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NON-TECHNICAL SUMMARY

Investigating how brain immune responses drive behavioural symptoms associated with mild and repeated traumatic brain injury

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Mild Traumatic Brain Injury, Concussion, Immune system, Inflammation

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 aim of the project is to understand how the immune system responds to a mild traumatic brain injury (mTBI) (also known as concussion), and repeated mTBI (rmTBI) and how it affects post-injury symptoms.

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?

Mild Traumatic Brain Injury (mTBI), often referred to as concussion, is the most common form of traumatic brain injury due to accidents, assaults, domestic violence, contact sports and war. The use of the term 'mild' to define this type of injury is currently under debate by clinicians, as this does not represent an accurate description of the consequences for some patients.

The immediate effects of mTBI are well recognised, such as a brief loss of consciousness, headache and even short-term memory loss. However, it is now understood that in the long-term, mTBI, and particularly repeated mTBI (rmTBI), can cause mental health issues such as anxiety and depression, or even dementia and neurodegenerative disease in later life.

We are beginning to understand that mTBI is more dangerous than we thought, though we still don't know why.

Animal models suggest that the immune system can play a pivotal role in both the damage and recovery of the brain after injury and during disease. However, much less is known of what specific role the immune system plays in mTBI. We hypothesise that an excessive immune response to mTBI and rmTBI is responsible for some of the long-term symptoms after head injury.

Here, we have an opportunity to understand the underlying biology of mTBI and rmTBI and pave the way for treatments in those suffering the long-term consequences.

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

Outputs from the project:

1) we will gain greater understanding of the nature of the immune response to mTBI and repeated mTBI (rmTBI) in a mouse model relevant to human injury.

2) we will understand if this immune response can be modified to improve behavioural symptoms after injury.

3) we will publish the results in open access, high-quality scientific journals and present the work at relevant conferences

4) we will share our results with collaborating scientists and ultimately make our data publicly available

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

As we are looking at a brain injury (concussion) that can result in mental health issues, such as anxiety and depression, the underlying biology will be of interest to immunology, neurology, neuroscience, psychology and psychiatry researchers.

In the long-term, researchers investigating treatment options for mTBI will benefit from the understanding of how the immune system contributes to injury and symptoms. Ultimately, this will benefit the patients themselves.

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

To maximise the outputs of this work we will:

1) Coordinate triannual meetings for our local institutes that cover immunology, neuroscience and barrier biology to update on our findings and receive feedback on the project. This will inform a wide range of immunologists, neuroscientists and clinicians working on brain injury and disease. We will organize biannual meetings with the local Concussion Research Group to disseminate findings to a wider scientific audience, but still within the realm of mTBI.

2) Disseminate data to a wider scientific audience and foster collaboration, by the traditional routes of national and international scientific conferences and seminar series, and use our active social media account to highlight our own and others' findings.

3) All findings will be published on open-access preprint servers (i.e. biorxiv), including negative data sets.

4) Any outputs will be assigned a digital object identifier (DOI) to facilitate sharing and accessibility of them.

Species and numbers of animals expected to be used

- Mice: 4500

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 will be using adult mice to investigate the biology of mild Traumatic Brain Injury (concussion).

Though it is clear that differences exist between mice and human brains in terms of structure, function and geometry, there is still substantial similarity in the physiology, including the immune and inflammatory response. The study of basic biology of mTBI is not possible in humans and raises significant ethical concerns in non-human primates. The ethical concerns in rodents have been considered and the overall benefits deemed appropriate. Lower species than rodents were considered, but due to the mouse brain's similarity to humans, and that measurement of behavioural symptoms is well characterised in mice and is a key part of our proposal, we have chosen this species.

Children and adolescents are also at risk of mTBI, however, the developing brain can be quite different from that of an adult. Therefore, this proposal will focus on the biology of adult mice after mTBI to ultimately understand the response in adult human injury.

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

Wild type and genetically altered mice will be used to investigate the immune mechanisms that may be important in brain injury. Whilst under general anaesthesia, adult mice will receive a single mild impact to the head, or repeated mild impacts to the head, referred to as a repeated mTBI (rmTBI), from a controlled pneumatic impact device. Mice will not undergo any surgical procedures relating to the impact of their heads. Anaesthetised mice will lie, unrestrained on a sponge platform, allowing free movement of the head after impact, to represent the situation in human mTBI. Each mTBI, including induction of anaesthesia takes no longer than 15 minutes and analgesia will be provided following the procedure.

The model has been refined over a number of years and is reproducible. Mice will be closely monitored following the procedure and returned to their home cage after recovering from anaesthetic.

Mice may undergo behavioural testing following sham (a control group that mimics all aspect of the mTBI procedure, except the impact itself), mTBI or rmTBI or naive controls to assess their neurological, emotional, and cognitive function in tests such as elevated plus maze and novel object recognition. Mice are habituated to the environments where these tests are carried out and are painless, minimally stressful and non-invasive.

To investigate the underlying biology, in wild type or genetically altered mice, substances may be administered prior, during or after the mTBI or rmTBI to manipulate the immune response to head injury.

Standard routes of administration will be used, such as oral, intraperitoneal, intravenous or subcutaneous but also direct injection to the central nervous system. This will require general anaesthesia and surgical exposing of the skull using aseptic technique. Different routes can help accessibility of administered molecules or drugs to the brain. Administration procedures requiring surgery will only be performed once per animal and analgesia will be provided during/following your surgical procedures.

To reduce the numbers of animals used we may also include imaging techniques such as magnetic resonance imaging (MRI) to investigate the brain without sacrificing the animal and therefore produce time course data in one animal, not multiple animals.

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

mTBI is typically associated with a brief depression in breathing, followed by a prolonged time to recovery from anaesthetic. Following waking from anaesthetic, animals may appear unwell (self isolate, reduce movement), which typically gets better within one hour. After this, animals appear normal. With a single mTBI, sensitive behavioural tests cannot distinguish animals that have had a mTBI compared to animals that have not. With repeated mTBIs, animals may show signs of behavioural change with sensitive testing, but they typically recover from each injury as mice do after a single mTBI.

Our previous data shows that mice usually lose no more than 5% body weight 24 hours after, which returns to normal after 2-3 days. This is not exacerbated by repeated mTBIs.

Our previous experience indicates that in a proportion of animals, breathing does not return to normal and the animal does not wake up from anaesthetic (<5% animals).

Injections to the cisterna magna, a fluid filled region at the back of the brain, are performed under anaesthesia, as a result animals have few associated adverse effects. Local bleeding is possible after surgical incision of the skin.

Animals will experience stress or transient discomfort during restraint for injection of substances or inhalation of gaseous anaesthetics (100% incidence).

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)?**

20% non recovery

30% mild

50% moderate

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?

We currently perform some of the most advanced neuroimaging in people with mTBI, yet we still do not have the ability to investigate basic biological mechanisms at the cellular level in the human brain. Obtaining brain tissue is not possible. We aim to understand how the immune system regulates complex behaviours after a brain injury, which can only be done in animals.

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

Cerebral organoids (cells grown in vitro to resembles an organ), were considered to assess cellular processes. Cerebral organoids, are three-dimensional (3D) clusters of human stem cells that mimic some aspects of the developing human brain, including cell types and tissue architecture. Whilst not a full alternative to the use of animals, we did consider use of non-protected species such as larval stage zebrafish and fruit flies. If we do find specific immune cell populations are responsible for modulating behaviour, we will develop human Induced pluripotent Stem Cells (iPSC) 'mini-brain' organoids and examine the direct effects of these populations on human neurons.

Why were they not suitable?

Cerebral organoids lack a blood supply, key immune cells and most complex functions. Importantly, they are not relevant to the study of behaviour. Some behavioural outputs are possible in fruit flies and zebrafish, but their immune systems are distinct from mammals and complex behaviour is limited.

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?

From analysis of our previous studies and consultation with a statistician and use of the National Centre for the Replacement, Refinement and Reduction of Animals in Research 'Experimental Design Assistant' (NC3R's EDA) has been used to calculate the number of animals needed.

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

Multiple read-outs will be measured in the same experiment, e.g. multiple behavioural tests measuring different aspects of behaviour in the same mice and a full range of tissue taken and analysis performed from the same animal after it is killed.

The NC3R's experimental design assistant has been used, and will continue to be used, to determine the minimum number of animals to provide sufficient power to analyse relevant effects sizes of treatments.

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

For breeding and maintenance, the number of animals born from genetically altered animals will be reduced by following good colony management strategies described on the nc3rs website, such as holding stock but not breeding, intermittent breeding when necessary. Behavioural studies will use an optimal number of tests to maximise the information from each animal. The number and order of these tests has been previously validated to ensure each parameter can detect change in behaviour without overstressing the animal.

Pilot studies will be undertaken when starting new experiments to inform experimental design, i.e. power calculations, again optimising animal use.

A continued effort will be made to share tissue from experimental animals.

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.

A mouse model of mild Traumatic Brain Injury (mTBI) and repeated mTBI (rmTBI) (often referred to as concussion) will be performed. Mice will be under general anaesthesia during the head injury and peri-operative pain medication will be administered which will reduce stress and pain for the animal. Mice will not undergo any surgical procedures relating to the impact of their heads.

Mice will be closely monitored following the procedure and quickly returned to their homecage with their littermates after recovering from anaesthetic to reduce stress.

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

Less sentient species than mice were considered, but due to the mouse brain's similarity to humans, and that measurement of behavioural symptoms is well characterised in mice and is a key part of our proposal, we have chosen this species. Terminal anaesthesia is not appropriate due to subtlety of signs requiring complex behavioural assessments.

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

We have now extensively refined our model from our previous licence, including secure anaesthetic facemask, reproducible impact site and adding multiple injuries without increase in adverse events. We

continue to provide close monitoring and pain management post injury. We always keep up to date on the latest guidance and will undertake training when required to improve how we handle and perform procedures on animals.

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

All relevant nc3r's guidance and updates will be engaged with, including sign posting to published studies, e.g. FRY, D. 2014. Chapter 8 - Experimental Design: Reduction and Refinement in Studies Using Animals. In: TURNER, K. B. V. (ed.) Laboratory Animal Welfare. Boston: Academic Press and the ARRIVE guidelines for reporting animal research (Kilkenny et al., 2010) - Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving bioscience research reporting: the ARRIVE and PREPARE Guidelines for reporting animal research. PLoS Biol 8:e1000412 and Morten et al 2001 for routes of administration and volumes: doi.org/10.1258/0023677011911345

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

Continued engagement with the nc3R's resources will be undertaken. In particular continued use of the EDA will facilitate regular assessment of the 3R's and recent developments whilst also enhancing experimental design. Monitoring the model/disease-specific scientific literature for advances that may improve 3Rs and be applicable to this project

Attendance of workshops organised by the animal facility will be undertaken as well as continued consultation with the nc3rs officer and the establishment 3Rs manager and NIOs.