatomy. It is not possible to use lower species to reproduce the same range of human behaviour and comparative brain anatomy and function.

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

As described above, we will monitor general physiology and behaviour of our animals closely both during and outside experimental phases. For recovery surgery procedures we will apply appropriate post-operative care and analgesia. We will adjust our procedures if required through our close monitoring of animals at all stages and seek advice from NVS and other animal unit staff as appropriate.

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

We will follow the standards set by PREPARE (experiment preparation and execution), LASA (aseptic surgery), NC3Rs (e.g., blood sampling) and the Joint Working Group on refinement (Morton et al., 2001 Lab Animal 35).

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

Training routes and updates are provided by our animal house staff regularly and recent advances in animal husbandry and care are communicated effectively to all users. All activities under this license will also be informed by the regular NC3Rs newsletter. Laboratory workers are normally members of established national (e.g., British Neuroscience Association) and international bodies (e.g., International Brain Research Organisation) that are active in promoting best practice for animal research.