Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted in 2018

Contents

1. Cross-talk between the brain and the immune system

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

Cytokine, inflammation, liver, microglia, astrocyte

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

It has become clear that events throughout the body play a significant role in controlling the response of the brain to injury and disease. The principal goal of the work described here is now to discover how inflammation both in the brain and in the rest of the body can affect brain function. We will build on our discoveries using rats and mice which showed that the injection of immune molecules into the brain results in a rapid response by the liver and spleen. This response by distant organs coordinates and control the body's response to brain injury. In our previous experiments suppression of the liver response reversed brain injury. Thus, targeting events in organs distant from the site of injury can change the outcome of brain disease. Under this licencewe will principally use rats and mice to continue to explore how, infection, diet, affect brain disease and how brain disease impact on the body's ability to cope with infection.

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.

What are the potential benefits that will derive from this project?

The results of our preceding in vivo work have been published in at least 50 peer-reviewed articles. Our collaborators, also working under our previous licence, have also published

widely. We expect the results obtained under this new licence to translate to new therapies, and we expect our work to lead to the development improved medical imaging for use in the clinic. Our on-going work has provided the first evidence that inflammatory responses in peripheral organs, such as the liver and spleen, after acute or chronic injury in the brain is responsible for controlling the magnitude of the brain inflammatory response and the associated depression-like behaviors. We have subsequently shown that liver-produced immune molecules can be manipulated with diet or therapy to improve behavior and we will use the new licence to better understand the underlying molecular basis of these observations. Under this licence we also expect to show that structures within the brain can be imaged with scanners to reveal 'invisible pathology' early in disease that cannot be seen with conventional imaging methods. We expect our work, under this licence, to generate new patents and translational discoveries. Animals used on my previous licence helped us to show that blood and urine analysis could be used to diagnose and monitor progression in individuals with multiple sclerosis; under this licence we will use our animal models to better understand the meaning of these molecular disease signatures. And finally, we have been able to show that it is possible to selectively deliver therapy to sites in the brain where tumours have spread. Here, we will continue to search for new agents that can be used to deliver vital therapy to the brain that is normally excluded by the blood-brain barrier. Thus under this licence we will continue to move our important work forward to generating tangible benefits for UK citizens.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Mice and rats and over the 5-year period. 10 scientists working on this project will expect to use approximately 1600 rats and 3000 mice. This is based on our ongoing experience of these experiments.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

It should be noted that we will use long acting local anaesthetics for all surgical procedures and we do not expect our investigations to induce anything more than mild flu-like sign in some animals. The use of imaging techniques reduces the overall number of animals required. Most of our models of human disease are clinically silent and we use these wherever possible. However, some animals might exhibit temporary weakness after the generation of a focal stroke-like lesion, but otherwise we expect no clinical signs. Some animals (where the chronic inflammation is targeted to the gut) might develop diarrhoea. The our chosen model of colitis (less than 2% of animals employed) the pathology is very mild and will expect to see no overt changes in behaviour; if animals appear overtly unwell they will be culled. The animals will all be killed at the end of our experiments.

Replacement

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

In the past we have established features of the host response to brain injury and disease in whole animals that could not have been predicted from existing knowledge or have been achieved in cell culture experiments. By the nature of this project it is important not to confound our data by using animals that are stressed or ill. Experienced licensees will gently handle animals and behavioural experiments will be performed in conditions where sound, light and heating are controlled. Many of the molecules that we know are important in the brain-immune system communication pathways are not expressed in fish or flies.

Reduction

Explain how you will assure the use of minimum numbers of animals.

We will use state-of-the-art imaging techniques, molecular biological that require less tissue and behavioural experiments to examine the effects of the inflammatory response in the whole body and in the brain on the evolution of pathology in the brain. Wherever possible we will try to sample tissue or image in a serial manner to reduce the number of animals required. We use established statistical methods to ensure that the most appropriate number of animals are used in our experiments and we use archival material or surplus animals wherever possible.

Refinement

Explain the choice of species 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.

The complex regulation of the immune system involves a neural component, which will we also be studying. Rats and mice can be used in our experiments to closely model the human immune responses to brain injury and disease, which is not possible in non-mammalian species. All animals undergoing surgical procedure will receive pain relief. Wherever possible, we will combine behavioural experiments with tissue collection for microscopy and molecular analysis in order to increase the amount of data that can be collected from one animals. Tissue will also be 'biobanked' and retained for experiments in the future. Most animals (75%) will be killed within 24 hours of any surgical procedure, and in those animals that survive for more than one month (about 10%) - such as those employed in our studies of multiple sclerosis pathology - we do not observe any overt behavioural changes that would enable them to be distinguished from untreated animals.

2. Analysis of mouse models of neurobehavioural and neurodegenerative disorders

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

brain, neurodegeneration, behaviour, epilepsy, psychiatry

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
- Required at inspector’s discretion

Objectives and benefits

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

What's the aim of this project?

The aim of this project is to analyse novel mutant mice to provide new insights into the molecular basis of neurological diseases.

Many people will suffer from a neurological disorder at some time in their lives. Some of the most common and devastating of these lead to cell death in the brain, including Parkinson’s Disease and Motor Neuron Disease. In addition, psychiatric disorders are very common with up to 1 in 10 adults suffering from a serious mental disorder in a given year. However, the fundamental processes behind many diseases of the central nervous system are still unclear and very few treatments are available.

One effective way of addressing this problem is to model these diseases in the mouse with the ultimate aim of testing new therapeutic strategies. Mice share many of the basic biological features of man; their brain structure is also very similar to humans, and

behavioural tests can be carried out to model features of psychiatric disease. Although rodent models exist for some neurological disorders, many have no corresponding mouse model or the specific gene mutations found in humans have not been studied.

Our in-vivo work is primarily based on mice. Using a simple battery of behavioural tests, new mutant lines that might display a neurological defect are identified. We also make new mouse models that contain the same change in their DNA as a patient with a neurological disease. We have a particular interest in movement disorders and mutants that show abnormal walking / gait are selected for further study. We then carry out a detailed pathological screen to identify abnormalities in the central nervous system and muscle that might explain the phenotype. We also use biochemical techniques to understand the function of the gene that causes the behaviour. Further behavioural testing may be carried out on these new lines, and we may also age the animals, with close monitoring, to model aspects of human diseases that occur in old-age.

A retrospective assessment of these aims will be due by 06 June 2024

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its 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.

What are the potential benefits that will derive from this project?

Under this project, the study of selected mice with mutations in both known and novel genes will provide valuable insight into the mechanisms of human neurological disease. These results can then be applied to benefit the identification of molecular targets for new therapeutic strategies. For example, sufferers of many of the most serious neurodegenerative disorders begin to show irreversible symptoms late in life. The study of new mouse mutants allows early pre-symptomatic markers or pathways that cause degeneration to be identified as potentially valuable targets for therapeutic intervention; these could not be discovered from cellular or human tissue experiments alone.

In addition, our findings will continue to be published in high-impact journals, not only benefiting scientists interested in neurological disease pathways but also to those that work on the mutated genes in other fields of biology.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Approximately 53,500 mice over 5-years.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

To model neurological diseases some of the mutants may display common behavioural features to humans; for example, progressive loss of neuronal cells in part of the brain that causes movement problems. Such mice will be carefully monitored throughout life. To quantify the behavioural changes and identify the earliest signs of disease, sets of tests will be carried out at several times during the animal's lifetime. These will include the analysis of gait and co-ordination as well as more complex behaviours such as the ability to learn and remember simple tasks. In addition, we will try to understand why stressful life events during pregnancy increase the likelihood of a child developing mental health problems, such as schizophrenia, later in life. To model these findings, we will put pregnant mice in situations that cause them to feel stress for a short amount of time, such as being placed in unfamiliar surroundings. We then study the offspring of these particular mice when they reach adulthood to determine whether they show aspects of behaviour that relate to human mental health disorders; for example, their ability to pay attention to a novel stimulus, such as a sound or flashing light. At the end of these studies, the mice will be humanely killed to collect tissue for pathological studies. The level of severity for these studies is expected to be moderate. We will also investigate the causes of epilepsy, a very serious neurological disorder that causes seizures; therefore we might see seizures in some of our mutant models to help us understand what is happening in the brain. The level of severity may be severe in some of these cases.

Other mouse models will be studied or generated to examine the function of a disease gene where noadverse effects are detected. The mouse model will still be a vital tool for the study of the gene in human disease, but the level of severity for these studies will be mild.

A retrospective assessment of these predicted harms will be due by 06 June 2024

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

The need for live animals is essential to develop effective models for human neurological disease and to study defects in complex behaviours such as learning and social interaction, which are important for understanding these disorders, but cannot be examined in cells or lower organisms such as flies or fish.

By using mice for pathology after behavioural studies are complete, we can avoid breeding unnecessary animals for both purposes independently.

In parallel with these studies, to understand the function of the mutant gene we actively carry out much of the work in mammalian cell lines. This is an efficient method, as genes can be manipulated more easily than in a whole organism; this way we can also reduce the number of mice that we use and carry out pilot studies prior to starting additional animal work.

A retrospective assessment of replacement will be due by 06 June 2024

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 you will assure the use of minimum numbers of animals.

By using mice for pathology after behavioural studies are complete, we can avoid breeding unnecessary animals for both purposes independently.

In parallel with these studies, to understand the function of the mutant gene we carry out much of the work in mammalian cell lines. This is an efficient method, as genes can be manipulated more easily than in a whole organism; this way we can also reduce the number of mice that we use and carry out pilot studies prior to starting additional animal work.

We will always use good principles of experimental design to ensure that we use the correct number of animals for each experiment to generate statistically meaningful results.

A retrospective assessment of reduction will be due by 06 June 2024

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

Explain the choice of species 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.

The mouse is a vital mammalian system used to model a wide variety of brain disorders. The anatomy of the mouse brain is close to that of a human to allow studies of specific structures or groups of cells that would not be possible in a lower species. This is important as many neurodegenerative disorders are characterised by loss of certain neuronal cell types. In addition, the mouse is also suited for behavioural testing and pharmacological interventions that are directly applicable to humans.

For behavioural testing, a single mouse might be tested a number of times in their lifetime to help us understand the progression of a disease model that will mirror the stages of a

certain human disease. Here, we will ensure that less harmful tests are carried out first, before undertaking more complex tasks if required, to minimise the stress to the animal. In addition, we will use the latest and most refined versions of these testing methods that that cause the least harm but still provide meaningful data.

For models of human neurological diseases such as epilepsy, we will use new video-tracking methods to observe mice over 24-hours and identify any potentially harmful behavioural features, for example seizures, that may otherwise be difficult to detect. This way, we can minimise suffering by defining when these harmful events happen; for example, if they occur at a particular age. Furthermore, combining these methods with measurements of brain activity (EEG) will allow us to detect small but important changes in brain function that we can still study and try to correct, but the sensitivity if the method means the mice will not necessarily display the potentially harmful effects of seizures.

Regular examination of the animals by trained staff and experienced technicians will ensure that steps are taken to minimise any distress or discomfort to the animals. Veterinary advice will always be sought where and when necessary.

A retrospective assessment of refinement will be due by by 06 June 2024

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?

3. Immune and therapeutic aspects of neurodevelopmental disorders

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants.

Key words

Neurodevelopmental disorders, animal models, therapeutic strategies.

Retrospective assessment

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

Objectives and benefits

Description of the project's 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 program of work is to understand how prenatal/early postnatal factors and exposure to drugs can induce and/or affect behavioural, genetic and molecular alterations that are found to occur in autism and comorbid disorders. Our primary aims are to understand:

The role of prenatal and early postnatal factors on autism-relevant phenotypes in rodents.

The functional role of genes relevant to autism (using genetically modified rodents).

The effects of drugs and environmental manipulations (e.g., environmental enrichment) on aberrant and normal behaviour in rodents.

If we can start to clarify these three dimensions we will be much better placed to understand ASD and comorbid disorders.

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.

What are the potential benefits that will derive from this project?

The aim of this project is to provide mechanistic explanations for behavioural and cognitive aberrations observed in autism and comorbid disorders. We will study normal, genetically modified and environmentally induced animals models and their reactivity to environmental challenges and drugs.

The ultimate goal of the project is to find interventions, treatments, and biomarkers that might identify, prevent, alleviate or reverse autistic behaviours.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

It is expected that no more than 600 mice and 120 rats will be used during the 5 year-course of this project (as described on the project, when possible and beneficial for the animals and project, some animals will undergo more than one protocol).

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

The experiments will involve testing the ability of rats and mice to perform behavioural tasks in which they have to learn and remember crucial information such as where a food reward is or how to escape from a pool of water. We also assess anxiety, for example, by measuring animals' preference for a new place versus a known, 'safe' location. We will examine the effects of genetic mutations, drug treatments and environmental prenatal/early postnatal challenges on these behaviours. The animals will readily learn what to do to get a tasty food reward or how to climb out of the water. The expected adverse effects would include brief periods of distress during some of the behavioural tests (e.g. after exposure to loud noises). There may also be transient pain and discomfort after injections, which will be controlled by appropriate practices The experiments are of moderate severity. Animals will be humanely killed at the end of the experiments.

Replacement

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

In order to show that a particular bit of the brain, or a particular chemical neurotransmitter or receptor, is important for the brain to work properly, it is necessary to remove or silence that bit of brain, or remove or block the neurotransmitter from working. This is not ethical (nor practical) to be performed in humans. We are, however, collaborating with other research groups that use simpler animals, like fish, and simulations; we will aim to use

always the simpler alternative. We will keep analysing our findings during the project, to seek, review and incorporate alternatives throughout the project

Reduction

Explain how you will assure the use of minimum numbers of animals.

We will minimize the numbers of animals used by making both the behavioural tests and the experimental manipulations (e.g. drug delivery, genetic modifications) as accurate and sensitive as possible. Furthermore, when possible, inbreeds animals will be employed to reduce bias. When no established information about a given approach is available, we will employ pilot studies to avoid larger studies. We will seek to have the most efficient use of tissue/sample/resources obtained from animals, working in an ethical and collaborative manner. The project planning and experience with statistical analyses are useful to reduce the number of animals used. Video recordings will also allow further exploration of data generated per animal.

Refinement

Explain the choice of species 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.

We work on rats and mice because they are the lowest vertebrate group which reasonably resembles the human behaviours we are seeking to analyse. Animals are constantly monitored for their health status and potential discomfort. Any potential suffering is avoided by using most refined tests and when necessary analgesia. We have very well stablished humane endpoints to minimize harm to the animals. Furthermore, animals are going to be handled by trained and experienced researchers and technicians.

4. Rodent toxicity, tumorigenicity and safety studies

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.
- 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 purpose (b)
- Protection of the natural environment in the interests of the health or welfare of man or animals.

Key words

Regulatory, Rodent, Safety, Toxicity, Tumorigenicity

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
- Required at inspector’s discretion

Objectives and benefits

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

What's the aim of this project?

The project aim is the determination of scientific and/or regulatory endpoints in rodent toxicity, tumorigenicity and safety studies for submission to regulatory authorities and/or for safety assessment purposes.

Regulatory approval is required to allow a new drug to be tested in human or veterinary trials, or for a chemical, agrochemical, food additive/substance or medical device/article to be marketed.

Studies are designed to determine specific toxicity or regulatory endpoints, and/or for safety assessment, ranging from single dose to 12 month repeat dose toxicity studies and

life-span tumorigenicity studies (which determine any changes in tumour profile). Tumorigenicity studies can also be performed over a shorter period using genetically altered mice and by using a short study method. Other protocols include the provision of body fluid/tissue samples, use of juvenile animals exposed in the womb, and validation/research and development studies.

A retrospective assessment of these aims will be due by 12 June 2022

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its 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.

What are the potential benefits that will derive from this project?

Governments require (and the public expects) that substances we are exposed to are safe or that their potential hazards are well understood and documented.

The data generated from the studies performed under this project will be used to inform decision making processes on substances under development and, where appropriate, to satisfy governmental regulatory requirements necessary to gain clinical trial approval, marketing authorisation or product registration.

This safety assessment is of immense importance along with other non-rodent and non-animal studies in demonstrating to governments and the public the safety of these substances or highlighting their known hazards and safe handling.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Rats: 111,600
Mice: 68200
Hamsters:10300

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

Administration of test substances may result, usually at the highest dose level in mild to moderate signs of toxicity, eg mild/moderate effects on food consumption, bodyweight,

clinical pathology parameters, neurobehavioural parameters and organ function tests. Studies involving therapeutic agents (i.e. pharmaceuticals, biologicals and veterinary/animal health products) may produce pharmacological or pharmacodynamic effects at all dose levels, but they are expected to be transient.

Experience shows that the majority (~65%) of animals are not expected to show any clinical signs of suffering (either no clinical signs or normal background signs expected of the rodent strain). A small percentage (~15%) may show transient subtle to mild clinical signs. Moderate signs of adverse effects may be seen in some animals (~20%), usually in the higher dose groups. Lethality and/or severe effects are not study objectives in any of the protocols within this licence, but for preliminary studies that may be the first animal studies with limited data available, a very small percentage of animals may inadvertently show severe findings before they are immediately and humanely killed.

Most of the dosing techniques, manipulations or investigations do not cause any lasting adverse effects, but a small number of animals may show temporary moderate distress due to, for example, withdrawal of blood.

A retrospective assessment of these predicted harms will be due by 12 June 2022

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

There is currently no regulatory and scientifically acceptable alternative to the use of rodents in these studies.

A retrospective assessment of replacement will be due by 12 June 2022

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 you will assure the use of minimum numbers of animals.

The regulatory guidelines usually indicate the number of animals in a study; otherwise, the number used is the minimum to achieve the aims of the study.

A retrospective assessment of reduction will be due by by 12 June 2022

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

Explain the choice of species 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.

The regulatory guidelines require toxicity testing in rodents, with the rat, mouse and less commonly, hamster being regulatory accepted test species.

Studies are performed in a stepwise manner, starting with preliminary studies using small numbers of animals where there is limited information. This gives the highest prospect of refining and optimising the programme to achieve the desired scientific endpoints and also resulting in the least pain, suffering, distress or lasting harm in the animals.

All animals are regularly monitored for signs of any adverse effects on their health or wellbeing, and to prevent unnecessary suffering, early humane end-points are applied under appropriate veterinary guidance (e.g. modification/withdrawal of treatment with the test item, or humane killing of affected animals).

A retrospective assessment of refinement will be due by by 12 June 2022

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?