Home Office

NON-TECHNICAL SUMMARY

Neural Circuits and Immunity in Psychosis

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
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Schizophrenia, Psychosis, Perception, Neural circuits, Immune system

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

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

This project is aimed at elucidating the biological mechanisms that give rise to psychotic symptoms. The goal is to develop new biological treatments for brain disorders such as schizophrenia.

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?

Psychosis is characterised by disturbances of perception and thought such as hallucinations and delusions. Psychotic symptoms are a typical manifestation of severe mental disorders including schizophrenia, bipolar disorder, depression and dementia. The prognosis of these disorders has not improved over the past decades, and the development of new treatments has been slow. One main reason for this stagnation is that we still do not understand the biological roots of psychosis.

We will study how brain and immune dysfunctions lead to psychosis-like behaviour. In particular, we will investigate nerve cells that are inside or connected with the striatum. The striatum is a brain region that is believed to be involved in psychosis. We will observe how different types of nerve cells signal during psychosis-like behaviour, and how changing signalling in these nerve cells reduces psychosis-like behaviour. This will allow us to identify new antipsychotic treatment strategies that target specific nerve cells. Moreover, we will study how immune signals lead to psychosis. While the exact causes for psychosis are unclear, a variety of genetic and environmental factors point to an involvement of the immune system. We will identify immune signals that are altered in patients with psychosis and induce these immune signals in mice. We will then observe how these immune signals induce psychosis-like behaviour, and test different strategies to block these immune signals to reverse psychosis-like behaviour. This will allow us to identify new treatment strategies that modulate the immune system.

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

This project will generate new scientific knowledge about the biological roots of psychosis. We will publish our results through publications in high-quality peer-reviewed scientific journals.

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

This project will advance our understanding of the biological roots of psychosis. We expect to generate fundamental insights into the mechanisms underlying behaviours relevant to psychosis. This will benefit future research on psychosis in the short-term. Moreover, by identifying new strategies to reverse psychosis-like behaviour, we hope to provide the basis for developing new treatments for psychotic symptoms. This will create opportunities for pharmaceutical companies to develop more targeted therapeutic compounds with less unwanted side effects. This will benefit people affected by psychotic disorders in the medium to long-term.

These benefits are substantial given the high individual, societal and economic costs of psychosis. Psychosis is a syndrome of various major mental disorders including schizophrenia, bipolar disorders, depression and dementia. Mental ill health is the single largest cause of disability in the UK, contributing up to 22.8% of the total burden, compared to 15.9% for cancer and 16.2% for cardiovascular disease (Department of Health and Social Affairs, 2011). The wider economic costs of mental illness in England have been estimated at £105.2 billion each year (ibid.). This includes direct costs of services, lost productivity at work and reduced quality of life. Moreover, because of the devastating interpersonal repercussions of psychotic symptoms, psychotic disorders pose a high emotional burden on families.

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

During this project, we will actively collaborate with colleagues from academic psychiatry who are working on related questions in humans. We will further engage ourselves in studies in psychotic patients. We will use our own and our collaborators' results to shape our project on an ongoing basis. We plan to disseminate the new knowledge through publications in scientific journals and through presentation at national and international conferences. We will further actively work on making the data generated in this project open-source so it can be used by other scientists in their future research.

Species and numbers of animals expected to be used

- Mice: 5000

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.

Mice are the species with the lowest sentience that is still suited for studying the brain and immune system. Therefore, mice have been extensively used in neurophysiological and immunological research. As a result, all the necessary assays and reagents for this project are available for mice. As psychosis typically manifests in early adulthood, we will focus on adult mice. However, as some of the biological risk factors affect the developing organism, we will study mice at earlier stages of life as well.

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

Animals will undergo behavioural experiments. During these experiments, animals will be presented with stimuli such as lights and new sounds. All the stimuli are below the pain threshold, and animals will be habituated to them over the course of days. In response to these stimuli, animals will poke their snouts into openings and receive water as a reward. In some behavioural experiments, animals will be unable to move their heads as they will be mechanically fixed. This is necessary to allow for measurements of brain function that require a still head. To make sure that animals are motivated to perform the experiments, water may be restricted outside the experiments. However, we will make sure

that animals maintain good health and weight by supplementing them with additional amounts of water outside the experiment or by providing them with free access to citric acid water. Such citric acid water is not as palatable but equally hydrating as normal water.

Some animals will receive injections of a neuroactive or immune-modulating substance. Animals will typically be injected a small volume of a substance under the skin of the neck, in the belly, or in the muscle. In some cases, injections will be given directly into the space along the spine that contains the fluid that bathes the spinal cord and the brain. For this procedure, a small cut of the skin in the neck may be performed under general short-term anaesthesia. These injections into the space along the spine are necessary to test the effects of substances that cannot otherwise reach the brain because of the barriers between the brain and the rest of the body. This procedure is similar to intrathecal injections used in humans to treat cancer and autoimmune diseases of the nerve system. Animals will experience the effects of the substances such as altered perception, increased activity and general sickness behaviour. These effects will be transient, and we will monitor and control for them. Animals will typically receive one injection per day and a total of two to twenty-four injections over the course of the experiment. In some cases, more than one injection needs to be given on one occasion. In these cases, injections will be performed under general short-term anaesthesia.

Some animals will receive immune cells from another animal. For this procedure, animals will first undergo radiation or chemical treatment to ablate their own immune system. This procedure leads to a transient suppression of the immune system, which is usually well tolerated. Some animals might experience infections or gut inflammation, which we will monitor for and treat. The procedure is similar to a stem cell transplant that is used to treat human blood cancer.

Some animals will undergo surgical procedures. A portion of the experiments will involve the implantation of a device such as a glass fibre or cannula into the brain. Some experiments will also involve the implantation of a small pump under the skin of the back. This small pump is used to deliver drugs continuously or on-demand without the need for an injection. This is similar to insulin pumps used in the treatment of diabetes mellitus in humans. To alleviate pain and prevent infections, the procedures will be conducted under anaesthesia and aseptic conditions. Postoperative pain and inflammation will be closely monitored, and animals will receive preventive pain killers during the surgery and when they show signs of distress after the surgery. Animals will be left one week to recover before undergoing any other experiments.

Experiments typically run for 3-12 months.

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

During the behavioural experiment, the animals will experience stress which we plan to minimise through gradually habituating animals to the new environments. Animals typically show signs of distress in the form of increased activity for around five to fifteen minutes after entering the experimental environment for the first two to three times until they get used to this situation. Water restriction outside the experiments might lead to thirst and weight loss, which we will minimize by ensuring a minimum water intake or giving free access to citric acid water. Animals may experience pain during injection of substances which typically lasts for minutes. Animals may experience effects of the neuroactive drugs which might induce agitation or sedation which typically last for hours. Animals may experience the effects of immuno-active substances which may include sickness behaviour, less movement and less

feeding and are typically mild and last for days. A portion (20%) of the animals undergoing immune cell transfer may experience infections or gut inflammation which typically spontaneously resolves after days. Animals will experience pain and distress after surgery, which we aim to minimise with pain killers and careful monitoring, and which typically resolves after a few days.

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 - 50%

Moderate - 50%

Severe - 0%

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

- Killed
- Used in other projects
- Kept alive

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?

Psychosis exclusively manifests itself through behavioural alterations, therefore a living and behaving organism is necessary to study psychosis. Because humans cannot directly be studied with invasive methods that enable to study the biological mechanisms in sufficient detail, studies in animals are necessary.

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

Following the PREPARE guidelines and RSPCA suggestions, we considered cell cultures, simpler non-vertebrate organisms, mathematical and computer simulations, and studies in human volunteers.

Why were they not suitable?

We will supplement our research with the identified non-animal alternatives to refine our hypotheses whenever possible, but our objectives cannot be achieved by non-animal alternatives alone. Isolated cell cultures are not able to describe behavioural alterations of a living organism affected by psychosis. Similarly, simpler non-vertebrate organisms are not suited to reproduce the complex behavioural

alterations related to psychosis. Mathematical and computer simulations are unable to recapitulate the unknown biological links between immunity, brain and behaviour related to psychosis. In human volunteers we cannot study the biological mechanisms underlying psychosis in sufficient detail because we cannot directly access the brain for experimental measurements and manipulations. The available methods for studying brain function non-invasively (functional imaging, electroencephalography, transcranial magnetic stimulation) lack the temporal, spatial and biological resolution required to achieve our objectives.

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?

We used our previous research to estimate the number of animals. When interventions are required, we expect that 5-10 animals per treatment group will usually be sufficient to obtain robust results. Experimental design is based on the PREPARE guidelines. When effect sizes are known, we will use power calculations to determine the number of animals needed. When effect sizes are not known, we will use the minimum number of animals to provide an adequate description or perform power calculations after the first experimental pilot animals, when expected animal numbers are comparably large (>10 animals).

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

To complement my statistical expertise gained during 15 years of research, I will consult with the biostatisticians at my institution whenever necessary for advice on sample size based on power analyses and pilot studies. I will use online tools such as the NC3R Experimental Design Assistant to adequately design the experiments with the minimum number of animals needed, whenever applicable. We will mainly use repeated-measures experimental designs, which will reduce the number of animals needed as compared to standard grouped designs.

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

To achieve our objectives, we will need transgenic mouse lines. Efficient breeding will minimize the number of animals during breeding. This will include breeding from homozygous breeders to ensure that all offspring have a suitable genotype as well as cryopreservation of embryos to enable breeding only when animals are needed. To further minimize the number of animals, we plan to minimize individual variability by using in-bred strains with genetically homogenous backgrounds. To further reduce animal numbers, animals will be efficiently used whenever possible without adverse effects on animal welfare. For instance, when animals have successfully undergone non-invasive behavioural

training in a task, they may be transferred to another protocol to test the effects of different experimental interventions on behaviour. This will reduce the number of animals required as compared to the alternative of establishing a behavioural task by training one cohort of animals and testing the effects of experimental interventions by training and testing a new cohort of animals. Moreover, we will minimize the number of animals by maximizing the amount of data gained from one animal whenever possible. For instance, we plan to use imaging or electrical recording methods that allow studying multiple nerve cells at a time, as well as molecular analyses that yield information about single nerve or immune cells. All experiments will be conducted in animals of both sexes, unless a scientific reason suggests the use of one sex. For instance, when a certain autoantibody is exclusively present in female psychosis patients, we will focus on female mice to investigate the biological role of this autoantibody in psychosis.

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.

Experiments will be performed in wild-type animals (around 60%) and genetically altered animals (40%). Genetic altered animals will carry mutations that help us to measure and manipulate specific kinds of nerve and immune cells. These animals are not expected to show any clinical signs or harmful phenotypes. In some cases, we will use genetic alterations will model genetic and immune alterations associated with psychotic disorders. These animals typically show mild behavioural changes as assessed by specialised tests but no signs of adverse effects that impact materially on their general well-being.

All animals included here will undergo behavioural experiments that capture psychosis-like behaviour. During some of these experiments, animals are trained to respond to sensory stimuli such as lights or sounds in order to get a water reward. In most cases, animals will freely move around during these experiments, which is associated with less distress as compared to head restraint. Head restraint will only be used when valid neural recordings cannot be obtained without it. To make sure that animals are motivated to perform the experiments, water will be mildly restricted outside the experiments. Mild water restriction is a method to motivate animal behaviour that is associated with less pain and suffering as compared to aversive motivations using painful punishments.

Some animals will receive repeated injections or be drawn blood through a needle. Although the harm of these procedures is transient, repetition can contribute to cumulative suffering. However, this repetition is necessary for the efficient experimental repeated-measures design which will reduce animal numbers (reduction). Some animals will be drawn very small volumes of cerebrospinal fluid through an inserted cannula under short-term general anaesthesia. This procedure is similar to a lumbar puncture that is routinely performed without anaesthesia in human patients in neurology and oncology. This procedure allows assessing the immune compartment associated with the brain.

Withdrawal of cerebrospinal fluid does not result in lasting harm and is associated with less harm than alternative methods involving the removal of brain membranes or tissue.

Some animals will undergo surgical procedures. Surgery is necessary to allow access to the brain. We will use the least invasive surgery method suited to answer our scientific question. For instance, to test the role of one brain region in psychosis-relevant behaviours, we will use drugs to transiently block signalling in that region instead of surgically removing that brain region. Good surgery techniques and pain killers after the surgery reduce the pain and suffering of the animals.

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

Psychosis affects patients of all ages with a peak in early adulthood. The investigation of the biological mechanisms underlying psychosis therefore requires the study of animals of all ages and cannot be exclusively performed at most immature life stages. Because psychosis affects patients as a whole behaving individual, psychosis-like behaviours cannot be studied under terminal anaesthesia. Moreover, for the work to be translatable to human patients, mammalian species are needed. Mice are well established in neuroscience and immunology research.

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

To reduce the stress associated with behavioural experiments, all the delivered stimuli are below the pain threshold, and animals will be frequently handled and habituated to the experimental setup over the course of days. To minimize thirst caused by the water restriction, we will either ensure that animals receive a minimum of 40ml/kg water (roughly equivalent to 1/3 of their body weight) or we will provide animals unrestricted access to citric acid water (less palatable but equally hydrating as regular water). Whenever possible, neural recordings will be carried out in freely moving animals using lightweight implants that are easily supported by the animal, but, in some cases, head restraint may be needed to enable valid results. In this case, animals will be habituated to the recording setup in incremental steps starting with short durations on the order of several minutes.

To reduce the stress associated with the injection or blood draws, animals will be habituated to being held in the hand of an experimenter. Moreover, injection volumes will be small, and single use of needles ensures a sharp and clean needle per animal. Suitably small needle sizes will be chosen, in accordance with current best practice. To reduce the stress associated with cerebrospinal fluid draws, animals will undergo this procedure under short-term anaesthesia.

To reduce the risk of infection during immune cell transfer, animals will be housed in clean facilities and closely monitored. If signs of infection are detected, a veterinarian will be consulted about the possibility of prescribing antibiotics.

Surgical procedures will be conducted under anaesthesia and aseptic conditions to alleviate pain and reduce the risk of postoperative infection. Postoperative pain and inflammation will be closely monitored, typically twice a day. Animals will receive preventive pain killers during the surgery and when they show signs of distress after the surgery. Animals will be left one week to recover before undergoing behavioural experiments.

Animals will be observed every day by a person experienced in animal husbandry to identify potential adverse events and ensure that humane endpoints are adhered. We will typically group-house animals and provide enrichment including nesting material to increase animal welfare.

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

Whenever applicable, we will follow the best practice guidelines provided by the NC3Rs (e.g. for blood draws or for husbandry). For surgical and non-surgical procedures, we will follow the recommendations of the Laboratory Animal Science Association (https://www.lasa.co.uk/wp-content/uploads/2018/05/Aseptic-Surgery.pdf) and of the Procedures with Care website (https://researchanimaltraining.com/article-categories/procedures-with-care/).

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

I constantly monitor best practices and new information available in the international literature and on the NC3RS and RSPCA websites (www.nc3rs.org.uk/our-resources www.rspca.org.uk/adviceandwelfare/laboratory). I am also subscribed to the newsletter of the NC3RS (www.nc3rs.org.uk), and follow the RSPCA twitter account dedicated at laboratory animal welfare (@RSPCA_LabAnimal).