Home Office

NON-TECHNICAL SUMMARY

Developmental Dynamics of Tissue Formation

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

developmental biology, spinal cord, central nervous system, vertebrate embryology

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

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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?

How are the right types of cells produced in the right place, at the right time, in the right numbers in a developing embryo? We study these questions in the central nervous system (CNS). Despite its complexity, the CNS is assembled in a remarkably precise and reliable manner. This precision is necessary for the wiring of nerves into the functional neural circuits that gives the CNS its function. Our research focuses on the spinal cord, which is the part of the CNS that allows us to sense our environment and respond to it by moving muscles. Our goal is to understand how the spinal cord forms during embryonic development by determining the mechanisms that produce and organise the cells involved.

A retrospective assessment of these aims will be due by 23 March 2027

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

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?

Understanding the embryonic development of the spinal cord will shed light on the formation and function of the adult spinal cord. Understanding the molecular and cellular processes of spinal cord development is important for developing stem cell based methods for the generation of artificial spinal tissue for use in regenerative medicine and disease modelling applications. Moreover, knowledge of normal spinal cord development will provide insight into the diseased and damaged nervous systems. Neurodegenerative diseases such as motor neuron disease, tumours such as paediatric glioblastomas, and congenital disorders such as spina bifidia all involve the spinal cord. Understanding how the spinal cord forms in embryos will help in the development of therapies and treatments for these severe conditions.

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

The main goal of this work is to advance our biological understanding of embryonic development and the outputs from the programme will include new knowledge and publications in peer-reviewed journals.

Expected outputs include:

1. A better understanding of how the actions of a protein, Shh, organises the pattern of gene expression in the spinal cord.

2. Knowledge of how gene regulation in individual cells allocates developing progenitors to one of several possible fates.

3. Insight into how gene activity is controlled by the actions of specialised regions of the genome.

4. Genetically altered lines of mice that contain reporters for specific molecular activities or specific mutations in defined genes. These may be of broad benefit to the research community as they can be used in numerous other projects.

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

Knowledge of the basic mechanisms of embryonic development will be of interest to scientists studying development and stem cell biology and is essential to understand the causes of dysfunction in disease states. The availability of this knowledge will lead to a better understanding of tissue function and is likely to be employed and extended by other researchers. These intellectual impacts have practical significance. First, it is now well established that, if deregulated, basic developmental processes can result in disease states that range from neurological disease, such as autism, neuromuscular diseases, to cancers such as gliomas. This will be of interest to clinical researchers studying these diseases. Second, the ability to direct the differentiation of stem cells to specific cell types will be a major impact of this project. Given their unique properties, stem cells are promising candidates for tissue engineering, cellular therapies and drug screening. A significant problem, however, is generating populations of desired cell types from initially pluripotent stem cells. We anticipate that based on new knowledge of the basic mechanisms of development uncovered in this project, improvements in the current state of the art approaches for stem cells will be forthcoming.

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

We will maximise the outputs of our work by the timely publication of primary research papers and the presentation of the work at both big international meetings and smaller workshops. Not only will the positive results be communicated, but where it is useful for other researchers, unsuccessful approaches will also be highlighted.

We collaborate widely, both internally and externally and this provides another avenue to share details of approaches that were ultimately sub-optimal and how methods were improved.

Where will release pre-peer reviewed versions of our work on bioRxiv to ensure its rapid dissemination.

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.

Our aim is to unravel the cellular, molecular and genetic mechanisms of embryonic development of a vertebrate tissue. Use of animals and tissue derived from animals is essential for this. In particular, the generation and analysis of genetically altered mouse embryos is necessary to test the function of specific genes and this requires the breeding of genetically altered mouse lines. The similarity between the human and mouse nervous system means that it is necessary to work with mice. Mice offer the unique advantage of offering sophisticated genetic tools that allow precise functional experiments and the ability to construct quantitative reporters of gene activity.

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

The vast majority of our regulated procedures involve the breeding of genetically altered animals or minor interventions such as injections, with minimal effects. Typically several hundred matings of genetically altered mice will be performed each year. These will be used either to maintain specific genetic lines or to produce embryos with specific genetic make up in order to study gene activity. Injections will be used to introduce substances that alter gene activity or label replicating DNA, these substances typically have little if any noticeable effect on the animals. Some of the genetic mutations we use may directly lead to mild effects on the animals, such as mice having more than the usual number of toes. Some procedures involve surgery. These surgeries are necessary for the creation of new genetically altered animals and involve transferring embryos into uterus of female adult mice. Such animals will be closely monitored, and anaesthetics, analgesics and/or other ameliorative procedures will be used as appropriate. In all cases, animals will be humanely killed if there are signs of pain, distress or suffering above agreed limits. We are careful about group sizes, using the minimum numbers of control and experimental animals compatible with robust conclusions, making use of statistics when appropriate.

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

For the majority of experiments we do not expect any adverse effects as we will be mainly working with genetically altered animals that have little if any noticeable negative effects on the animals carrying the genetic alterations. Some the genetic mutations we use to study the spinal cord may also lead to mild effects on the animals, such as mice having more than the normal number of toes or small eyes. These malformations are lifelong but do not cause distress or suffering. Some of our procedures involve surgery, these are carried out under appropriate anaesthesia. Post-operative pain is managed with analgesics and full recovery is usual within one or two weeks.

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)?

Moderate severity is expected for about 5% of mice. For particular genetically altered strains, between 5-10% of genetically altered mice die suddenly, without showing any prior health concerns. As the reason for death is unknown, we cannot exclude any suffering prior to the event, those mice will be deemed to have had a severe experience. The rest of the mice will reach a maximum of mild severity.

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

- Killed

A retrospective assessment of these predicted harms will be due by 23 March 2027

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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 have been studying vertebrate embryonic development for 25 years and have gained tremendous insights into the mechanisms of tissue formation and the molecular and genetic control of cell behaviour from working in model tissue culture systems. However, in order to be able to investigate tissue formation in embryos, we need to perform experiments in animals.

We use mice because, as a mammal, their embryonic development closely resembles that of humans. Moreover, sophisticated genetic and transgenic tools are available that make it possible to generate mutant or transgenic lines in a highly precise and efficient manner. To minimise the number of procedures performed on animals deemed to be sentient, we do most of our work with embryos before they have reached 2/3 of their gestation time. The only work that will be done on adult animals is biopsying for genotyping, administration of substances to control gene expression and surgical procedures necessary in the generation of new transgenic lines.

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

We complement our in vivo analyses with the use of tissue culture models and organoids. Our studies are likely to further validate these in vitro systems and promote their use with other researchers. Nevertheless, in the development and validation phase it will be necessary to carefully compare and benchmark these methods with normal embryonic development.

Why were they not suitable?

We gain a certain amount of information from in vitro systems, but there are limitations. It is not currently possible to replicate the complexity and precision of mammalian tissue development in culture models. The complexity of embryonic development, which arises from multiple interactions between different cell types, involving short and long range signalling molecules, and complex morphogenetic events over time, requires in vivo analyses

A retrospective assessment of replacement will be due by 23 March 2027

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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 calculate the numbers of animals required based on the numbers of mutant and transgenic lines that we are currently maintaining, plus those that we need to generate to be able to achieve the aims of the project. We carefully design experiments to be sure that we use the minimum number of animals required to give clear scientific answers. We also make extensive use of in vitro assay, in particular cell culture, and in silico mathematical modelling and simulation. This greatly helps experimental design and reduces our use of animals. We also use several hundred chick embryos every year, from embyonated eggs before 2/3 of the gestation period. The accessibility of chick embryos allows us to do experiments that would otherwise have to be performed in mouse embryos and require the termination of the pregnant female. This results in a substantial reduction in the number of animals we use.

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

We regularly review our mutant and transgenic stocks and cull any that are no longer required. We freeze sperm and/or embryos to archive the line. Thus, we will only maintain breeding lines that we are actually using in on-going experiments. Through exchanges with other labs, in the UK and elsewhere, we are able to minimise the number of mutant and genetically modified strains that we keep. Moreover, the stocks of adult animals that we keep are mostly heterozygotes carrying recessive mutations and are phenotypically normal. In addition, because we share and exchange mouse lines with a number of other labs, we ensure we decrease the number of animals in use.

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

We will aim to keep as few mice as possible by careful monitoring our mouse colony and good practice. To minimise breeding, lines under sporadic use are maintained at lower levels, and frozen whenever practicable. Lines will be maintained in collaboration with other licensees wherever possible to minimise redundant breeding.

Our mouse lines are routinely maintained by keeping 2-3 breeding pairs, with around 3-4 litters/year - total 75-100 animals per strain/year. For crosses to enable characterisation of specific phenotypes, in general 5-6 breeding pairs will be kept with 6-8 litters/year - total 350-400 animals per strain/year.

We use local expertise as well as consult with collaborators to optimise the number of animals used.

Whenever possible and when there are no harmful phenotypes we maintain genetically altered mouse lines in appropriate genetic combinations of alleles to reduce the numbers of animals required.

A retrospective assessment of reduction will be due by 23 March 2027

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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 use mice as a model for mammalian development, as mouse embryos share key features with humans and can be manipulated genetically. Our embryological work is preceded by in vitro and/or in silico studies to test the approach or experimental design before introduction into animals. Failure at this preliminary stage is taken as final and no in vivo work will take place until this step is successful. In addition, we minimise suffering by maintaining a high health status of the animal population, by attention to feeding regimes and environmental enrichment of cages. We check all stock daily and cull any that show signs of significant illness or deformity. Where surgical or other potentially distressing procedures are required, these are performed under appropriate anaesthesia with analgesia both pre and post operation. Any animals showing signs of distress on recovery from a surgical or other procedure are killed promptly by an approved method.

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

The majority of our work is carried out on embryos prior to 2/3 of gestation and these are not considered sentient. However, to produce these embryos breeding of adult mice is necessary and the generation of new mutant or transgenic lines entails surgical operations followed by recovery of the operated animal. We use mice as a model for mammalian development, as the development of the nervous system and other organ systems of mice shares key features with humans.

For some experiments we use chick embryos. In many cases the similarity in molecular and cellular mechanisms between different vertebrate species allows experiments to be performed on non-mammalian species and the results can be extrapolated to mammals. Nevertheless, in some cases the use of mice is unavoidable.

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

We try to minimise any possible adverse effects. Surgical procedures will be performed aseptically with suitable anaesthesia and animals monitored post-surgery to ensure that they recover well. We will also use suitable analgesia for all surgery.

We choose well-established protocols, known to have minimal harmful effects, whenever possible. Animals produced in this project are not expected (<5%) to exhibit a moderate phenotype. However, it is not possible to fully predict the nature or severity of any potential defect and for all types of mice there will be careful monitoring for possible side effects. Animals exhibiting any unexpected harmful phenotypes will be killed using an approved method, or in the case of individual animals of particular scientific interest, advice will be sought from the Home Office Inspector.

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

We are aware of NC3Rs. We also discuss with colleagues in other research groups and the BRF new improvements that lead to refinement.

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

We will stay up to date via regularly communication with BRF staff, other scientists in the field and regular visits to the following website https://www.nc3rs.org.uk/3rs-resources

A retrospective assessment of refinement will be due by 23 March 2027

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?