NON-TECHNICAL SUMMARY (NTS)

Project Title	Injury mechanisms and therapeutic targets for perinatal brain injury
Key Words	brain injury, inflammation, therapy, neuron production, vascular development
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(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 aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

The overall aim of this work is to understand normal developmental processes in the brain, and how these processes are perturbed by inflammation or other injuries. It will identify mechanisms of injury during gestation or in the perinatal period that have long-term effects on brain function and animal behaviour. We will model mechanisms of injury observed in the clinical to identifying and test novel therapeutic agents.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

This work will identify process of brain injury that can be targeted with drugs to improve the lives of significant numbers of babies and their families by reducing the severity of injury. We are also asking novel questions about how the brain develops and the processes by which it may be injured. This will be of substantial interest to all researchers in the field of brain development. In addition, we aim to identify sensitive and specific biological markers of injury (e.g. with MRI), that will aid detection of injury and selection of patients for appropriate future therapy. In the laboratory, this work will ultimately reduce the number of animals required for experiments, as each animal will be able to be imaged at multiple time point. Currently animals need to be killed at all time points so that the results of the MRI can be compared and verified by other methods.

What types and approximate numbers of animals do you expect to use and over what period of time?

A maximum of 8000 mice will be bred for this project, both transgenic and wild type, and either the pregnant animals or the newborn pups used for experiments. Up to 1000 pregnant mice will have either treatment of the mother or foetus directly. The effect of these interventions on the foetal (and early postnatal) brain development will be assessed hours or days after the intervention. Up to 6800 of the breed animals will be used postnatally, with interventions occurring over the first three postnatal weeks, and with outcomes measured within minutes to hours through to adulthood. Preterm brain injury will be modelled by inflammation and oxygen deprivation.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

In our maternal inflammation models, mothers will typically receive one or two injections and experience mild ‘flu like symptoms. Alternatively, the mothers will be anaesthetised and the foetuses will receive an injection directly to the brain. The maternal surgery is quick, and pain relievers will be used. In both cases, the mother and the pups will be killed by Schedule 1 methods either before or after birth, possibly after a second injection of a potential therapeutic agent. The overall severity of both these protocols is moderate. In newborn mice, at different times over the first week of life, mechanisms of injury and therapy will be explored in by either peripheral or direct brain administration of inflammatory agents or other brain signalling molecules, or by reduced blood supply to the brain. If a direct brain administration route is required, or for producing reduced blood supply, surgery will be performed under anaesthesia and with pain relief and is expected to only cause short-term pain and distress. The overall severity of this is moderate.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Our project aims to understand the mechanisms involved in early life infectious/inflammatory and oxygen deprivation-induced brain injury and to test neuroprotective strategies. As such, we need models that allow us to mimic human brain injury that occurs before or at birth, in systems where we can modulate the genetic and environmental stressors of injury. In particular, our experiments take a whole body approach, considering how multiple cell types interact over development and injury, both within the brain and influenced by the rest of the

body. Experiments are required that cannot be conducted in humans for ethical and scientific reasons. In addition, for pharmacological studies, distribution of the drug in whole organisms, with functioning organs, is key to inferring potential clinical use.

Where possible we will replace whole animals studies with primary cell preparations or experiments in appropriate cell lines (e.g. for neuronal stem cells, microglial, oligodendrocytes, endothelial cells) for our preliminary and proof-of-concept studies.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Our intention is always to use as few animals as possible for ethical reasons. At the same time sufficient numbers have to be used to ensure accuracy of results obtained. Through our previous work, we have optimised the conditions for the proposed experimental models, therefore ensuring the reproducibility of the injury and reducing the number of animals needed for each experiment.

Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

There are other animal models we could use, some in which the brain structure is more similar to that of a preterm or term human (foetal sheep, primates, piglets). However, we have decided to replace the use of such animals with mice, without detriment to the science. This specie is considered to be less sentient, easier to handle, breed easily and have a short generation interval. Importantly, mice share several important features with the human brain with regard to brain complexity and injury response in white and grey matter and thus can be considered a valid model in which to deliver the objectives of the project.

While mice are small, and there are difficulties in performing complex surgical procedures in these animals, they have the advantage of responding well to anaesthesia and pain relief measures and recovering quickly from interventions. Our team has sufficient expertise in small animal surgery to ensure procedures are as minimally invasive and quick as possible to reduce the cost to the animals.

The brain injuries produced are mild and there is no evidence (from monitoring normal behaviour and interactions in their home cage) that the mice experience distress or pain subsequent to the initial injury induction.