Home Office

NON-TECHNICAL SUMMARY

Understanding the mechanisms involved in network formation and function in the developing brain

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

neurodevelopment, brain, neurons, glia, neurodevelopmental disorders

Animal types	Life stages
Mice	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?

The main aim of the project is to understand how brain activity can impact cellular development, distribution, maturation and interactions of brain cells in the developing brain.

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?

Neurodevelopmental disorders like autism spectrum disorder (ASD) affect more than 1 in 100 people. According to the World Health Organisation, ASD affects approximately 1% of the population worldwide. Individuals with ASD often experience significant challenges in social communication and behaviour, and many also have co-occurring conditions such as intellectual disability, epilepsy, anxiety and sleep disturbances. These challenges can substantially impact quality of life, education attainment, employment opportunities and long-term independence, placing a considerable burden on affected individuals, families and health care system. Despite its lifelong neurodevelopmental condition, currently, there are no known treatments that can cure or reverse these conditions. ASD is usually diagnosed only after a child is older, which makes it hard to step in early and change how the condition develops. One major reason for this is that we still don't fully understand how the brain normally develops. In this project, we aim to: (1) better understand how the brain develops over time, (2) identify both internal and external factors – such as cell behaviour, daily rhythms or environmental influences – that can change brain development, and (3) learn how these changes might lead to conditions like ASD.

Brain building is a protracted process that lasts for several weeks in rodents but years in humans. In our previous research, we discovered that pyramidal cells (the main excitatory neurons and the "message-sending" nerve cells in the brain) help control microglia, which are the brain's immune cells, during development. Now, we want to explore how other brain cells are involved too. This includes: (1) astrocytes, which support and protect brain cells, (2) oligodendrocytes, which help signals travel faster in the brain, and (3) interneurons, which act as the brain's "brakes" to balance activity.

Here, we want to study how things like sleep cycles, environment, or other influences affect how these cells grow and communicate with each other, and how that shapes brain wiring and function. Finally, we hope to uncover how disruptions in these processes might lead to difficulties seen in conditions like ASD, such as challenges with thinking, learning and memory.

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

This project aims to uncover how conditions such as ASD arise by studying how the brain develops. We will look at how different factors – such as genes, environment and even daily sleep-wake cycles – might disrupt brain activity during development and lead to similar outcomes, like learning difficulties or sleep problems.

We believe that small changes in how brain cells communicate while the brain is forming could have long-term effects. From this proposal, there are several key outputs including:

(1) identify key stages where brain cells grow and connect

(2) understand how genetic and environmental factors influence this process

(3) reveal how these different factors can lead to similar changes seen in ASD.

While this research is not expected to lead directly to immediate clinical treatments, the findings will provide important foundational knowledge about early brain development. More specifically, the knowledge obtained from this project can:

(1) explain why people with ASD show certain traits

(2) support the identification of early warning signs (biomarkers) for quicker diagnosis

(3) inform the new treatment strategies that target brain development early on.

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

Currently, there is no reliable way to diagnose or treat conditions like ASD early in life. To change this, we need a better understanding of how the brain normally develops and what goes wrong in these disorders. By uncovering what disrupts brain development, we may be able to find new ways to detect problems sooner and create treatments that reduce the social and cognitive challenges people with neurodevelopmental disorders face.

In the short term, this project will (1) identify key genetic and environmental factors that shape how brain cells grow and communicate, (2) reveal how different influences – like genes or environmental disruptions – can lead to similar difficulties with social interactions, learning and sleep seen in many neurodevelopmental disorders. This work will generate new knowledge, tools, and data that will support researchers both within and beyond the field and spark new collaborations.

In the long-term, these findings could help doctors and scientists in applying these findings to human development. Furthermore, these findings could help identify new diagnostic tools, discover biological markers of risk and design treatments that intervene before long-term difficulties take hold. Finally, as sleep and circadian rhythm problems are common in neurodevelopmental disorders, understanding their role and impact will be a key part of this effort.

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

We will disseminate the outputs of the project (both positive and negative results) through the following ways: (1) publications and preprints, (2) conferences and scientific visits through talks, poster

presentations, (3) collaborations with other scientists, (4) collaborations with clinicians working in neurodevelopmental disorders, (5) deposition of data (e.g., transcriptomic, proteomic datasets) and tools (e.g., codes and protocols used in analysis) in online repositories or other data platforms and (6) social media (e.g., Bluesky, Linkedin).

Additionally, if any of the procedures or techniques developed through this work contribute to the refinement of animal procedures, the replacement of animal use, or the reduction in the number of animals required, we will ensure that these advances are communicated to the wider scientific and regulatory community. Dissemination will occur through peer-reviewed publications and preprints, presentations at conferences and scientific meetings, collaborative visits, and engagement with established networks and Animal Welfare and Ethical Review Bodies (AWERBs).

Species and numbers of animals expected to be used

- Mice: 8000

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.

In this project, we use normal and genetically modified mice, to study how brain cells develop and interact with one another. We will use both male and female mice.

The mouse is an ideal choice as

1) brain development in mouse is similar to humans

2) the mouse brain contains a similar complement of cell types compared to humans

3) the availability of genetically modified mice that allows for cell-type specific manipulations and the induction or deletion of specific gene of interest.

This project will use mice at all life stages as we will study how the brain develops from embryonic and postnatal developments and study how any alterations can impact brain function in the adult brain.

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

The bulk of the animals in this protocol will not undergo any further procedures beyond the normal breeding and maintenance protocol (60%) of genetically altered mice, which is expected to be no more than mild.

The remaining animals will undergo at least one (20%), two (15%), or up to three (5%) of the following procedures listed below

1. Breeding and maintenance of mice in light-controlled cabinets where the day-night cycle is shifted (e.g. lights on at 11 am and lights off at 11 pm, or lights on at 11 pm and lights off at 11 am).

2. Administration of substances through food, water or brief injections under the skin or into the belly (e.g., abdominal cavity). Substances will be administered maximally for 7 days, twice daily.

3. Administration of substances directly into the brain of embryos or neonates. These procedures will not last more than 30 minutes.

4. Behavioural tests that aim to measure the different aspects of behaviour that are typically associated with neurodevelopmental disorders (e.g. cognition, compulsion). The maximum length of time the animals will spend performing these behavioural tests will not exceed 24 days.

5. Changes to sensory input (e.g., changes in the light/dark cycles and their ability to perceive their environments through whisker stimulation or deprivation) that aim to elucidate how sensory experiences can impact brain cell development. The maximum length of time these sensory alterations will be applied to the mice is three weeks.

6. Environmental alteration models that aim to elucidate how changes in the environment can impact brain cell development. This includes changes in the feeding behaviour of the pups by altering the size of the litters and introduction of mild stress by separating the pups from their mothers for a short period of time. These models will be performed maximally for 3 weeks up until weaning.

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

Previous experience has shown that these procedures do not typically induce no more than moderate effects. Below are some of the possible adverse effects that may arise in this project.

1. There is a low risk of death occurring from anaesthesia or complications during surgery (<1%).

2. In very rare occasions, electroporation (e.g., The use of mild and controlled electric pulses to make tiny pores in brain cells' outer skin. The pores allow us to slip things like DNA into cells. Right after the electric pulses, the pores will resealed immediately) may cause sudden death to the dams (<1%) and pups (<1%).

3. Other adverse effects following surgery or substance administration may include weight loss for more than 24 hours, even after palatable food has been offered, of up to 15% of total body weight in adult animals compared to untreated controls. In neonates and juveniles, this may instead present as a failure to gain weight, defined as a 15% difference compared with age matched siblings. These situations are expected to be rare.

4. Low risk of pups being rejected by their mothers or foster mothers after administration of substances into the central nervous system (<1%). The administration of substances into the central nervous system itself will not induce no more than transient pain and discomfort.

5. Weight gain/loss from altered rearing conditions (e.g. altered light-dark cycles, changing litter sizes) due to changes in their metabolism.

Furthermore, while each procedure listed above may produce a mild and transient pain/discomfort on its own, the cumulative effect of the multiple procedures may increase the severity limit.

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)?

The expected severities of the animals in this project will be mild (70%) and 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?

The main aim of the project is to understand 1) how different genetic and environmental factors can contribute to brain cell development and interaction and 2) how these factors may converge into similar molecular mechanisms (e.g. changes in neuronal activity) that impact cellular development and numbers. In the first few weeks of mouse postnatal development, the developing brain undergoes substantial changes. These dynamic and complex physiological changes are important in dictating how these cells develop, interact and mature. Consequently, the use of mice is necessary for the following reasons: (1) the dynamic changes in the cytoarchitecture of the mammalian cerebral cortex at different developmental stages (e.g., the structure and organisation of cells in the outer layer of the brain) and (2) the lack of suitable in vitro and in silico models that captures this dynamic and complex changes.

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

To investigate non-animal alternatives, I have conducted searches over the past five years using databases, websites and journals such as PubMed, Google Scholar, Alternatives to Laboratory Animals (ALTA), and Replacing Animal Research (https://replacinganimalresearch.org.uk) using terms including "in vitro model for brain development", "3D models for brain development and function" and "in vitro assay for brain function." The most recent searches were carried out in November 2025 during the time of writing. I also receive weekly Google Scholar alerts for these keywords, which keep me informed of the latest peer-reviewed studies, preprints and relevant reports such as press releases. In addition to database searches, I have actively discussed potential non-animal alternatives during conferences, research visits and through professional networks such as the MRC National Mouse Genetics Network.

From these searches, I have considered the use of 3D in vitro culture models such as organoids and spheroids as potential non-animal alternatives. These are lab-grown, self-organising structures made from stem cells that can mimic early stages of brain development in a controlled environment.

Why were they not suitable?

There are several limitations to the use of organoids/spheroids in this project for the following reasons:

1) Inaccurate replication of the cytoarchitecture of the brain. The mammalian cerebral cortex is a six-layered cortex that is specified by other inputs from other brain regions such as the thalamus. In the organoid/spheroid system, there is a lack of distinct/correct cytoarchitecture.

2) Lack of complex and dynamic environment. In mice, the development and maturation of neurons occur right up to two weeks after eye opening as the visual inputs from the retina are required for the appropriate development and maturation of the pyramidal cells. As organoids/spheroids are unable to capture such changes in the environment, they remain in an immature state.

3) Lack of cellular diversity. The brain consists of different cortical cell types that originate from different lineages and areas during development. Organoids/spheroids however are grown from stem cells originating from the same lineage or with limited mixed lineages. Consequently, this will preclude the study of cell-cell interactions from multiple lineages.

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?

I have estimated the number of animals required based on my previous experience and power calculations using current preliminary data. Most of the necessary data will be obtained through ex vivo analyses, allowing the same samples to be used across multiple experiments to address different scientific questions. The majority of animals in this project will be generated through breeding to obtain the appropriate genotypes. These estimates are based on Mendelian inheritance ratios, as multiple breeding rounds will be needed to produce animals with the desired genotypes. Mendelian inheritance ratios describe the predictable patterns by which parents pass traits on to their offspring. They explain why, when two parents with certain traits are bred, their offspring tend to inherit those traits in set proportions rather than at random.

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

I have more than 20 years of experience using rodents as a model system. Coupled with tools such as NC3Rs experimental design assistant, I have minimised the number of animals used when testing our hypothesis by including the use of factorial experimental design. Furthermore, before the experimental design, I have also taken the online course (www.3rs-reduction.co.uk) and went through the PREPARE guidelines (https://norecopa.no/prepare) to minimise the animal used while ensuring high reproducibility.

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

To optimise animal use, all lab members will complete an online course focused on the principles of reduction (www.3rs-reduction.co.uk).

For animal breeding, we will implement efficient colony management by maintaining detailed breeding records. This will allow early identification of any issues (e.g. poor breeder performance), enable timely rotation of breeders on a strict schedule, and ensure the replacement of non-productive breeders. Additionally, we will cryopreserve any mouse strains that are not currently in use to avoid unnecessary breeding.

We will also establish a centralised brain database to record all collected brain samples along with relevant metadata (e.g. experimental use, age, sex). This will ensure that each brain can be used by the entire lab and potentially by other researchers. Given that the mouse brain yields a substantial number of slices, this approach will maximise tissue use. These tissues can also support pilot studies, such as testing novel reagents (e.g. antibodies).

Together, these strategies will minimise the overall number of animals used, while promoting resource sharing within the lab and across the university.

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 genetically modified mice to study how specific genes influence brain development. Some procedures in this project involve targeting gene manipulation to specific cell types. For neonatal procedures involving brain injections, special care will be taken to prevent mothers from rejecting their pups. For example, we have established that gently rubbing the pups with nesting material before returning them to the cage significantly reduces the risk of maternal rejection. This not only improves animal welfare but also reduces the number of animals needed by preventing unnecessary loss. For embryonic procedures involving brain injections, special care will be taken to ensure minimal animal suffering through the application of anti-inflammatories/analgesics and post-operative care.

When possible, we use local rather than systemic administration of substances to minimise side effects and reduce discomfort. All procedures involving genetic manipulation will be carried out with strict attention to animal welfare, including the use of appropriate anaesthesia, analgesia and careful monitoring during recovery to ensure that any discomfort is kept to a minimum.

To study behaviour relevant to neurodevelopmental disorders, we have selected tests that assess features that are common for most disorders such as short-term memory disruption and social interactions (e.g., preference for social vs non-social stimuli). In choosing behavioural tests, we have deliberately prioritised methods known to cause the least stress – for example, selecting land-based tasks rather than water-based ones whenever possible.

We will also study how sensory experiences influences brain development through controlled changes in sensory input, such as adjusting light-dark cycles or brief periods of whisker stimulation or deprivation. In these models, we use the least stressful conditions that can still achieve scientific validity. For instance, in continuous light exposure experiments, animals will be kept under dim rather than bright light, as this is known to significantly reduce stress levels.

Finally, some environmental manipulation models will be used to study how early-life experiences can affect brain development. These include mild changes in litter conditions or feeding patterns that are known to influence key developmental signalling pathways and neuronal activity. These approaches were chosen because they are effective while still allowing us to minimise stress and avoid invasive methods such as the use of ketamine in increasing neuronal activity.

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

The project aims to understand the molecular mechanisms driving brain development. Currently, the mouse remains the best option as there are no (1) suitable in vitro/in silico models that can mimic the in vivo environment, (2) less sentient animals such as drosophila, worms and zebrafish lack a cerebral cortex (e.g., the outer layer of the brain that is seat of higher learning and function), (3) some species do not have the same complement of cell types (e.g. drosophila lacks microglia) and (4) the brain development process in mice is similar to those in humans.

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

All animal handling and care will be carried out in ways that minimise stress wherever possible, including the use of low-stress techniques such as tunnelling. We will also aim to reduce distress during behavioural testing. In our previous application, we proposed using the Morris Water Maze to assess spatial cognition and learning. However, in this proposal, we have opted for the Barnes Maze, a dry-land task that is less stressful for the animals. For the rotarod task, where transient stress may occur when animals fall from the rotating rod, we will minimise stress and prevent injury by padding the surface beneath the apparatus.

Special care will be provided following all surgical procedures. This will include increased post-operative monitoring, provision of analgesics when required, and efforts to reduce the duration of surgical interventions. We will also minimise the time pups are separated from their mothers to reduce distress. All new lab members will receive training on animal cadavers until they demonstrate

competence to perform surgeries. Furthermore, whenever possible, animals will be gently handled and habituated before behavioural experiments to further reduce stress.

Refinement will also be applied to the selection of animal models. Full knockout animals, in which the gene of interest is removed systemically, can sometimes develop multiple organ dysfunctions that compromise their health and welfare. When possible, we will instead use genetically modified mice that enable gene manipulation in specific cell subpopulations. This targeted approach will help mitigate the adverse effects often observed in full knockout models.

When appropriate, mice will be housed in an enriched environment that includes cardboard shelters, chew sticks, and plastic toys. Because environmental enrichment has been shown to increase cortical activity, additional enrichment items will be withheld in experiments that require reduced cortical activity. In such cases, standard housing conditions will be maintained, including nesting material and cardboard tubes, but no further enrichment will be provided.

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

We will follow the guidelines and advice provided by the NC3Rs establishment manager and Named Information Officer (NIO) to ensure that the experiments are conducted in the most refined way. Furthermore, we will follow the guidelines published on the home office websites (https://www.gov.uk/guidance/research-and-testing-using-animals), N3CRs (https://nc3rs.org.uk/3rs-resources) and Morton et al. *Laboratory Animals* (2001) 35(1):1-41.

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

We will work closely with the establishment 3Rs manager and Named Information Officer (NIO), who will advise us on the recent advances in 3Rs and implement these advances effectively during the lifespan of this project. Furthermore, before starting any experimentation, all lab members are required to undergo a 3R training course provided by the NC3Rs (https://nc3rs.org.uk/e-learning-resources). Here, they will learn about animal welfare assessment, euthanasia, and anaesthesia. In addition, all lab members are expected to keep up to date on advancements in the 3Rs through literature in journals such as ILAR Journal and Journal of Applied Animal Welfare Science.