Home Office

NON-TECHNICAL SUMMARY

Cellular homeostasis and brain development

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

Key words

neurodevelopment, Brain, Neurons, microglia, neurodevelopmental disorders

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

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

The main aim of this project is to understand the role of pyramidal cells, the most abundant excitatory cell in the cerebral cortex, in regulating the development, distribution and maturation of microglia, the resident immune cells, in the developing cerebral cortex.

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 such as autism spectrum disorders (ASD) affects at least 1% of the population. Currently, there are no known treatment. Furthermore, ASD are only reliably diagnosed late in development, consequently preventing earlier intervention in halting disease progression. The lack of treatment and earlier diagnosis is largely due to our lack of understanding as to how the brain develops. This work aims to remedy this by (i) increasing our understanding as to how brains are formed during development, (ii) identification of a critical time window during development in which the brain is susceptible to perturbations and (iii) providing insights into how neurodevelopmental disorders such as ASD may arise during development.

How does this work aim to achieve this? Neurons are the basic working unit of the brain in which information is transmitted and processed from the periphery to the brain. Pyramidal cells, in turn are the most abundantly found excitatory neurons present in the cerebral cortex. In individuals that have been diagnosed with ASD, pyramidal cells have been known to behave aberrantly (e.g. altered levels of neuronal activity). We hypothesised that despite the different factors contributing to ASD, these factors converge onto a similar molecular mechanism, namely alteration of neuronal activity during early postnatal development. These changes can have an impact on how brains are built, especially on cell types such as the brain immune cell, microglia. Microglia are known to be involved in different aspects of brain development, more specifically in how neurons communicate with one another. Consequently, alteration in how microglia develop, mature or even the amount that are present in the brain can have a significant impact on how the brain functions. In this work, we aim to understand how changes in pyramidal cell activity can impact microglia development and how deviation from normal microglia development may contribute to some of the impairments observed in ASD such as alteration in cognitive function.

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

Overall, this proposal aims to find new methods of earlier autism spectrum disorder (ASD) diagnosis and intervention by increasing our understanding of the molecular mechanisms involved in brain formation. This will be achieved by demonstrating that although different factors (e.g. genetic mutations, environmental insults such as maternal infection during pregnancy, pollutions) may contribute to ASD, these factors however, converge onto a similar molecular mechanism, namely alteration of neuronal activity during early postnatal development. We hypothesised that this alteration of neuronal activity has a significant impact on how brains are built, especially on cell types such as the brain immune cell,

microglia, which are known to modulate cortical function and behaviour. The discovery from this proposal will be key in (1) identifying the role of neuronal activity in modulating microglia development and number, (2) identifying the critical period during development in which the developing mice are susceptible to changes and (3) convergence of molecular mechanisms leading to the phenotypes typically observed in individuals with ASD. These discoveries will increase our basic understanding as to how brains are built during development and provide insights as to what happens during ASD and possibly other neurodevelopmental disorders.

In the long term, application of the knowledge obtained from this project onto human development will allow for (1) understanding as to how alteration of neuronal activity during early development can impact microglia development and number which in turn may explain some of the impairments observed in ASD, (2) earlier diagnosis of ASD based on changes in neuronal activity observed during gestation or during the first few months of life using resting-state fMRI and (3) new therapeutic avenues that might prevent or reduce the impact of changes of neuronal activity during early development.

To ensure that these discoveries and information will be disseminated as far and wide as possible, we will publish these discoveries in peer-reviewed scientific journals and also in talks and posters during conferences (both positive and negative results). Furthermore, the project involves the generation of transcriptome datasets, which illustrates the changes in multiple gene expression within the microglia population in reaction to altered neuronal activity. This data set will be made readily available through online repository. We will also summarise our findings for the general public and will communicate our discoveries through press releases and also via social media (e.g. Twitter).

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

According to the latest statistics from the UK government, neurodevelopmental disorders such as autism spectrum disorder (ASD) affects at least 1% of the population. Unfortunately, this is likely to be an underestimation due to the difficulty in diagnosis, especially among young children. Currently, the earliest detection of ASD in humans are approximately 2 years old. More often however, individuals with ASDs are typically diagnosed much later in life. Earlier detection and diagnosis of ASD will enable earlier intervention that may reduce social and cognitive deficits. One possible avenue that may allow for earlier diagnosis and intervention is by increasing our understanding on the aetiology of ASD.

In the short term, the outputs from this project can be seen in the advancement in basic sciences. The discovery from this proposal will be key in (1) identifying the role of neuronal activity in modulating microglia development and number, (2) identifying the critical period during development in which the developing mice are susceptible to changes and (3) convergence of molecular mechanisms leading to the phenotypes typically observed in individuals with ASD. These discoveries will increase our basic understanding as to how brains are built during development and provide insights as to what happens during ASD. The people benefiting from these outputs will include researchers from within and outside the field where they will be able to utilise the scientific discovery made and the training provided (e.g. knowledge gained from the insights and the direct use of the data that will be made available via publication and online repository). Furthermore, new collaborations may be formed based on the data produced by this proposal.

One of the long-term objectives of the lab is to collaborate with clinicians in order to determine how these findings can be applied onto human development. Application of the knowledge obtained from

this proposal will allow for better understanding as to the aetiology of ASD and the possibility of an earlier diagnosis among children. Currently, there are no known treatments available for ASD. Identification of new therapeutic targets that can prevent significant alteration in neuronal activity during development may not necessarily cure ASD, but it will halt the deterioration of further impairments that can occur during development. Consequently, earlier diagnosis and prevention will allow for better management of the symptoms.

Finally, if the outputs from this project can be successfully applied onto human development, individuals with high risk of developing ASD (e.g. siblings with known ASD diagnosis) in the long-term, will benefit from this work as earlier detection of this disorder will allow for more efficacious treatments and preventive measures to be done earlier during development. To this end, we are currently consulting with clinicians in order to determine how the discovery from this proposal can be applied onto human development. Together, this will allow for earlier diagnosis and potentially earlier intervention in individuals that have a high risk of developing ASD.

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

To maximise the outputs of this work, we will disseminate the output of the project through publications and preprints. We will also disseminate outputs (successful and unsuccessful approaches) through talks, poster presentations and informal discussions during conferences and meetings. We will also look into building new relationships with clinicians in order to apply this knowledge onto human development. Furthermore, the output of this work can also be further maximised by the formation of new collaborations, both in the field of neurodevelopment and beyond, as the knowledge generated through this project can be easily applied to other diseases, organs and organisms.

Species and numbers of animals expected to be used

- Mice: 5700

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 use genetically modified mice, both males and females, in this proposal in order to study cell-cell interactions in the developing brain. For this purpose, mice are an ideal choice as

1) brain development in mouse is similar to those in humans

2) the mouse brain contains the same complement of cell types as in humans

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

This project will use animals in all life stages. This will allow us to investigate how the brain develops during early postnatal weeks and also to study the consequences of altered brain development in the adult animals.

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 procedures (70%).

The remaining animals will undergo at least one (10%), two (15%) or up to three (5%) of the following procedures listed below

1. Administration of substances peripherally (e.g. via their diet/drinking water, orally, subcutaneously (e.g. under the skin) or intraperitoneally (e.g. into the abdominal cavity)). Substances will be administered maximally for 7 days, twice daily.

2. Administration of substances directly into the central nervous system of neonates (e.g. intracerebral injections). This procedure will not last more than 30 minutes.

3. Behavioural tests that aims 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.

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 any adverse effects. Nonetheless, listed below are 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) Other adverse effect from surgeries and substance administration may include weight loss in excess of 15% of total body weight or excessive loss of coat (e.g. piloerection) in rare situations.

3) Low risk of hypothermia from Morris water maze. This will be mitigated by the use of heating cabinets and by maintaining the water and room temperature appropriately (<1%).

4) Low risk of pups being rejected by their mothers or foster mothers after administration of substances into the central nervous system (<1%)

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 at the end of 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 the formation of the brain and elucidate the role of neuronal activity in shaping the development and maturation of the various cell types in the developing cortex. The developing brain undergoes tremendous changes during the first few weeks of mouse postnatal development. Due to the complexity and ever-changing physiological environment during development, the use of mice is necessary as the tissue architecture plays an important role in influencing this process. Currently, it is inconceivable that we will be able to generate computer models that will allow us to study the cell-cell interaction in an ever-changing three-dimensional structure that is required for this study. For the same reason, this precludes the majority of in vitro models such as primary slice cultures. Nonetheless, we will use organotypic slice cultures whenever possible to reduce the severity of the procedures. In this instance, we aim to use organotypic slice cultures in order to test the efficacy of our pharmacological candidates before testing these drugs in vivo.

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

As a non-animal alternative, I have considered the use of organoids as a potential in vitro system. Organoids are self-organising three-dimensional multicellular in vitro tissue. Organoids are derived from stem cells that are meant to recapitulate the developmental progression of an organ in vitro.

In addition, I have checked the FRAME website (www.frame.org.uk) and currently there are no other suitable replacement available for this current project.

Why were they not suitable?

The use of organoids has several major limitations in these key areas as they lack:

1) the correct cellular structure and layers (e.g. the presence of inputs from brain regions outside of the cerebral cortex such as the thalamus is lacking in organoids)

2) the complex environment is required to study brain immune cell behaviour and neuronal interaction. It has been previously shown that brain immune cells do not fully mature into the adult state when grown outside the organism (e.g. cell culture and organoids).

3) the brain consists of many different cell types that originate from different lineages during development. Organoids however are grown from cells originating from a single lineage. Consequently, it is currently not possible to study the interactions between cell types from different lineages using organoids.

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 used based on my previous experience and the current preliminary data obtained where I have performed power calculations. As most of the data required can be obtained from ex vivo analysis, we will be able to use the same samples for multiple experiments in order to address different scientific questions. The bulk of the animals estimated in this project will come about through the breeding of mice for the appropriate genotypes. The number of animals estimated have been calculated based on Mendelian genetics in order to obtain the desired genotypes. Consequently, multiple breeding will be required to obtain animals with the appropriate genotypes.

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

I have 10 years of experience using mice as a model system for studying neurodevelopment. I have used this experience together with tools such as the NC3R's experimental design assistant to minimise the number of animals used when testing our hypothesis, including the use of factorial experimental design. Furthermore, prior to 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) in order 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 the number of animals used, all lab members will be required to take an online course designed to minimise the number of animals used (www.3rs-reduction.co.uk).

With regards to animal breeding, we will ensure efficient mouse breeding by maintaining a detailed record of the breeding and colony management. Record keeping in this instance will allow us to identify problems with the colony early on (e.g. the performance of the breeder, rotating breeders on a strict

schedule, replacing non-productive breeders). Furthermore, we will also cryopreserve any mice strains that are not in use.

We will also have a brain database to ensure that every brain collected will be recorded together with the relevant information (e.g. experiments conducted, age, sex) to ensure that it can be used by the entire lab in addition to other researchers beyond the lab. We anticipate that as the mouse brain is relatively large and quite a substantial number of brain slices can be obtained per animal, this will allow for the maximal use of the tissue collected. In addition, the tissue collected can be used for pilot experiments in which we will test novel reagents such as antibodies. Together, this will reduce the overall usage of mice in our experimental procedures while sharing the resources with everyone in the lab and in 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 the function of specific genes in the formation of the brain during development. Some of the procedures that will be used in the project aims to manipulate genes in specific cell types. In the case of neonatal manipulation, we will provide special care to ensure that no pups are deserted after intracerebral injections. In particular, we have previously found that rubbing pups with the bedding reduces the probability of mothers rejecting their pups. This method not only serves as a refinement measure but also lessens the number of animals used, due to a reduction in animal lost. Furthermore, localised administrations and manipulations reduce the adverse effects that are typically associated with systemic manipulations where multiple organs and cell types are affected at the same time. For all genetic manipulations, animal welfare is our topmost priority and care will be given in terms of anaesthesia, analgesia and recovery monitoring to ensure that any discomfort and suffering will be minimised.

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

The aim of this project is to study brain development which involves a complex choreography of different cell types in an ever-changing environment. Currently, the mouse remains the best options as there are (1) no suitable in vitro experiments that can mimic the in vivo environment, (2) less sentient animals such as drosophila, worms and zebrafish lack a cerebral cortex and in some species (e.g. drosophila) microglia and (3) the brain development process in mice is relatively 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 handing and care will be conducted in a manner that minimise stress whenever possible. This would include using techniques such as tunnelling. We also aim to minimise any distress in animals while performing behavioural tasks. In the Morris water maze, animals are subjected to 5 trials per day over 7 days where the aim of the test is for the animals to learn the location of the hidden platform. Each trial last for 1 minute followed by a 30 second interval in which the animals are left on the platform. To minimise distress, the water and room temperature will be maintained appropriately and any animals that failed to find the platform after the 1-minute trial will be guided directly to the platform. For rotarod, transient stress may occur when the animal falls from a short distance from the rotating rod. We will minimise stress and avoid potential injury by padding the surface in which the animals will fall onto.

Special care will be given to all animals after a surgical procedure or prior to any animal behaviour experimentation where they will be gently handled and habituated in order to minimise stress inflicted by the procedures. This will include increased monitoring after any surgical procedure which includes post-operative care and analgesic if required. In addition, we aim to reduce the duration of all surgical procedures. We also aim to minimise the time pups are kept separated from their mothers in order to reduce their distress. All new lab members will be trained on animal cadavers until they are competent to perform the surgeries.

Refinement can also be achieved in the type of mice we used. In full knockout animals, the gene of interest is systemically removed in the entire organism. Sometimes, the global removal of certain genes can induce multiple organ dysfunction that can impact the health and welfare of the animals. By using, specific genetically modified mice in order to manipulate genes in a specific subpopulation of cells when possible, we can mitigate the potential adverse effects that are observed in full knockout animals.

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

We will follow the guidelines and advices provided by the NC3Rs regional manager 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) and the N3CRs (https://nc3rs.org.uk/3rs-resources).

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

We will be working closely with the NC3Rs regional manager who will be able to advise us on the advances in the 3Rs and implement these advances effectively during this project. Furthermore, prior to the start of any animal experimentations, all lab members are required to undergo a 3Rs training course provided by the NC3Rs (https://nc3rs.org.uk/e-learning-resources) which will provide training on animal welfare assessment, euthanasia, and anaesthesia. In addition, all lab members are expected to be up-to-date on advancement in the 3Rs through literature in journals such as ILAR Journal online and Journal of Applied Animal Welfare Science.