Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 3

Projects with a primary purpose of: Basic Research – Nervous System

Project Titles and keywords

1. **Analysis of neurological mouse mutants**
    - brain, neurodegeneration, psychiatry, behaviour, movement disorders

2. **RNA methylation in neurodevelopmental disorders**
    - RNA methylation, Synaptic plasticity, Fragile-X syndrome, Autism, Schizophrenia

3. **Autoimmune diseases in the central nervous system**
    - Multiple sclerosis, autoimmune disease

4. **Misfolded proteins in neurodegenerative disease**
    - Misfolding, neurodegenerative diseases, proteins

5. **Mechanisms underlying neurodegeneration**
    - Neurodegeneration, Alzheimer's disease, therapeutic, mechanisms

6. **Imaging Agents to Investigate Epileptogenesis**
    - Epilepsy, Imaging, Radiotracer

7. **Genes and lifestyle influences on brain ageing**
    - Diet, brain, genotype, ageing, inflammation

8. **Investigating Motor Neuron Disease and Dementia**
    - Paralysis, dementia

9. **Nervous system injury and repair strategies**
    - Hypoxia-ischaemia, neonatal encephalopathy, CNS injury, PNS injury, oxidative stress

10. **Development and nervous system repair in zebrafish**
    - brain, spinal cord, injury, regeneration

11. **Mechanisms of Neuronal Dysfunction in Neurodegeneration**
    - Neuronal function, neurodegeneration, nitric oxide toxicity

12. **Brain mechanisms for learning and decision-making**
    - Learning, decision-making, prefrontal cortex, reward

13. **Development of novel translatable measures and therapeutics for pain disorders**
    - Pain, rodents, analgesics, biomarkers, drug discovery

14. **Mechanisms of neuronal plasticity**
    - Alzheimer's disease, schizophrenia, synapse, genes

15. **Presynaptic function in health and disease**
    - Neurotransmitter, neurone, synapse, vesicle, disease

16. **Neural plasticity in health and disease**
    - Learning, memory, neural development, neurodegeneration

17. **Development of new models of FTLD/MND**
    - Neurodegeneration; dementia; transgenic; mouse; disease

18. **Roles of electrical activity in brain maturation**
    - Brain, activity, plasticity, development, olfaction

19. **Studies of brain development in the mouse**
    - Mouse, brain development

20. **Bioelectronic Medicines**
    - Electrophysiology, implantable devices

21. **Tissue isolation for electrophysiology, neurochemistry and pharmacology**
    - Neurodegeneration, pain, native, drug discovery

22. **Investigation of genetic forms of neurodegeneration**
    - Frontotemporal dementia, motor neuron disease, amyotrophic lateral sclerosis, C9orf72

23. **Autophagy and neurodegeneration**
    - Autophagy, Neurodegeneration, ataxia, signalling pathways

24. **Inter-connected studies of prion and other neurodegenerative diseases**
    - Prions, Creutzfeldt-Jakob Disease, transgenic, therapeutics

25. **CNS glial development and demyelinating disease**
    - multiple sclerosis, demyelination, neuroinflammation, neurodegeneration, neuropathology

26. **Mechanisms of synaptic transmission and plasticity in the central nervous system**

- Synaptic Transmission, Learning and Memory, Ageing, Neurodegeneration

27. The role of glia in nerve regeneration and cancer

- Nerves, cancer, regeneration, glia, microenvironment

28. Impact of exercise and NAD levels on brain and fat tissue

- Voluntary exercise, healthy ageing, epigenetics, NMNAT1, stem cells

29. Dorsal horn circuits in normal and pain states

- Interneurons; circuits; acute pain; chronic pain; mouse

30. Assessment of vascular cognitive impairment in rodents

- Dementia, cognition, vascular risk factors, neuroimaging

31. Neural mechanisms of pain and homeostasis

- Nociception, Neuroscience, Autonomic Nervous System, Behaviour

32. Mechanisms of acute and chronic pain

- Inflammation, arthritis, neuropathy, diabetes,

33. Understanding Cortical Plasticity in Health and Disease

- Memory, schizophrenia, parietal cortex, prefrontal cortex, mouse

34. Triggering and propagation of neurodegeneration

- Neurodegeneration; Parkinson's disease; ALS; synuclein; RNA-binding proteins

35. Cortico-thalamo-cortical interactions in primate cognition

- Cortex, Thalamus, Learning, Memory, Decision-making

36. Using transplanted neural tissue to understand neurological disease

- Neuron, development, brain

37. Experimental Molecular Medicines for Restorative Neuroimmunology

- Molecular therapies, inflammation, brain, stem cells, brain repair

38. Mechanisms of brain function and dysfunction

- Neurons, Glia, Receptors, Glutamate, Dopamine

39. Drug Development in Tuberous Sclerosis Complex

- Tuberous sclerosis, drug development, seizures, tumours, cognition

40. Neuron-g lial-pericyte-vascular interactions

- neuron, astrocyte, oligodendrocyte, microglia, pericyte

41. Synapses and plasticity in health and disease

- Alzheimer's disease; learning & memory; diet; improved mouse models

42. **Genome involvement in brain function, disease and development.**
    - Disease, genetics, gene regulation, transgenic, mouse

43. **Regeneration and cancer in the nervous system**
    - Neural stem cells, glioblastoma, tissue environment, injury, repair

44. **Establishing connectivity in the brain**
    - Brain development, neurodevelopmental disorders, vision

45. **Genetically defined neuronal networks regulating sleep, wake and circadian rhythms**

46. **Mechanisms underlying Huntington's disease**
    - Behaviour, sleep, circadian rhythms, EEG

47. **Signalling and Neurodegeneration**
    - Parkinson's disease, Alzheimer's disease, disease mechanism, finding new targets for treatment

48. **Information encoding for memory storage and retrieval in mammalian brain circuits**
    - Brain, memory, perception, Alzheimer's Disease

49. **Pathogenesis and treatment of Huntingdon's Diesease**

50. **Investigating and modulating cortical network activity**
    - Dementia, Epilepsy, Neurons, Brain Circuits

51. **The function of the neural circuitry of the olfactory bulb**
    - Olfactory bulb, smell, neural circuit

52. **Information processing in neural networks**
    - Brain, Neural Code, Multisensory, Perception, Memory

53. **Preclinical research of novel pain therapeutics**
    - Chronic pain, Analgesia, Hyperalgesia, Allodynia, Therapeutics

54. **SUMOylation accelerates supply-rate depression**
    - Epilepsy, SUMOylation, Synapsins, Levetiracetam

55. **Mechanisms of brain plasticity in health and disease**
    - neuron, plasticity, learning, memory

56. **Neurogliaform cells in health and disease**
    - interneuron, neocortex, neural circuits, neurogliaform cells, synaptic neurotransmission

57. Mechanisms controlling vertebrate development

- Neuronal development, axon guidance, vision

58. Organisation and function in cerebellar and pre-cerebellar circuits

- Cerebellum, movement, learning, Neurophysiology, motor control

59. Interactions Between the Developing Visual and Nervous System

- eye development, brain development, eye disease, sensory guided behaviour

60. Role of Gut-Brain axis on brain and behaviour

- gut-brain, casein, early-life

61. Understanding brain function and dysfunction using stroke models

- Brain, stroke, blood flow, ageing, MRI

62. Mechanisms underlying neural development

- Brain, Stem cells, Neurogenesis, Gliogenesis, Transcription factors

63. Neural circuit mechanisms for animal behaviour

- Neuron, Brain, Cognition, Behaviour, Navigation

64. Understanding and treating neurodegenerative disorders

- Neurological diseases, Mechanisms of disease, New Therapies

65. Molecular determinants of cortical development

- cortex, development, neurogenesis, migration, circuits

66. Metabolic and cardiovascular regulation by GLP-1 and the autonomic nervous system

- diabetes, obesity, neurophysiology

67. Regulation of the circadian clock and sleep homeostasis

- Sleep, Circadian, Clock, Neuroscience

68. Cortical structure and information processing

- brain, neurons, synapses, neuronal networks, sensory physiology

Project 1	**Analysis of neurological mouse mutants**	
Key Words (max. 5 words)	brain, neurodegeneration, psychiatry, behaviour, movement disorders	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to analyse novel mutant mice to provide new insights into the molecular basis of neurological diseases. A significant proportion of the current burden on healthcare resources is accounted for by diseases of the central nervous system (CNS). In Europe alone, the total cost of these CNS disorders, including treatment, caregivers and lost productivity is over £600 million a year – twice the estimated cost of cancer. 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 CNS 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 work is primarily based on mice generated by a large-scale screen at MRC Harwell in which many animals containing random single gene mutations are generated. Using a simple battery of behavioural tests, new mutant lines that might display a neurological defect are identified. 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 CNS and muscle that might explain the phenotype. Genetic studies are then used to identify the causative mutation prior to functional studies of the mutant gene. 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.
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)?	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.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, 31 000 over 5 years
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	To model severe 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

will happen to the animals at the end?	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. Other mouse models will be studied or generated to examine the function of a disease gene where no adverse 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.
Application of the 3Rs	
1. 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.
2. 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.
3. Refinement Explain the choice of species and why the animal model(s) you will	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

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.	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. 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.

Project 2	**RNA methylation in neurodevelopmental disorders**	
Key Words (max. 5 words)	RNA methylation, Synaptic plasticity, Fragile-X syndrome, Autism, Schizophrenia	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The brain contains billions of neurons which are capable of communicating via chemical and electrical signals and this communication is critical for memory formation and learning. The detailed molecular mechanisms that allow these memories to be formed and stored are very poorly characterised, but are important to understand as many human neurological disorders such as Intellectual Disability (ID) and Autism Spectrum Disorders (ASDs), may result from genetic mutations that disrupt them. By studying genetic function we aim to characterise these important molecular mechanisms in more detail. This will help establish routes to therapy for a range of neurodevelopmental disorders.	
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)?	We aim to shed light on the molecular pathways that enable these important aspects of neuronal function. By doing so we will also create knowledge which will aid in drug choice/design for therapeutic use in patients where these processes are disrupted. To this end we will utilise transgenic mouse models harbouring disruptions in genes known to be disrupted in human patients with syndromic forms of ID and ASDs, in order that their function in regulating	

	neuronal responsiveness can be determined.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice for a period of five years. We calculate that we will require 1800 mice for completion of the studies.
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 genetically altered animals will have a moderate phenotype which will consist of reduced body weight and impaired neurological performance such as a sub-optimal capacity for learning and memory formation. Ear notching and tail snipping to obtain samples for necessary genotyping will be performed, In addition a proportion of the mice will be subject to injections for the induction of transgenes. Other than these, no other procedures will be performed on the mice. Mice will be killed at the end of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In these experiments it is absolutely essential that animal models are used. This is because in neuronal cell cultures the synaptic networks required for higher-order neuronal communication to manifest are not present and thus the appropriate investigations cannot be made. Due to the relevance to human neural function, synaptic plasticity studies investigating genetics are almost always performed in transgenic mice, and thus, to relate our work to what is previously known, and to help design the relevant experiments, mice are the only realistic option for the proposed studies. Wherever transgenic mice are to be used we will strictly adhere to a programme of reduction and refinement.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will further optimize experimental design by performing statistical analysis to determine the number of mice needed to yield meaningful data in the most efficient manner.
3. 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	A mammalian model is needed to make our findings relevant in humans, and mice represent a robust option for this purpose due to the ease of genetic manipulation. All mice will be housed in state-of-the-art, environmentally controlled, purpose-built facilities and routine housing and husbandry procedures will be performed by trained and competent staff. Pharmacological agents used in the study will not be

(harms) to the animals.	administered to living mice but instead will be applied to tissue ex vivo. In our experiments once brain tissues have been harvested, they can then be bathed in appropriate neuro-pharmacological media to elicit acute neuronal responses under study. This optimises animal welfare, whilst ensuring the benefits of the research are at their maximum potential.

<table>
<tr><td>Project 3</td><td colspan="2">Autoimmune diseases in the central nervous system</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">multiple sclerosis, autoimmune disease</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 yrs</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>x</td><td>Basic research</td></tr>
<tr><td>x</td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
<tr><td></td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
<tr><td></td><td>Preservation of species</td></tr>
<tr><td></td><td>Higher education or training</td></tr>
<tr><td></td><td>Forensic enquiries</td></tr>
<tr><td>x</td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">Multiple sclerosis (MS) is a serious autoimmune disease, afflicting around 1 in 1000 people in the UK, which causes gradual disability and often causes a lot of pain. Susceptibility to MS comes from both inherited and environmental risk factors (viruses, vitamin D, smoking), however how they trigger the disease remains unknown. Consequently, the only treatments which are currently available for patients will only modestly help disease symptoms and do not prevent the disease from occurring, or delay the time a patient takes to become permanently disabled. In parallel with data obtained from analysing patient and healthy control blood samples, the first part of this project aims to use genetically modified mice to investigate how inherited risk genes change the immune system in a way that makes them either more susceptible to disease, or changes disease severity.

Very little information is available to allow us to determine why MS gets worse over time and cannot be treated with drugs that stop the immune system. Our understanding of the later stages of disease is far from complete and therefore requires a lot more research before new drugs can be designed. The second part of our project therefore aims to further</td></tr>
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	understand the pathways that lead to disease progression and disablement. Pregnancy in MS patients has shown to dramatically reduce disease symptoms in the last trimester, followed by a sharp increase in relapses after giving birth. Identifying the pathways, which control the disease during different stages of pregnancy, offers a unique opportunity to understand specific aspects of the disease, which could be targeted for future treatments. Using both MS patient blood samples throughout different stages of pregnancy, as well as analysing the effects of pregnancy in genetically modified mice, we aim to explore and identify new protective disease pathways.
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)?	1. **Identification of disease-causing pathways** - The benefit of using genetically modified animals to understand the causes of MS is their ability to allow us to understand how individual risk factors affect an entire organism in their normal state and during disease, in a manner which cannot be obtained by other methods. Such information is imperative to understand gene function and how or whether it is sensible to target such pathways in patients. Therefore, while this is a basic biological research project, it is expected that information generated in these studies will have future impact on therapeutic design, and will help to inform clinical practice on whether certain disease pathways may be worthwhile targets. 2. **Understanding why MS worsens over time** - As yet there is limited information on why the disease progresses in MS patients, despite treatments being able to reduce the relapse frequency. Therefore, in addition to trying the understand how a genetic risk factor changes disease susceptibility, we also aim to use our animal models to understand how this changes the disease course and, if possible, to understand what determines whether the disease worsens over time. This biological information is crucial if we are to understand why the disease progresses. Disease progression occurs in almost all MS patients, for which there are currently no treatments. 3. **Understanding how pregnancy changes disease symptoms** - we aim to explore the dramatic effect that pregnancy has on women suffering with MS, where their disease symptoms can significantly

	reduce in the last trimester. Understanding what controls such a change in the immune system during pregnancy will allow us to investigate parallel immune functions in disease, with the aim to identify novel targets for future drug therapies, which will be applicable to both women and men.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use both genetically modified and wild-type mice for our experiments. Up to 10,000 transgenic mice will be bred and maintained over a 5 year period. Approximately 2000 mice will be used for disease studies over 5 years, 500 mice will be used for studies on immune cell function and 500 mice for investigating novel pathways, which control the immune system throughout pregnancy. Again, both over a 5 year period.
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?	Most mice will be bred and maintained under a mild protocol and therefore are expected to develop no adverse symptoms. Mice which are allocated towards experiments where they develop MS-like disease will become partially disabled, typically experiencing complete loss of the ability to use their hind legs for an average of 2-4 days. Mice are intensively monitored however in some cases, they may experience weight loss of up to 20% within this time. All mice under experimental conditions to induce disease will have a severe severity level. In a very small number of cases, mice will have hind leg paralysis for longer periods of time (up to a maximum of 3 weeks) in order to investigate the progressive, chronic stages of disease. However, in all cases, mice which develop any additional symptoms, such as excessive weight loss, weakness in their front legs, or look like they are experiencing pain, will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We continually strive to find alternative approaches to restrict the requirement of animal models. We use several experiments using human cell lines, blood samples from healthy and MS patients, and post mortem tissue, whenever possible, to test the role of risk genes. However, MS is a complex disease that is the result of several interacting pathways and organs, which simply cannot be modelled by any other means in their entirety. It is also unclear how the disease progresses, and therefore cannot be recapitulated in a non-animal system.

2. Reduction Explain how you will assure the use of minimum numbers of animals	All preliminary data for a specific project will be obtained by first analysing samples from healthy donors, patient samples, or cell lines, whenever possible. The requirement to use animals for any project will be decided by the inability to test the specific question within that project by any other means. We have a statistician working as part of our group who performs high-throughput analysis of risk genes, which have been identified through whole genome sequencing. This allows several gene candidates to be excluded or validated statistically before committing any animals to a specific research project. Preliminary studies using other non-animal techniques will also ensure that any experiments requiring animals are dedicated and specific to cell types or pathways within the disease and therefore will require fewer animals overall to answer the scientific questions posed.
3. 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.	Mouse models of MS closely reflect the clinical and pathological symptoms of disease in humans and can allow us to study complex pathways which attribute to disease manifestation and progression. The development of a number of drugs used to treat MS patients today were achieved using the mouse MS model, reflecting its suitability, including from our own research. We have several years of experience working with the MS disease model in mice and within this time have developed several refinements to minimise animal suffering at all stages of every experiment. The housing environment of mice with disease is a key focus of our daily management of sick mice. We use soft bedding for comfort and reduction of sores, provide access to food supplements on the cage floor, which consists of infant formula mixed with food used for weaning mice, and house mice in smaller numbers to allow easier movement around the cage when mobility is reduced. We have recently investigated the efficacy of purchasing pre-made immunisation solutions to induce disease, which creates disease in 100% of mice, which is very uniform, and can be modified such that the level of disease severity is reduced in experiments where this does not effect the scientific question. In the majority of cases, only a single disease episode (monophasic) will be sufficient to answer our scientific question and therefore, while disease progression forms a part of our research

	strategy, this reflects only a very small percentage of animals that will experience disease for longer periods. Whenever possible, mice which are pregnant will be treated with immune-modifying drugs before mating so that the minimum amount of distress is experienced by expecting mothers. Similarly, all pilot studies be performed using non-pregnant mice, whenever possible, again to restrict the amount of distress pregnant animals experience.

Project 4	**Misfolded proteins in neurodegenerative disease**	
Key Words (max. 5 words)	Misfolding, neurodegenerative diseases, proteins	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	✓	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	There are several chronic diseases of the brain such as Alzheimer's disease (AD) and Parkinson's disease (PD) characterized by the presence of aggregated and accumulated proteins. As life expectancy increases and the population is getting older, these neurodegenerative diseases are showing an increased prevalence. Current treatments do not slow down or reverse the progressive course of these diseases and most of the novel pharmacological compounds that have reached human clinical trials failed to achieve significant benefits. Reasons for this failure include lack of understanding of the mechanisms of disease progression, difficulty with measuring such progression and the inability to diagnose patients until the disease reaches an irreversible state. Therefore, an earlier diagnosis combined with a clearer fundamental understanding of the mechanisms underlying these neurodegenerative diseases should provide a good opportunity for therapeutic interventions. Hence, our objectives are: to identify at least 2 early diagnostics markers tauopathies and synucleinopathies and to identify at least 2 novel treatments disease-modifying therapies for these diseases.	

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)?	The main benefits of this project can be divided in two very different areas. The first one would be related to basic research to understand how the misfolded proteins involved in neurodegenerative diseases are acting inside of the cells. And moreover, to discern whether they are a cause or an effect of the pathology in the cell. Because these are progressive diseases that spread through different areas of the brain, we need to understand the specific mechanisms allowing such a progression. The second benefit would be a translational one. Our research may generate a clinical test capable of diagnosing AD or PD at an earlier stage than is currently possible. Additionally, we will target the pathological mechanisms aiming to obtain disease modifying therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	We are going to use mice. We will use approximately 8,000 animals over 5 years.
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?	We do not expect to have adverse effects in the breeding protocol of transgenic animals. In the experimental protocols the level of severity, the majority of the time, will not exceed moderate Some animals will have chronic low levels of discomfort from the administration of substances. Other animals will have a transient higher level of discomfort derived from surgery. However, a particular line of transgenic animals that model AD will develop motor and behavioural deficits as a consequence of a genetic mutation or following a neurotoxic insult to the brain. In these animals the level of harm is likely to be such that it is considered severe, because approximately 10% of the animals are expected to experience persistent distress by the end of the experiment caused by paralysis of their hind limbs. This is the time where the toxic aggregated proteins become detectable and it is therefore important to keep the animal until this point in order to evaluate the effects of drugs that potentially alleviate this pathological sign. At the end of all the experimental procedures the animals will be humanely killed and postmortem tissue used for analysis.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are currently using many experimental in vitro technologies that are allowing us to make great progress in understanding the pathological mechanisms of the aggregated proteins involved in neurodegenerative diseases. We use commercial cell lines for the early steps of our research to select potential therapeutic pathways and compounds. Afterwards we use cultures obtained from our animal models to study the selected drug treatments after neurotoxic to minimize the number non effective drugs that will move forward to tests in animal models. However, cell cultures can only provide a limited replacement, as cells represent a two-dimensional system, and lack trophic support of surrounding cells present in the brain, as well as protein function can differ when overexpressed in cells in vitro. Hence, it is not currently possible to accurately re-create a functioning nervous system that mimics the complex interaction between different neuronal cell types, tissues and organs. Therefore to explore the full therapeutic efficacy of relevant drugs a whole-animal model is needed.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals is kept to the minimum by carefully monitoring the colony size and breeding, and matching these to the demands of the experiments. By applying statistics to the experimental design we will obtain meaningful results that will prevent experiment repetitions. Additionally, we plan to share tissues between multiple users and generating a tissue bank from animals, maximizing the use of each mouse.
3. 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 are using mice because they have a nervous system that is sufficiently similar to that of humans and that the biology of neurodegeneration is almost the same. In addition, mice behaviour is well characterized and our mouse models mimic the behaviour in the human clinical condition. Furthermore, mice can be genetically manipulated, allowing molecular hypotheses to be tested. When animals are used in an invasive procedure, potentially painful, analgesia and anaesthesia will be used to minimize the suffering of animals. Extra monitoring will be provided to those animals for at

	least 24h after the procedure and under close supervision of the establishment veterinary surgeon. Many of the drugs tested under this licence are already present on the market, so toxicology data, dosage and best delivery routes information are available. Thus allowing the selection of the most appropriate experimental design for the animal. For the drugs for which that information is not known, extensive cell culture work will be performed before testing in the animals. Additionally, refinements for this licence are likely to be establishing good endpoints to obtain the data you require, but the mouse suffers as little as possible.

Project 5	**Mechanisms underlying neurodegeneration**	
Key Words (max. 5 words)	Neurodegeneration, Alzheimer's disease, therapeutic, mechanisms	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to understand the biological events underlying the development and progression of neurodegenerative diseases such as Alzheimer's disease. This information may identify, and enable the development of, new therapeutic strategies for treatment.	
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)?	Neurodegenerative diseases occur primarily in mid-late life and include Alzheimer's disease and related dementias. While there is a small percentage of genetic heritability in these diseases, the vast majority of cases are sporadic, meaning that they have no known cause. In all neurodegenerative disorders there is progressive and relentless nerve cell death, with degeneration of brain regions giving rise to the characteristic clinical symptoms of disease. Dementia, including that caused by Alzheimer's disease, is the most common neurodegenerative disease. Current estimates show that dementia affects more than 35 million people worldwide and, with an ageing population, the prevalence of this disease is expected to double every 20 years. Despite intensive research the cause of most dementia remains unclear. There are few drugs available to treat dementia, and none that effectively slow or prevent disease progression. The ultimate	

	goal of this research project is to increase our understanding of the biological events that underlie neurodegenerative diseases such as dementia in order that these findings might aid in the development of novel disease-modifying treatments for these ever more prevalent disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use a maximum of 1550 genetically altered and normal mice, and 100 normal rats per year. This amounts to 7750 mice and 500 rats over the 5-year licence period.
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?	We are using mice that have been genetically modified to develop adverse effects of neurodegenerative disease seen in dementia eg problems with locomotion, thinking and learning capacity, grip and uncertainty in novel situations. We will study the biological events associated with these disease features as mice age. In addition, we will treat mice to modify important disease pathways. This will enable us to determine the effects of treatment on mouse behaviour and brain chemistry. Mice and rats will also be used to collect nerve cells from the brain and these cells are kept alive in culture for additional experiments. Although experiments are carefully planned to minimise the risk of suffering, this work may result in some physical or psychological adverse effects. Animals will be humanely killed at the end of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is essential that we use mice and rats for this work since mouse models show measurable progressive changes in brain chemistry and behaviour over their life-span that mirrors the development of human neurodegenerative disease, and that cannot be modelled in “lower” animals such as flies, worms and fish. We also use non-animal derived cell lines for some aspects of our work. However, many important cell processes differ significantly between these cell lines and mammalian brain cells. Therefore, to ensure that our work is relevant and valid to human disease, it is necessary that we culture brain cells from rats and mice for some experiments. se human nerve cells, but these cannot replace animals for all of our studies because it is known that other brain cell types are important for dementia and methods to derive human cells of this type are not yet well established. To

	determine the effects of these other brain cell types on disease pathways we must culture nerve cells and other brain cells from the same animal together.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have carefully planned all of our experiments so that we can answer our experimental questions using as few animals as possible. We also use cultured cells and tissues for many experiments. Enough neural cells or brain slices can be cultured from a single mouse or rat brain to allow analysis of many experimental conditions or treatments, considerably reducing the number of animals that we need to use. When animals are treated, multiple features of disease are assessed in a single animal (such as behavioural changes and alterations in brain chemistry) and in a single brain (where multiple methods of assessment must be used). Again, this considerably reduces the total number of animals used in our work.
3. 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 use mice and rats since they are the lowest animal species that is sufficiently similar to humans to allow us to answer our research questions. We will use genetically altered mice that are considered to be the most appropriate and relevant for each experiment. These include mice in which: 1. Genes have been removed to enable us to understand their function for health and disease. 2. Human gene mutations have been introduced into mouse genes to allow us to determine how the mutation causes neurodegenerative disease. 3. An entire human gene has been introduced at low levels to allow us to investigate how these genes cause neurodegeneration as mice age. 4. More than one human gene has been introduced. Mice only develop the key features of Alzheimer's disease if more than one human gene is expressed. Animal harm in our work has been minimised by: 1. Our selection of animal models that develop only mild-moderate features of human neurodegenerative diseases. We do not use animals that show the harmful features of terminal disease stages.

	2. The use of brain cell and tissue cultures as an alternative to studies using live mice. This considerably reduces the number of animals that are aged until they develop disease signs and that are used for treatment studies. 3. Using the least adverse methods that are compatible with the aims of the study. For example, we preferentially study ages of mice in which there are changes in brain chemistry, but no psychological or outward physical signs of disease. 4. When we treat animals, we use the least invasive and least repeated methods of administration e.g. we prefer to give treatments in drinking water or food rather than by injection.

Project 6	**Imaging Agents to Investigate Epileptogenesis**	
Key Words (max. 5 words)	Epilepsy, Imaging, Radiotracer	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Epilepsy is a devastating condition affecting over 50 million people worldwide, 30% of which cannot be controlled by current epilepsy drugs, and is associated with increased mortality and stigmatization of affected individuals. The limited knowledge on the processes leading to epilepsy (i.e. epileptogenesis) dramatically hamper the development of new treatments. The aim of this work is to use novel non-invasive imaging techniques to (i) reveal mechanisms and targets of epileptogenesis, (ii) identify predictive biomarkers for epileptogenesis, and (iii) to provide tools to assess the efficacy of new antiepileptogenic strategies. Ultimately the aim of this work is to assess the efficacy of new antiepileptogenic strategies for use in humans.	
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)?	Developing new techniques to image epilepsy is likely to achieve a better understanding of causes and mechanisms of epilepsy which, ultimately, could be crucial for the development of new antiepileptic drugs. Additionally, as preclinical imaging protocols can be easily translated to clinic, this project could results in better tools to diagnose and follow-up epileptic patients.	

What species and approximate numbers of animals do you expect to use over what period of time?	We will use rats and over the five years we will use a maximum of 500 animals.
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?	As we are modelling epilepsy, our animals will be induced to fit/seizure for a short period of time under strict monitoring. Drugs and constant care will be provided to ensure that the animals stop these fits/seizures after this time period and do not undergo any undue suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Epilepsy takes place in the brain, one of the most complex organs, and the disease is believed to result from a combination of numerous factors. In vitro methods do not reflect this complexity. For this reason, it is necessary to further investigate the adequacy and clinical feasibility of new imaging protocols in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	It is possible to calculate the numbers of animals required for experimentation based on previous data. Imaging lets animals be used as their own control, allowing paired comparisons, and imaging is inherently sequential, using fewer animals to achieve the same statistical power as conventional designs. In all cases we ensure that we have calculated the minimum number of animals required for the experiment to give us useful data. This approach also reduces the likelihood that the animal experiment would have to be repeated.
3. 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 animal model to be used has been refined significantly in recent years, and we will employ these procedures in order to minimise animal suffering and reduce the mortality rate. We also regularly monitor body weight, body condition, food and fluid intake of animals as a measure of disease; we set strict limits to ensure that there is limited harm to the animals used.

Project 7	**Genes and lifestyle influences on brain ageing**	
Key Words (max. 5 words)	Diet, brain, genotype, ageing, inflammation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	There is currently a lack of information regarding the molecular-mechanisms underlying the increased risk of cognitive decline in “at-risk” individuals (high fat/high sugar diet, APOE genotype, insulin resistance, sedentary lifestyle, etc.). Dietary patterns (calorie restriction, ketogenic diet, intermittent fasting, etc.) and dietary compounds (polyphenols, polyunsaturated fatty acids, isothiocyanates etc.) may hold the potential in delaying the onset of degenerative and metabolic disorders through the modulation of key signalling pathways critical in maintaining optimal neuronal function and cognitive performance. However, research linking “at-risk” dietary patterns, genotype and nutrients are currently lacking. This program of work will therefore strive to broaden our understanding of the role that genetic factors, dietary patterns/nutrients play in the prevention of human brain ageing and the enhancement of a range of cognitive abilities. We will strive to understand 1) How selected genes and lifestyle factors affect cognitive functions, neuroinflammation and synaptic plasticity; 2) Can the effects of genes and lifestyle factors be improved or rescued by drugs/dietary compounds acting on its pathways or by restoring the metabolic balance and 3) To identify if selected genes	

	affect specific metabolic pathways and the distribution of drugs/dietary compounds to the brain and peripheral tissues.
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)?	The benefits of the proposed research may be considered both in terms of the potential clinical applications of our findings and the advancement of fundamental knowledge regarding the influence of diet, genetic and metabolic factors on cognitive functions in both normal and pathological ageing. The studies will provide the background for new and promising preventive treatments for tackling cognitive decline and are aligned with a move towards the provision of personalised/stratified preventative strategies, in particular in individuals identified as being at “high-risk”.
What species and approximate numbers of animals do you expect to use over what period of time?	Wild-type and/or genetically altered mice and rats will be employed in our programme of work. The number of animals used in the entire programme will be minimised, and the breeding carefully planned to meet our research needs with respect to number, uniformity and health. Over the 5-year period, it is anticipated to use about 3000 mice and 350 rats.
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?	Procedures involved in this project are expected to result in no more than transient, moderate pain and no lasting harm. Surgical procedures will be carried according to the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2010) and severity limits greater than mild will be controlled by use of general anaesthesia and analgesic treatments. In the unlikely event of post-operative complications, the development of any adverse reaction to a treatment or if any animal fail to recover and exhibit signs of pain, distress or of significant ill health, testing will be discontinued and animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cell cultures, human tissue assays or computer modelling cannot adequately model the complete array of molecular, cellular, physiological and behavioural interactions necessary to fully understand how genetic and metabolic modifications result in normal or abnormal brain function. For example, it is difficult in cell cultures to reveal the interactions between all the various cells types as they are normally situated within a whole tissue, the immune system that normally affects them, or the effects of

	many other factors and signals that exist in a live, whole organism. Furthermore, when dealing with dietary supplementation studies, it is important to address bioavailability, tissue distribution and metabolism of such compounds. Therefore, there is no feasible alternative that would entirely replace the use of a living animal that would allow the objectives to be met.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When designing experiments we rely on 10 years past experience, literature searches, consulting the institute statistician and statistical analyses to ensure the minimum number of mice per group that will be informative are used. As a general principle, for quantitative experiments, sample sizes will be set using power analysis. Generally, the significance level will be 5% and the power 80%. The exact numbers of animals required will vary with the particular experimental design, the estimate of variation in response, etc. For qualitative experiments, the amount of material required will be the minimum necessary to provide an adequate description. To maximize the information from a single animal and to minimize suffering, we will aim to collect samples post mortem and we will share tissues with other appropriate scientific colleagues for use in their work.
3. 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.	Mice and rats have been selected for our research programme because: (1) the anatomy and physiology of their brain is similar to those of humans and we intend our data to have direct application to the human domain; (2) we require an animal that is omnivorous, capable of rapid learning and (3) a considerable body of general knowledge concerning the anatomy, neurochemistry and physiology is already available. There are no suitable, less sentient alternatives available for these studies which would allow us to fulfil the above requirements, and provide rapid theoretical development or clinical application. Animals will be kept in social groups, and in enriched environments that allow them to behave in a normal manner. No pain or distress is expected from the diet treatment. At all stages of our research, any animal showing pain or distress will be humanely killed. When chronic drug administration is required, implantation of osmotic minipumps will be preferred following general anaesthesia. On completion of each procedure, our results will inform our decision about the most fruitful direction to follow while minimizing

	the number of animals used, treatments within a procedure, and exposure to the more severe procedures.

Project 8	**Investigating Motor Neuron Disease and Dementia**	
Key Words (max. 5 words)	paralysis, dementia,	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	✓	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to improve our understanding of the cell processes that lead to motor neuron disease and frontotemporal dementia. Work will focus on understanding what cell processes may cause the diseases to start, and what then causes them to develop from the early mild symptoms to the devastating disorders we see in patients, including how the disease may pass from cell to cell. We will also investigate why some groups of nerve cells are more vulnerable to these diseases than others. New information obtained will then be used to identify and assess new targets and potential therapies for drug development.	
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 enhance our understanding of what causes brain and spinal cord cell degeneration in motor neuron disease and frontotemporal dementia. We will learn more about what cell processes may be involved in disease, and how, and also understand how a disease that starts in one specific region of the body may spread out to affect the whole as the disease progresses. This will inform us both of basic biological processes, and also how they can go wrong. Research conducted in this project may also have relevance for other similar disorders, such as Alzheimer's disease, as many of the cell processes in	

	these diseases are likely to be similar. In addition, this research will allow us to identify new targets for the development of drugs. In addition, if assessment of a new therapy proves promising, work done in this project may lead to the progression of the drug into clinical trials to improve patient outcomes.
What species and approximate numbers of animals do you expect to use over what period of time?	A maximum of approximately 7,500 mice over 5 years.
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?	This project will model motor neuron disease in mice, hence some animals are expected to develop symptoms of the disease, including problems with walking possibly coupled with weight loss as the disease progresses. Most animals will not be kept beyond this point, but a small number will be allowed to progress to a more severe disease state, which could include muscle weakness and some paralysis. This will allow direct comparison between these mice and the end stage human patient tissue available for study. All animals will be humanely killed at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Motor neuron disease and frontotemporal dementia are diseases that affect the nerve cells of the brain and spinal cord. These are complex organs where cells communicate with each other and with muscles often over long distances. Although non-animal research can be used to address some of the basic questions about what might go wrong in cells in the case of disease, only animal research can currently provide the complex network of cells that are affected in these diseases. However, where possible, specialised cell cultures will be used in preference to a whole animal. These cultures provide a simplified version of the cell network found in live animals, and serve as an excellent replacement for live animals when investigating some of the more simple cellular processes and disease progression mechanisms to be investigated in this project.
2. Reduction Explain how you will assure the use of minimum numbers	The work planned in this project will make sure that the maximum information possible is obtained from each mouse used, with the minimum harm, so that total numbers can be kept to a minimum. For

of animals	example, when testing for problems with moving or memory, each animal will undergo tests for both, and then brains and spinal cords will be collected from the same animal, allowing one animal to answer multiple questions about the disease process. Also, where possible, the same animal will be assessed at several different ages (timepoints), rather than using a new animal each time. This enables data progression to be monitored in an individual animal and enhances the nature of the data obtained. In addition, early studies will be done in petri dishes, to ensure that only the most relevant questions are progressed for investigation in live animals. The use of specialised cultures prepared from the tissues of mice used in this project will also help to reduce future total animal numbers. One mouse can be used to generate several cultures, which can be used to address different questions, thus reducing the total number of animals needed to answer many of the questions regarding the role of different cell processes in disease, and how the disease progresses from cell to cell.
3. 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.	Motor neuron disease and frontotemporal dementia affect the brain and spinal cord, and although more simple species, such as the fruit fly or the zebrafish, can be used to address some questions regarding cell processes in these diseases, only mammals have a similar brain and spinal cord structure and network to humans. The mouse is the best model to address our questions regarding how and why some nerve cells are preferentially killed by these diseases, and how the disease moves from one cell to the next. This is because the mouse is the least sentient animal that can be used to address these questions, and also allows us, via genetic alteration, to investigate specific pathways and processes of interest to motor neuron disease and dementia. This project will predominantly use models that are well established and known to reflect either healthy or mutant versions of cell proteins that are known to be involved in motor neuron disease and frontotemporal dementia. A small selection of other mouse models will use proteins that have only very recently been associated with disease, but these models are crucially important in helping us to understand which underlying cell processes are most important in

	causing these disorders. Animals will be housed in small social groups, and given bedding and in cage housing to provide an enriched environment, and all animals will be monitored daily for any symptoms. Once they develop motor neuron disease animals will be provided with wet mash food at floor height, so they can easily feed themselves. No sick animals will be bred. Symptomatic animals will only be kept if they are needed for a specific experiment. All animals will be humanely killed if they develop a paralysis that prevents them from readily accessing their food.

Project 9	**Nervous system injury and repair strategies**	
Key Words (max. 5 words)	Hypoxia-ischaemia, neonatal encephalopathy, CNS injury, PNS injury, oxidative stress	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Injury to the human brain, whether it occurs around birth, during childhood or in the adult, frequently leads to devastating consequences. In particular, it causes impairments in the ability to communicate, move normally and lead an independent life. The main sources of brain injury are physical damage (trauma), infection and insufficient supply of oxygen and nutrients known as hypoxia/ischaemia. This insufficient supply can be due to global lack of oxygen, as in cardiac arrest, obstruction of the umbilical cord, or placental dysfunction, for example in pregnant diabetics. However, it can also be due to occlusion of just one vital brain vessel by a blood clot, as in stroke. Whatever the cause, the recovery of neurological function, such as speech, movement or perception, after any form of brain injury is complicated by the low potential for neural repair and, perhaps more frighteningly, by secondary or delayed brain damage. The mechanisms leading to secondary brain damage and the low potential for repair are poorly understood. In the current project, we aim to throw light on these mechanisms by employing several strategies, in particular the use of genetically modified mice. Genetically modified mice provide a very powerful tool to dissect contributing molecular	

	pathways, either by removing potentially harmful genes or by turning on the expression of potentially beneficial genes. Treatment with genetically engineered viruses or stem cells also offers a second alternative to limit brain damage and replace cells killed by the first or second wave of brain damage. We will also use chemical drugs that have been shown to have a beneficial function in the test-tube, to see if these could be used to reduce damage and improve recovery in animals with brain injury from trauma, infection or hypoxia. Interestingly, animals at different stages of development respond in different ways to different forms of brain damage, meaning that different strategies may need to be used to treat newborn, young or adult brain. Therapeutic success will be monitored by a range of neurological tests to evaluate memory, coordination and perceptive functions. However, we will also use histology to look in depth at the cellular processes such as death of nerve cells and inflammation, which underlie disturbed function. Overall, this project serves as a crucial step not only to understand the mechanisms important in brain injury and repair, but also to be able to provide better treatment for human patients suffering from these conditions.
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 aims at reduction of the incidence, severity or prevention of neonatal hypoxic-ischaemic brain damage thus saving babies' lives and reducing the socio-economic burden to the affected individuals, families and healthcare system. This project also aims at helping people with brain damage through better understanding and development of potential treatment for patients suffering from bacterial meningitis, head trauma, hypoxia and stroke and improvement of their recovery of mental, sensory and motor functions.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 9400 over 5 years Rats 1500 over 5 years
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?	Our proposed research involves breeding of genetically modified mice with mild and moderate severity. We will be modelling birth asphyxia and maternal infection often occurring as a complication and reason for premature delivery and subsequently resulting in neonatal brain injury. We will be also modelling neonatal and adult nerve regeneration, as

	well as excitotoxicity, neurotoxicity and adult asphyxia. As the animals will be exposed to surgical procedures, we expect them to experience some pain, but that will be maintained through application of analgesia and we would expect the animals to recover.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Work in cell culture has played a pivotal role in many aspects of the projected work, in the identification of neurotrophic substances, excitotoxicity, and the intracellular cell death and differentiation pathways. However, in vitro studies deal with simplified systems, and cannot in the final analysis predict, whether a specific molecule has a detrimental, beneficial effect OR, just as importantly, simply no effect in the complex organism. To answer these specific questions, one cannot avoid doing these experiments in vivo.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In our projected experiments, we will use power analysis and to pare down on unneeded group size and avoid duplication of control groups, using box analysis and ANOVA testing several experimental groups against same group of controls.
3. 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.	All surgical procedures will be done under anaesthesia and aseptic conditions. Experimental animals will be limited to mice and rats. The animals will be subjected to postsurgical daily monitoring of recovery, including ability to eat, drink and keep a well-kempt fur, the passing of urine and faeces and for any signs of distress. Animals showing signs of distress will be subjected to schedule 1. Animals will also be closely observed during behavioural testing and if they show distress the experiment will be stopped.

Project 10	**Development and nervous system repair in zebrafish**	
Key Words (max. 5 words)	brain, spinal cord, injury, regeneration	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	An injury to the human central nervous system, including brain and spinal cord, as well neurodegenerative diseases (e.g. motor neuron disease), in which specific populations of nerve cells perish, lead to irreparable loss of brain functions. In contrast, zebrafish have an extremely high capacity to replace lost nerve cells and their connections. We aim to better understand how zebrafish accomplish this feat and elucidate the mechanisms of this repair. Ultimately, that will contribute to understanding why regeneration in the nervous system of humans is so poor.	
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)?	The adult nervous system in vertebrates, which include humans and zebrafish, is highly complex. There is a large number of different nerve cell types and specific interconnections of these, such that repair of this structure is extremely complicated. The basic research interest of this study is to elucidate how the zebrafish is capable of repairing its complex nervous system after loss of specific nerve cells or after a spinal injury and how this may lead to a return of swimming function. During the period of the previous licence, we found that specific signalling molecules dramatically augment regeneration of	

	nerve cells in the zebrafish. The cell types and signalling molecules of interest are similar to those that are lost in human neurodegenerative diseases and spinal injury, such that emerging concepts from our zebrafish research may ultimately inform therapeutic strategies in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	This basic research project exclusively analyses development and regeneration of the nervous system in zebrafish, based on the expertise in our group and our previous findings in this powerful model of regeneration. This application is for 87250 animals. It is important to note that 86% of these animals will be used for breeding and generating new lines of fish. These individuals are likely to not experience any adverse effects throughout their lifetime.
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?	Those animals that do undergo experimental procedures, for instance a spinal cord injury will be paralysed for a couple of weeks from the injury site downwards (classified as “severe”). Fish in which the optic nerve is injured will be blind on one eye for a similar time period (classified as “moderate”). For some experiments it is necessary to remove one eye. Blindness on one eye is permanent, but compensated by the fish within minutes, as indicated by quick return of normal swimming behaviour and normal feeding. Fish in which specific nerve cells are killed, may experience difficulties in movement coordination (classified as “severe”). At the end of the regeneration period, all experimental animals will be humanely euthanized by a home office approved method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex environment of the central nervous system in vertebrates cannot easily be mimicked in non-animal alternatives and therefore there is currently no alternative to using adult fish. However, many aspects of the adult fish central nervous system already exist in larval zebrafish. Larvae have a simple nervous system and are not recognized as complex animals that are protected under the Act. Hence, wherever possible, we replace experiments in adult fish with those on early larvae.
2. Reduction Explain how you will assure	Based on our previous experience, we know what kind of animal-to-animal variation we can expect,

the use of minimum numbers of animals	such that in a statistical procedure, called power analysis, we will calculate the appropriate number of animals necessary to obtain meaningful results suitable for publication in the scientific literature. This will ensure that the scientific knowledge base on how nerve cells can potentially be replaced in a vertebrate is increased, without using excessive numbers of animals.
3. 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 use zebrafish, because they are capable of repairing their central nervous system. Moreover, we are held by the government to use the species of the lowest perceived sentience, which is appropriate to our research. A lot of regeneration research is done on mice, but arguably, zebrafish are the vertebrate model of the lowest perceived sentience. We take great care that animals experience the minimal harm during procedures, by closely checking animals after an operation. For example, after spinal injury animals are singly housed in covered tanks to minimize perturbance. An anti-fungal/anti-bacterial agent is added to their water and fish are individually fed and checked daily. In the rare instances the fish show signs of undue stress, such as not eating or increased breathing frequency, they will be immediately presented to the Vet and, if necessary, humanely euthanized to prevent them from experiencing further harm.

Project 11	**Mechanisms of Neuronal Dysfunction in Neurodegeneration**	
Key Words (max. 5 words)	Neuronal function, neurodegeneration, nitric oxide toxicity	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This programme aims to investigate cell signalling pathways involved in neurotoxicity and disease to identify putative targets for therapeutic intervention(s). Dysfunctional neuronal signalling has been implicated in several neurodegenerative diseases such as Alzheimer's (AD), Huntington's (HD) and Parkinson's disease (PD), but the exact mechanisms of neuronal death are still not fully understood. One of the common pathways across many diseases is the abnormal regulation of nitric oxide levels, which can lead to neuronal cell death via multiple mechanisms. The proposed studies try to help understanding the changes of neuronal function in response to altered nitric oxide signalling. This understanding and the identification of pathways involved in nitric oxide toxicity may open up new possibilities to tackle dysfunctional signalling in neurological diseases.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could	The results will provide fundamental knowledge about how neurons in a brain co-operate to perform physiological functions and what the mechanisms are when neurodegenerative diseases occur. The studies will examine how changes in the brain are achieved at the most fundamental levels. Our main focus is on the	

benefit from the project)?	signalling related to nitric oxide which is strongly associated with several neuronal dysfunctions and this will lead to a better understanding how interconnections between neurons decay during processes of neurodegeneration.
What species and approximate numbers of animals do you expect to use over what period of time?	In addition to wild type mice (~750), the breeding of genetically altered animals (usually mice), most of which will be used for *in vitro* experiments (breeding a mutation into a specific mouse strain takes many generations), will require ~1000 mice to provide ~200 with a genotype suitable for specific experiments. Wild type mice will be killed in a humane manner so that tissues can be removed for *in vitro* experiments (approximately 750). This project is planned for a period of 5 years+.
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 main technique used to isolate the mammalian brains is by decapitation which allows a very fast isolation of the brain with minimal suffering of the animal due to instant death. Decapitation will occur in both conscious and anaesthetised animals. Phenotypes of the GA mice bred on this PPL are expected to have nil or only low and transient adverse welfare effects. In the unlikely case that breeding of transgenic animals has a potentially harmful or abnormal phenotypes, the off spring will be killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our experiments aim to understand the role of nitric oxide in specific physiological functions and diseases, and so we need to explore the potential interactions of synaptic signalling molecules in a 'native' neuron within a real brain. Our initial investigations will use the *Drosophila* NMJ synapse and N2a and SH-SY5Y cell lines to explore some basic signalling pathways. Later studies on mouse brain slices are planned to confirm findings form initially used models and extrapolate to mammalian '*in vivo*-like' systems (slice preparations).
2. Reduction Explain how you will assure the use of minimum numbers of animals	Apart from the use of other model system (cell culture, *Drosophila*) we are currently sharing brain tissue with other groups. This tissue is used for immunocytochemistry studies and there is no need for using extra numbers of mice in our hands. In physiology studies we are using a minimal number of animals as we can generate an 'N-number' resulting from a single neuron recording. Nevertheless, we need

	a minimum of (only) 3 animals from which the slices will be taken to satisfy requirements for publication.
3. 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.	All experimental conditions will be tested initially in non-mammalian animal studies (again: cell culture and *Drosophila* NMJ) to avoid wasting time and animal numbers (such as in pilot studies). The main purpose for using mouse brain slices is to corroborate findings from above studies to extend the gained knowledge to mammalian and eventual human brain function. This is important for the translational aspect of the proposed work. All handling of animals will be done with minimal distress and the methods of humane killing will be chosen again to minimise suffering.

Project 12	**Brain mechanisms for learning and decision-making**	
Key Words (max. 5 words)	Learning, decision-making, prefrontal cortex, reward	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Humans and other primates with damage to the prefrontal cortex (PFC) are disorganized, impatient, make poor decisions and exhibit socially inappropriate behaviour. Further, dysfunction of PFC is associated with neuropsychiatric illnesses impairing choice, including depression, addiction, schizophrenia, obsessive-compulsive, anxiety and attention-deficit hyperactivity disorder. Our experiments are carried Out in normal, healthy non-human primates (NHPs), and are designed to provide mechanistic understandings of how PFC and interconnected areas support the high-level cognitive processes - including learning and decision-making - that are often disrupted in these patients. These studies will have major implications in understanding decision-making in health and disease, and will aid the development of novel therapies for PFC pathology.	
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)?	The overall aim of the project is to understand the neural basis of learning and decision-making mechanisms in the brain. Our research will provide insight into the behavioural and neural mechanisms which support how information is sampled, combined and prioritized to make a decision, as these processes are disrupted in patients with prefrontal cortex damage or in psychiatric disorders. Our research will also	

	establish the neuronal mechanisms supporting different reinforcement learning mechanisms which will provide important insight into mechanisms of habit formation, such as in addiction. Finally, we aim to identify neural coding patterns that could provide relational structure of information, thus providing a neural code for how information is organised into knowledge.
What species and approximate numbers of animals do you expect to use over what period of time?	Eight non-human primates (NHPs) with be used in 4 different experiments (2 NHPs per experiment) over 5 years. In the rare event of health- or behavioural-related problems with any of these 8 NHPs which preclude their use in an experiment, a NHP may be purchased to complete the experiments (this contingency is unexpected; zero cases in past 5 years).
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?	All non-human primates (NI-IPs) are routinely pair-housed and are provided with an enriched environment and have home cages, exercise pens and forage areas. They interact regularly during the day with investigators and husbandry staff. The protocol involves a number of stages for preparing NHPs for recording neuronal data while performing behavioural tasks. This includes a number of separate and well-spaced surgeries under general anaesthesia. These are carried out under full aseptic conditions and involve a full regime of pre- and postoperative analgesia. All general anaesthetic procedures are carried out by our Named Veterinary Surgeon (NVS) and all surgeries are performed under NVS supervision. Behavioural testing sessions involve NHPs sitting in an enclosed testing chair, with access to a joystick or touchscreen monitor for interacting and responding to the experimental task. The NI-IP's head may be immobilized to allow infra-red tracking of eye movements and safe collection of neuronal data from both brain hemispheres while the NHP performs its trained task. Neuronal data is collected from very fine, delicate electrodes that are advanced into the brain, which cause little or no harm to the brain, which cause little or no pain to the subject as the brain lacks pain receptors, and which are now routinely used in human neurosurgery without complications. Neuronal recordings are usually taken from multiple cortical and subeortical sites. Subjects may be on a scheduled or controlled food or fluid regimen to reward and maintain task motivation and dietary fitness. These experiments typically last 2-3 years, though the neuronal recordings typically last for

	only 15-45 days of the entire project. The most common adverse effects are minor bite/scratch wounds from their cagemate, and minor infections in and around the implant. Such infections are quickly detected and appropriate antibiotics are used. In rare cases, the nature of the procedures could cause unexpected adverse effects (e.g., intracranial infection, cerebral haemorrhage, seizures) and thus we set the expected level of severity as Severe to account for the worst possible situation. At the end of this procedure, the NHPs are humanly killed by an overdose of anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project explores the neural basis of learning and decision- making which is known to require the prefrontal cortex (PFC). The PFC of the non-human primate (NHP) brain has considerable anatomical and functional overlap with human PFC, but is virtually non-existent in other mammals, necessitating the use of NFIPs in this project. Further, the cognitive and sensory-motor requirements of these tasks require a highly developed PFC and basal ganglia, and such requirements could not be met in other species such as rodents.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use advanced experimental techniques which allow us to record more data simultaneously from different brain areas in a shorter time from a single subject. This directly leads to fewer animals being used, but with far better data as neuronal activity patterns can be directly compared in the same subject in the same physiological and behavioural state, thus allowing the most direct comparison of functional specificity.
3. 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 use of a non-human primate (NHP) model is essential given NHP and humans both share a prefrontal cortex which is absent in rodents. Rodent models could not be used for these experiments due to substantial anatomical differences and their inability to perform the complex tasks outlined in this License. Optimisation of animal welfare is achieved by a variety of approaches, which include: 1) Positive Reinforcement Training (PRT) of animal to cooperate with the procedure so as to reduce stress. 2) When necessary, giving animals a small dose of oral sedative for the first few days after new

	procedures are first introduced. 3) Use of appropriate pre- and post-operative analgesic and antibiotic regimes. 4) Use of additional NC3 Rs approved refinements to improve the outcome of the experiment and improve animal welfare.

Project 13	**Development of novel translatable measures and therapeutics for pain disorders**	
Key Words (max. 5 words)	Pain, rodents, analgesics, biomarkers, drug discovery	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Chronic neuropathic arid inflammatory pain conditions remain a substantial clinical problem for society. More effective, safer analgesics are still urgently required to help patients. This project aims to gain greater understanding of the mechanisms by which chronic pain conditions arise and persist, so that drug treatments that fulfil unmet medical needs for pain disorders can be identified and developed. A second critical aim of the project is to determine objective biological markers of the pain state experience, such that direct relationships can be inferred between findings in patients and experimental subjects (in this case, rats and mice).	
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 greatest success of this project will be to identity a novel analgesic that provides more pain relief in partly responsive patients, or provides pain relief in conditions that are currently poorly responsive to existing analgesics, or is free of the sometimes serious side effects that some existing analgesics possess. In this process of drug discovery, insights into the mechanisms of pain perception will be gained, most importantly with regards to the correspondence of the underlying mechanisms between humans and rats.	

	This work will directly challenge the utility of many different aspects of rodent pain research to determine which aspects hold the most predictive validity for findings in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice will be used for this work over a period of 5 years. Approximately 1000 of each species are likely to be used annually.
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 basic premise of this project is to use rats and mice to develop models of pain that relate to the human experience, measure both behavioural and physiological endpoints from these animals, and to determine how novel treatments influence these endpoints. Adverse events can arise from these three main objectives: induction of pain, measurement of endpoints, and administration of novel treatments. The intention of this work is to deliberately induce a controlled level of pain in rats and mice — these models will be chosen and limited such that they remain with Mild or Moderate level of severity. Measurement of physiological endpoints, such as EEG or brain neurochemistry requires invasive surgery to implant probes or electrodes into the brain — again this will be limited to remain at Moderate level of severity. The administration of novel treatments normally remains within a Mild limit of severity, although there is the potential for an unexpected adverse effect to occur which could raise severity to Moderate. Upon completion of study, or before completion of study if humane endpoints have been reached, animals will be killed by a Schedule I method. In some studies, animals may be killed by appropriate non-Schedule 1 methods for purposes of collection of tissue samples.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The experience of pain is a systems-level experience of an organism that is not currently possible to deconstruct in a meaningful way into lesser parts that avoid the use of awake animals. Further, understanding the balance between efficacy and adverse effects of novel treatments essentially requires the integrated physiological systems of a whole animal to maximise the predictive validity of the data.

2. Reduction Explain how you will assure the use of minimum numbers of animals	All studies will follow Institutional guidelines on best laboratory practice, where statisticians will be consulted to guide best study designs. Poorly performing assays or models showing large variability or inconsistent effects will be halted as soon as this is detected. For novel drug treatments, pharmacokinetic data and/or measures of target engagement (i.e. how well the drug interacts with its target at different doses) are collected before testing for in vivo analgesic efficacy. This obviates the need for dose- finding" studies and prevents animals being used for studies where inappropriate doses have been chosen.
3. 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.	While the intent of this project licence is to be able to model different forms of chronic pain in rodents in an attempt to replicate pain states in humans as closely as possible, the use of each model shall be defined and limited by expected humane endpoints. Any escalation of endpoint beyond these pre-defined criteria would result in immediate killing by a Schedule I procedure. The investigation and quantification of behavioural correlates of pain states requires active, healthy animals if reliable and meaningful results, free of experimental errors are to be obtained. The behavioural endpoints being measured in this project involve sensorimotor testing, naturalistic behaviour or food-rewarded operant behaviour, none of which can be reliably measured in severely stressed animals. These endpoints are extremely sensitive to pain, suffering or distress and any abnormal response (e.g. trial omissions, lengthy response latencies) will prompt immediate review, revision and/or termination of the study as appropriate. Elevation of the severity limit of our studies to severe (whether acute or cumulative) at any point in any animal effectively invalidates the behavioural endpoints and the whole purpose of this program of work. Rats and mice will be used since there is an abundant database of the behavioural, physiological and pharmacological characteristics of these animals. Rats and mice can reliably model clinically relevant aspects of the pain experience, i.e. hypersensitivity and allodynia. Gross anatomy of pain processing structures is broadly comparable between rodents and humans, and descending modulation of spinal cord activity is

	preserved across species. As a result, rats and mice have become dependable experimental systems for modelling different pain states and predicting clinical analgesic efficacy of test compounds.

Project 14	**Mechanisms of neuronal plasticity**	
Key Words (max. 5 words)	Alzheimer's disease, schizophrenia, synapse, genes	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cognitive impairment, resulting from dysfunction of memory storage mechanisms in brain regions such as the cerebral cortex and hippocampus, is a severe clinical problem, contributing to diseases such as Alzheimer's disease and schizophrenia. These memory storage mechanisms require specific co-ordinated changes in the activity of "synapses" – the points at which pairs of brain cells contact each other and pass on information. This process is known as neuronal plasticity. However, the mechanisms allowing synapses to change their function, and the extent to which these cause the disease symptoms to appear, are not understood well. This research programme aims to increase our understanding of the physiological and pathological processes resulting in synapse change in these brain areas, and of the role of specific genes in sustaining the changes, in the healthy situation and in disease models.	
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)?	The research will improve our understanding of the mechanisms allowing synaptic changes in the brain, and provide insight into how the processes go wrong in diseases such as Alzheimer's disease and schizophrenia. As a result, the research may also illuminate novel ways of alleviating these neurological diseases.	

What species and approximate numbers of animals do you expect to use over what period of time?	Rats (~ 250) and mice (~5000) over 5 years.
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 vast majority of these studies involve drug treatments or minor genetic manipulations that may produce subtle behavioural signs, but are not expected to lead to substantial welfare issues. None of the procedures are anticipated to produce overt suffering for the animals. Some of the procedures may reproduce aspects of psychiatric or neurological diseases in the rats and mice which could be associated with a degree of emotional stress. However, whether the emotional impact of such diseases in rodents is equivalent to that in humans is not clear, and the studies in any case are not predicted to reproduce the complete range of neurobiological change associated with these diseases. The animals will be humanely killed at the end of the studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The majority of the research uses in vitro systems such as cell culture models. However, these neurological diseases are complex, and result from subtle changes in the communication between interconnected groups of brain regions. Hence the hypotheses generated, based on the results from the cell culture models, generally need to be tested in animals at some stage.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Much of our research is conducted using tissue samples (e.g. biochemical tests). Furthermore, the use of cultured systems derived from tissue enables the preparation of multiple cell based tests from a more limited number of animals.
3. 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.	Rats or mice are used - there are good models of these diseases in these rodents. The vast majority of these studies involve minor genetic manipulations that are not associated with any welfare issues. None of the procedures involve any overt suffering for the animals. Studies are designed to minimise animal use, and maximise the amount of information obtained from each study involving animal use.

Project 15	**Presynaptic function in health and disease**	
Key Words (max. 5 words)	Neurotransmitter, neurone, synapse, vesicle, disease	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Communication between neurones occurs at specialized junctions called synapses where chemical neurotransmitters are released from one side of the synapse (presynapse) and recognised by the other (postsynaptic). Efficient and reliable neurotransmitter release from the presynapse is critical for correct brain function. Evidence is accumulating that a number of human neuronal disorders have at their core a defect in neurotransmitter release. These defects, sometimes imperceptibly small when compared to normal neurotransmitter release, can result in altered brain communication and ultimately in some cases neuronal death. This includes both neurodevelopmental disorders (such as X-linked intellectual disability, autism, Downs Syndrome and epilepsy) and neurodegenerative conditions (such as Alzheimer's, Parkinson's and Huntington's disease). Evidence for this comes from an ever increasing range of genetic mutations found in a series of presynaptic proteins that have been discovered in patients displaying these disorders. Thus it is a matter of urgency to identify the essential mechanisms by which neurotransmitter is released, in order to see how this process can be either preserved or restored in these debilitating disorders. To achieve this goal we must (a) understand how	

	neurotransmitter release is controlled during normal brain function and (b) how it is altered in specific animal models of human neurodevelopmental and neurodegenerative disorders. Thus central to this licence application is a need to understand how changes in presynaptic function lead to dysfunctional neurotransmitter release and ultimately precipitate neurodevelopmental and neurodegenerative disorders. The key areas for investigation in this project are therefore: (1) to determine the roles of specific molecules in presynaptic function and (2) to assess whether and how presynaptic function is altered in neurones from rodents with genetic modifications that model both neurodevelopmental disorders and neurodegenerative disease.
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)?	The benefits of the research outlined in this licence application will be the identification of new targets for drug intervention which ultimately could lead to the development for new therapeutics in the longer term to treat both neurodevelopmental and neurodegenerative disorders. Importantly, this approach examines specific processes that are controlled by individual molecules in neurotransmitter release. The advantage of this strategy is that it should allow a greater diversity of interventions in brain processes that are either parallel to, or are integrated with, the events that are affected in many neurodevelopmental and neurodegenerative conditions. The major beneficiaries will be the other researchers in same field, however as stated above there is potential for the developments of new therapeutics in the longer term.
What species and approximate numbers of animals do you expect to use over what period of time?	It is calculated that 10000 mice and 2000 rats will be required to achieve the aims outlined in this project.
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?	This project will utilise neurones taken from genetically modified rodents (mice and rats) for study on either glass coverslips or test tubes, for which there are no expected adverse effects, because animals with genetic alterations that may cause distress later in their life will be culled before symptoms arise. In some cases this will mean culling the mice as embryos. The likely/expected severity would be mild, with the

	exception of the audiogenic seizure model, where rodents will be challenged with a high decibel noise for up to 2 minutes to determine if they are more susceptible to epileptic seizure activity, which has moderate severity. In most cases culling will be performed by schedule 1 methods, but occasionally by conscious decapitation will be performed in instances where the release of stress hormones may affect the experimental outcome.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All of the work in this project will be performed *in vitro*, examining basic molecular processes. As the field currently stands there is no alternative to using neurones from animals. Cell lines currently cannot mimic the intricate series of molecular events observed in primary neuronal culture and in most cases require a series of interventions to make them "neuronal". Therefore it is unlikely that this system will accurate mimic the events that occur at the human synapse. Our research group are currently establishing assays of presynaptic function in human stem cells from patients suffering from neurodevelopmental / neurodegenerative disorders. It is hoped that in the medium term a number of our experiments will be able to be translated across to these human neurones thus replacing the need for neuronal cultures from animals in some aspects of our research programme. In some cases this will still not be possible however. In the case of synaptosomes (which are isolated presynapses from adult rodent brain) and brain slices (which retain relatively intact neuronal circuits and connections) there is currently no suitable alternative that accurately mimics that level of either maturity or complexity.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For primary tissue culture each animal provides at least 5×10^5 neurones in embryonic preparations 1×10^7 in postnatal preparations. These cultures will be shared among the research group to permit as many experiments as possible to be performed per animal. This is also the case with both synaptosomes and brain slices, where multiple experiments can be performed from the same tissue.

	We will use established statistical methods that we have applied to these types of data over the past 10 years, and that have been subject to scrutiny during peer-review. Primary neuronal culture is reproducible system to examine meaning we can reduce the number of animals required to reach statistical significance (usually less than 6 animals). This number is slightly higher for synaptosomes and brain slices (normally 8–10 animals are required for each experiment). There is a chance that novel genetic manipulations may lead to greater variance in a particular phenotype. Power analysis calculations can be performed to accurately estimate the number of animals required to complete the aims with statistical significance in this regard. Finally, all of the *in vitro* systems in the application are extremely well characterised by both ourselves and other research groups, meaning that almost all animals will be utilised in a productive manner. Wherever possible both male and female animals will be used in our experiments, again reducing the number of unnecessary deaths.
3. 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.	Rodents are the most appropriate species for our work not only because they are amenable to genetic manipulation but studies have identified that the key molecules we are investigating are very similar in both rodents and humans. Furthermore some aspects of the research programme require investigation of region-specific effects in mammalian brain (e,g, striatal dysfunction in Huntington's Disease). Therefore we have a system that is built in the same general manner to humans is key to this programme. Also, genetically modified rodents accurately recapitulate the genetic disease state in individual neurones, providing a refinement not possible with other alternatives such as lesion studies. Another further refinement is the expertise and experience of care and research staff within the laboratory and animal facility. Rodents will have access to a well-resourced and well-equipped modern facility with IVCs and barrier systems in use for maintaining SPF status/health. Where appropriate to animal health and wellbeing colonies will be provided with environmental enrichment. In most cases culling of animals will be performed

	using schedule 1 approaches. The GM mice we plan to use initially display only mild phenotypes. For any known age-dependent adverse effects of genotype animals will be culled before any distress is known to occur. Novel GM animals that may become the subject of investigation will have their health monitored closely.

Project 16	**Neural plasticity in health and disease**	
Key Words (max. 5 words)	Learning, memory, neural development, neurodegeneration	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	Basic research
	√	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	√	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim of this project is to gain insight into how the brain develops, and how it adapts during learning and memory. The key questions to be addressed are: 1) How do the connections between brain cells develop? 2) How do they change during learning? 3) How can these connectivity changes store memory? These questions will be addressed in normal animals as well as in animal models of human disease, such as autism spectrum disorder, schizophrenia and dementias.	
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 is expected to give us better understanding of how networks of the brain are formed and how they change in response to environmental demands. Such insights are required in order to understand what goes wrong in developmental neuropsychiatric disorder, such as autism and schizophrenia, as well as what causes memory impairments in dementia, such as Alzheimer's disease.	
What species and	The project will use mice and rats, including	

approximate numbers of animals do you expect to use over what period of time?	genetically altered animals. We expect to use approximately 8,000 animals over five years for breeding and tissue harvesting, and up to 9,750 animals for experiments.
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?	Most of the experiments will be done on anaesthetised animals. The level of anaesthesia will be carefully monitored, and in case of complications, the animals would be humanely killed. Some animals will be food or water restricted to motivate them to complete learning and memory tasks, and food/water is a reward for successful completion. In some experiments, the animals will undergo surgery under anaesthesia, involving for example placement of cannulae into the brain, through which drugs can be delivered, then recover before further behavioural tests are carried out. In other experiments, the animals will be stimulated with electrodes in the brain during the tasks. Although complications to such surgery are rare, the animals will be followed closely for signs of ill health, and if such complications of more than a mild nature were to occur and could not be promptly remedied, the animals would be humanely killed. All animals will be humanely killed at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals are necessary for these experiments because we want to gain insight into brain mechanisms of memory. Since this research requires recording from the brain through the skull or preparation of live brain tissue, human experiments are not ethically justified. No computer model is currently available that can replace the use of animal tissue for our objective, as there is insufficient knowledge of the mechanisms involved. Nevertheless, computer models will be used to assist in the interpretation of the data obtained in experiments from animal tissue.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will ensure that the minimum number of animals will be used by optimising the experimental design and by using appropriate statistical methods.

3. 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 will use rats and mice for this project. Rats and mice are evolutionarily, genetically and physiologically sufficiently close to humans to make the experiments relevant for human disease, and they are much used in behavioural studies of learning and memory. This enables us to build upon a rich body of research already carried out in the past. Moreover, genetic technology in mice enables more precise targeting of specific cell types, making the experiments easier to interpret thus reducing the number of animals required. Most experiments will be done on tissue slices taken from the brain of animals that have previously been killed under terminal anaesthesia. This is the most refined model that can be used for the study of developmental processes and memory-related plasticity. Animal suffering will be minimised by using such simplified models. However, these are still models, and may not always represent the mechanisms that operate in intact animals. Therefore, we need to confirm the results in a small number of behaving animals.

Project 17	**Development of new models of FTLD/MND**	
Key Words (max. 5 words)	neurodegeneration; dementia; transgenic; mouse; disease	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To develop novel mouse models of frontotemporal lobar degeneration (FTLD)/motor neuron disease (MND) in which to study mechanisms of disease and find new treatments. There is significant overlap between FTLD and MND with ~15% of patients presenting with both diseases. MND is a devastating disease, causing progressive paralysis and loss of life typically within just 2-5 years of onset. There are currently no treatments for these diseases. To generate the mice we will introduce a genetic modification that has been identified as being present in one out of every twelve patients diagnosed with FTLD/MND (i.e. we will be creating transgenic mice).	
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)?	The primary potential benefit of this project is the generation of novel models of FTLD/MND that can be used to develop new treatments. Detailed studies of disease mechanisms are not possible in humans and therefore new mouse models of FTLD/MND would greatly improve our knowledge in this field. There are no disease-modifying treatments at present for FTLD/MND and therefore potential future benefits could be significant if this new knowledge helps to identify novel ways of treating the disease that could be tested in patients.	

What species and approximate numbers of animals do you expect to use over what period of time?	All studies will be in mice. Over the five year period of the project the maximum number of mice we expect to use is ~2500 mice. The majority (~2000) of these will be for breeding purposes and for maintenance of transgenic lines with around 500 animals to be used in actual experimental 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?	We will be assessing behavioural changes in the mice to find out if these are similar to what is observed in FTLD/MND patients. In addition we will assess pathological changes in the brain of the transgenic mice. The tests to be used are quite straightforward and do not involve any stress or harm to the animal. They will be used to assess co-ordination, learning, social interaction and motivation. Wherever possible we will use tests that rely on normal mouse behaviours such as nest building and burrowing. No surgery or other invasive procedures will be used. Animals will receive some simple injections and will occasionally have blood taken. Since we are generating completely new transgenic mice we do not know at this stage what adverse effects will be observed. However as we are trying to model a severe human disease like MND it is possible that the animals will be affected quite badly, for example showing problems with movement and eventual paralysis. Based on very recent studies in mice with this gene related modifications effects range from none, the mice being completely normal, to hindlimb paralysis and reduced survival starting at 20-40 weeks. We will monitor all our animals very closely and immediately stop studies if there is any indication of adverse effects that are causing the animal pain, suffering or distress. The primary humane end point will be when animals show extreme weakness in both hindlimbs, defined as the inability to dorsiflex (bend).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Studying mechanisms involved in neurological diseases such as FTLD/MND is extremely complex and in addition to pathological changes in the brains of FTLD/MND patients, the disease is characterised by profound changes in behaviour, which it is not possible to study *in vitro*. The proposed animal studies are complementary to a much greater programme of work on FTLD/MND using human samples, cell lines and model organisms such as the

	nematode *C.elegans*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Pathological and behavioural end points proposed in this project are well established in studies of dementia and other neurological disease and experiments are therefore planned based on previously published data or our own experience. We will use the minimum number of animals that can answer the desired scientific objectives and will extract all relevant information in the data by using appropriate statistical analysis. Studies will be designed using the newly released Experimental Design Assistant (EDA) from the NC3Rs (https://www.nc3rs.org.uk/experimental-design-assistant-eda).
3. 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.	Although it is possible to use animals with a lower degree of neurophysiological sensitivity (e.g. zebrafish, drosophila, *C.elegans*) as models of dementia these organisms do not allow one to mimic fully the brain pathology and behavioural changes associated with FTLD/MND. In contrast mice allow one to model the disease more closely, hopefully leading to a better understanding of disease mechanisms and the discovery of new treatments. All animals will be closely monitored for adverse effects caused by the genetic modification. Should there be severe behavioural changes then animals will be humanely killed immediately.

Project 18	**Roles of electrical activity in brain maturation**	
Key Words (max. 5 words)	Brain, activity, plasticity, development, olfaction	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The brain functions through electrical activity that occurs in its constituent cells, or neurons. This activity is not only present in the adult brain, but is also an important feature of the developing nervous system. Our project aims to uncover the roles played by this electrical activity in brain maturation: in a process known as 'neuronal plasticity', how does ongoing activity in a neuron sculpt its development?	
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 is a basic science project that will primarily produce enhanced understanding of brain function. However, understanding plasticity in the normal brain is of enormous potential benefit for human health. The brain has limited capacity for self-repair, and by itself cannot counteract degenerative brain diseases such as Alzheimer's disease or Parkinson's disease, or trauma caused by stroke or injury. In the future it may be possible to repair damaged brain tissue by replacement therapy, introducing new, immature neurons that will eventually mature and restore brain function. This approach, however, requires detailed knowledge of how neurons mature naturally in the normal brain: when attempting to produce new neuronal networks in human patents, we first need to understand the mechanisms the brain uses during its own maturation.	

What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use around 6000 mice over the course of this 5-year project. The vast majority of these (5000) will be used for breeding purposes.
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 most common adverse effects are expected to be surgical complications such as post-operative infection, which will occur very rarely (<1% of animals), and will be closely monitored and treated in consultation with veterinary staff. All procedures will be of moderate severity at most. At the end of all protocols animals will be humanely killed; in many cases their brain tissue will then be used for experimental purposes.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no alternatives to using animals in this research. We aim to produce basic new knowledge concerning brain development in vivo, the kind of knowledge that simply cannot be obtained from current non-animal alternatives such as cell cultures or computer models. However, we will use cell culture approaches in parallel with proposed protocols to inform our experiments, thus minimising animals used. We will also make our data available to computer modellers to minimise future animal use in this area.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers will be minimised by 1) using previous and preliminary experiments in in vitro systems to identify the most appropriate analyses and necessary sample sizes in vivo. 2) Combining multiple measures of brain structure and function within individual animals, and often within individual cells. 3) Maximising data quality for each animal through stringent welfare controls and optimised experimental design.
3. 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.	Mice will be used throughout the project because they represent the simplest organism with appropriate neuronal maturation, and because genetically-modified lines allow us to ask powerful and crucial experimental questions. All protocols are refined to involve the least possible suffering: recovery surgery will take place in a minority of cases, using minimally invasive methods coupled with pre and post-operative care to minimise pain, distress or discomfort.

Project 19	**Studies of brain development in the mouse**	
Key Words (max. 5 words)	Mouse, brain development	
Expected duration of the project (yrs)	Five years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The process of development within the embryo of mammals is a complex one that is normally tightly controlled by means of a number of interacting chemical signals. Of all the organs in the body, perhaps the most complex is the brain, whose development involves millions of nerve cells making satisfactory links with each other. If connections are not made, or wrong connections are made, then the brain may not operate correctly; 'errors' occurring in specific regions of the brain may lead to a loss of specific functions, for example of hearing or of seeing. We are particularly interested in the establishment of connections between the eye and the brain, that allow the passage of visual signals from the retina (at the back of the eye) to the brain where they are processed. To do so, we will anaesthetise animals and administer a very small volume (micro-litres) of fluorescent tracer dye into the cells of the retina. This dye will then spread along the nerves, and we will be able to identify the connections made by these cells in the brain when the animals are killed and the brains were removed. This type of analysis enables us to investigate whether the connections between the eye and the brain have been made correctly. If they have not, we may be able to identify which genes	

	are responsible for this failure of development. We have already identified a class of molecules which plays a role in this process which we would like to investigate further. These tracer studies cannot be undertaken in dead animals; we have previously tried to do so but the results were very poor. Also the study of neural interactions in culture can be a first step but can give only very few information and requires the study of this process in the intact animal.
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)?	A fundamental challenge in neuroscience is to identify and to understand the molecules and mechanisms that ensure that developing neurons form the correct connections. The deregulation of processes which control neural circuit formation are very likely involved in the aetiology of neurodevelopmental disorders. For a better understanding of these disorders it is crucially important to first understand the normal development of neural circuits. We will study here the development of the axonal projection between the retina of the eye and the superior colliculus in the brain.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use about 5000 mice over a period of five years
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?	Mice used in these studies are very likely not to show any adverse phenotypes. However, in the event of animals showing an adverse phenotype, they will be killed using a schedule 1 procedure or the Home Office inspector will be notified immediately.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The analysis of axonal projections, in particular the projection from the retina to the SC, cannot be done in vitro as there are no in vitro assays or culture systems available which can reproduce the complex projections of axons seen in vivo. There is one co-culture system available developed in the lab of Patricia Gaspar (Nicol et al. Nat Neurosci. 2007,10, p340) which however only very superficially can mimic the targeting of axons but is by no means suitable for the experiments we would like to perform that is investigating also small changes in mapping. The analysis of these projections can therefore only be performed in living animals.

	Mice will be used because of the availability of suitable genetically altered mice, enabling us to look at the role of particular molecules in axon guidance processes and development of brain architecture.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will keep the number of mice as low as possible. Generally, the success rate of labelling a fraction of retinal axons with DiI is about 60–70%. We will need about 100 animals for the analysis of the projection pattern of a genetically altered mouse line, as we have to analyse the projection pattern of axons from a few (<5) different sites of the retina. These numbers are based on previous experiments, and experiments performed by other groups performing similar experiments, that is to analyse the projection pattern of retinal axons from one particular site in the retina, about 20 animals are needed anticipating that a same number of control mice is used.
3. 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.	Mice will be used because of the availability of genetically altered mice, enabling us to look at the role of particular molecules in particular axon guidance and developmental processes concerning brain architecture.

Project 20	**Bioelectronic Medicines**	
Key Words (max. 5 words)	Electrophysiology, implantable devices,	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The purpose of this project is to understand how implantable devices and electrical signals can be used to regulate the nervous system to treat disease and organ dysfunction. To do this we must first gain a better understanding of the anatomy and function of the nervous system, and how it exerts control of organ function. Secondly we must ascertain whether electrical regulation of the nervous system can be accomplished safely and effectively.	
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)?	Currently medicines treat a wide range of ailments in billions of people. However, there are a multitude of side effects and treatment resistant populations. Although in general successful, current treatments are expensive, socially limiting, and in most cases only a treatment and not cure. The potential for Bioelectronic medicine is broad, as all organs are controlled by the nervous system. Through implantation of devices that regulate the nervous system, and in turn organs, one can potentially reverse organ dysfunction and disease states completely.	
What species and approximate numbers of animals do you expect to use over what period of time?	Pig (250 over 5 years) and Sheep (250 over 5 years) and Goats (100 over 5 years).	

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?	Work will involve anaesthetised animals that are euthanised at the end of the study before they recover from the anaesthetic. Beyond the induction of anaesthesia these animals will not experience any pain of suffering. In addition, these animals will give us the information we need to more effectively and safely move to the next step of investigating treatment in animals with disease. Animals will undergo surgical implantation of devices. There are no expected adverse effects with implantation and treatment as this is a terminal procedure. All animals will be killed by a schedule I method or will be perfused to allow tissue to be analysed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	A limited amount of testing has been done without using animals to give confidence nerve stimulation may treat disease. The science cannot be advanced further without using animals. Only a whole body system biology approach will give conclusive evidence and understanding that manipulation of the nervous system can be an effective treatment of disease. A computer model does not yet exist to test nerve stimulation as a treatment of disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Pilot studies in small numbers of animals will be used to develop optimal methods, assess feasibility and outcome measures, and will define a go/no go criteria for further studies. Statistical advice will be sought for study design to ensure adequate animal numbers are used. The number of animals used in the studies will not exceed the study size required by statistics to ensure reliable significance and result confidence.
3. 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.	Pigs, sheep and goats will be used for all experiments because they are most appropriate species to determine efficacy and safety with respect to the devices tested. Their neuroanatomy and physiology is very similar to that in humans. We will work with manufacturers and academic experts, to ensure a continued refinement approach is adopted for all implantable devices, electrodes and leads. We will work toward fully implantable devices as advancement to external wires and head caps.

Project 21	**Tissue isolation for electrophysiology, neurochemistry and pharmacology**	
Key Words (max. 5 words)	Neurodegeneration, pain, native, drug discovery	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The work will lead to improved understanding of the biological pathways leading to chronic pain and neurodegeneration and the creation of improved in vitro models that better reflect the disease states.	
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)?	We will test new drugs that, if successful, could advance into clinical trials and may lead to new drugs for treating these diseases. This would allow people to be more independent, live longer and healthier lives and this should also reduce care burden and nursing home costs.	
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice will be used for this work over a period of 5 years. In total, approximately 10000 animals are likely to be used over the duration of this project.	
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?	Very occasionally (<0.1% incidence), administration of novel compounds and use of novel transgenic animal models can result in unexpected adverse effects that might require animals to be immediately and humanely killed, for instance seizures or respiratory distress. Should any adverse effect occur the animal will be immediately and humanely killed. At the end of the studies the animals will be humanely killed and tissues used for biochemical and histological assessment to	

	understand the model and the effects of treatment on key molecular pathways.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although recombinant cells can provide much information about the pharmacological properties of compounds, the nervous system is such that single cell preparations cannot predict the outcome of drug exposure as systems become more complex. Therefore, tissue derived from rodent nervous system will be used to determine the pharmacological activity of novel compounds at targets that modulate nervous system function.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This work will involve the minimal number of animals needed to achieve a statistically significant estimate of the mean and variance of key parameters. A professional statistician will help in determining statistical power and reproducibility, thus minimizing animal numbers.
3. 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 rat is predominantly used because of the large amount of information known about the biological basis of its behaviour. Mice may also be used when, for example, the target in those species more closely resembles the human form. Mouse tissue might also be required when a target protein has been under or over-expressed or where a mouse model of a disease exists.

Project 22	**Investigation of genetic forms of neurodegeneration**	
Key Words (max. 5 words)	Frontotemporal dementia, motor neuron disease, amyotrophic lateral sclerosis, C9orf72	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
	No	Regulatory use and routine production
	No	Protection of the natural environment in the interests of the health or welfare of humans or animals
	No	Preservation of species
	No	Higher education or training
	No	Forensic enquiries
	✓	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) (also known as motor neuron disease) are two related and devastating neurodegenerative disorders for which no effective therapies exist. We will investigate genes that cause FTD and ALS to determine how they lead to the death of brain cells. We will also investigate whether drug treatments can reverse these effects. This is the first stage of developing new treatments for FTD and ALS.	
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)?	The potential benefits are a greater understanding of FTD and ALS and the development of potentially new therapies.	
What species and approximate numbers of animals do you expect to use over what period of time?	We will use a maximum of 12,000 mice over the five year period.	
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	All procedures are either mild or moderate in severity. The most likely adverse effects are signs of neurodegenerative disease caused by the mice having genes that cause neurodegeneration. It is essential that the mice develop some symptoms of disease in order for us to study and treat the disease.	

end?	All mice will be killed at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The diseases we study involve multiple cell types, not just neurons, and appear over time, progressively. There is no cell culture or computer model that is able to replace a whole mouse for investigating the complex interactions that occur between different cell types and organs over an entire lifespan.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We work out before each study the number of animals required to give us statistically meaningful results – by using statistical calculations for known effects, where we can, and by comparing planned experiments with studies carried out previously, as necessary. We then plan our experiments accordingly with the minimum possible number of mice. We also regularly check our mouse colonies to ensure efficient colony management. Wherever possible, we use the same animals in multiple tests with the assurance that there are no additive adverse effects in animal cohorts.
3. 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 currently the only mammalian species that we can use to examine the full complement of parameters that are measurable in behavioural and physiological changes, cellular and molecular changes arising from neurodegenerative disease, in concert with our ability to tailor the genome of these animals to maximise the information gained from each mouse. We will minimise harm by implementing high standards of care for each mouse, by ensuring that the maximum severity for all protocols is either mild or moderate and by using well defined humane end-points.

Project 23	**Autophagy and neurodegeneration**	
Key Words (max. 5 words)	Autophagy, Neurodegeneration, ataxia, signalling pathways	
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Ataxias are a class of rare movement disorders, many of which of genetic origin, which are neglected by major research streams but devastate individuals and their families, leading invariably to death. Because of genetic inheritance these disease often manifest in more than one family member including children as young as 3. We aim at finding a cure for one of these ataxias called Dentato Rubro Pallido Luysian Atrophy. Our current understanding involves the cellular process of autophagy and a signalling pathway as key processes.	
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)?	We expect to refine our knowledge of Dentato Rubro Pallido Luysian Atrophy to the point of attempting treatment in animal models at the end of this work. Also we expect to deliver major knowledge advancement of how autophagy is regulated in nerve cells by the pathway identified, which will be relevant to many neurodegenerative diseases beyond Dentato Rubro Pallido Luysian Atrophy, like Alzheimer and Parkinson.	
What species and approximate numbers of animals do you expect to use	We plan to use mice as the least sentient species with a good anatomical representation of the cerebellar nuclei involved in this ataxia. We expect to	

over what period of time?	use approximately 3800 animals in the next 5 years
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?	All procedures are at a moderate level. The ataxic mice develop a neurological phenotype and we have established humane end point to minimise pain and distress in the latest stages of the disease. All animals are either transferred between protocols or humanely killed at the end of terminal procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are an ideal example of replacement. Our findings started in the fruitfly *Drosophila melanogaster* and whenever possible we use these invertebrate organisms instead of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Thanks to our integration with *Drosophila* we are able to reduce greatly the number of mice required for experiments as we only test finalised hypotheses in animals and use flies for the generation of new models and ideas, which requires larger numbers.
3. 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.	Mice have been chosen for our work as they have the lowest neurophysiological sensitivity while still having a nervous system of comparable complexity to man. When analysing knock out mice we use inducible technologies whenever possible, to minimise the number of mice affected by degenerative pathologies. In the cases, where a mouse has developed the neurological aspects of the disease, we apply a set of criteria to define the humane end-point and ensure that suffering is kept to a minimum.

Project 24	**Inter-connected studies of prion and other neurodegenerative diseases**	
Key Words (max. 5 words)	Prions, Creutzfeldt-Jakob Disease, transgenic, therapeutics	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	✓	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Prions are unique among infectious diseases by appearing to lack their own genes and consisting of misshapen forms of one of the body's own proteins, the prion protein. These abnormal proteins cause incurable brain diseases such as variant Creutzfeldt-Jakob disease (vCJD) (commonly known as mad cow disease). Our research programmes are highly multidisciplinary and are broadly aimed at understanding the basic biology of these diseases, which will intern give us pointers for intervention with candidate therapeutic compounds.	
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)?	Fundamental questions on how prions kill cells remain, and our future research is geared toward making treatment of patients a reality. Prion diseases are an important model for several other brain disorders such as Alzheimer's disease because it appears likely that very similar processes may be involved, making any therapeutic drugs found for prion diseases of wider benefit to public health.	
What species and approximate numbers of animals do you expect to use over what period	Mouse and Hamster. We expect to use 147,000 mice and 2000 Hamsters over the 5-year period.	

of time?	
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?	All procedures are either mild or moderate in severity. The most likely adverse effects are associated with microsurgery and intracerebral inoculations. All animals will be monitored closely for any adverse effects and appropriate refinement measures taken promptly. No animals will be allowed to exceed moderate severity before being killed humanely. All animals will be killed at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	While we have cell culture facilities for studying rodent prions in the test tube, there are at present no cell lines for studying human prions. We have parallel projects aimed at developing cell lines for studying human prions. However, until this test tube work has been successful hopefully in the near future, key prion disease parameters such as clinical duration and features, behavioural changes, brain damage and the spread of the abnormal protein within the body leading to fatal brain damage, can only be studied in an animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use our scrapie cell assay extensively and this has enabled us reduce the number of mice we would otherwise use in our studies. For all our research we will ensure that the smallest number of animals will be used consistent with achieving a clear experimental result. We also regularly check our mouse colonies to ensure efficient colony management.
3. 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.	By using anaesthesia and pain killers as necessary, and through careful monitoring, animal suffering will be reduced to an absolute minimum. We will minimise harm by implementing high standards of care for each animal, by ensuring that the maximum severity for all protocols is either mild or moderate and by using well-defined humane end-points.

Project 25	**CNS glial development and demyelinating disease**
Key Words	multiple sclerosis, demyelination, neuroinflammation, neurodegeneration, neuropathology
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;

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

Multiple Sclerosis (MS) is the commonest cause of neurological disability affecting young adults in the western world, occuring in approximately 100,000 people in the UK and 2.5 million worldwide. It is a truly devastating condition for those who receive the diagnosis, which usually occurs between the age of 18 and 40. Although there are now some effective drugs to reduce the number of MS attacks, there is no effective drug treatment to stop patients becoming disabled over the long term. The main objectives of our programme of research are: 1. to discover some of the molecules that allow the brain to repair itself following the type of damage caused by MS; 2. to find out what is responsible for causing this repair mechanism to fail later on in the course of MS; 3. to identify the molecules produced by cells of the immune system that cause death of neurons in the MS brain, which leads to a build up of disability; and 4. to use the information from the studies above to design and test new therapies that stop the death of neurons caused by immune cell molecules.

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)?

There is a great need for a better understanding of the disease processes involved in progressive MS and for the development of well tolerated drugs that can halt or substantially slow the progression of neurological disability. Knowledge of how individual proteins and cell-cell interactions are changed during the process of tissue damage and repair in demyelinating diseases such as MS is a prerequisite to

identifying potential targets for therapy. The end result of our research is expected to be a number of compounds (proteins or chemicals) that can be tested for therapeutic efficacy in early stage clinical trials in people suffering from progressive MS.

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

This research is expected to use approximately 1,100 adult rats and 600 adult mice over the next 5 years.

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?

MS is a debilitating neurological disease that affects young adults. The progressive build up of symptoms results in the patient being confined to a wheelchair during their third or fourth decade. One of the main aims of our programme of study is to test the idea that toxic molecules build up in the cerebrospinal fluid that surrounds the brain and this will inevitably give rise to a range of clinical symptoms that reflect the location and extent of the pathology. In order to be able to propose new therapeutic targets and test pharmacological agents that interact with those targets, thereby halting or slowing progression, it is necessary to mimic the human disease as closely as possible. For MS this inevitably means that the animal models will have to model the loss of motor and sensory function over longer periods of time that is characteristic of chronic MS. Thus some of the models will be classified as severe. At the end of the experiments the animals will be terminally anaesthetised and the brain and spinal cord tissues taken for detailed analysis.

Application of the 3Rs

Replacement

The purpose of this programme of research is to investigate the mechanisms by which patients accumulate neurological disability in the complex inflammatory neurological disease, multiple sclerosis (MS). MS involves the interplay of multiple body systems (immune, endocrine and nervous systems). Individual features of the complex disease can be studied using simpler cell culture systems once the overall mechanisms have been worked out. How all the steps interact and lead to clinical disease can only be studied using a living dynamic system that mimics the human disease. We are already using human post-mortem tissues and cultures of brain slices to identify individual molecules that might be involved in the disease process, but testing our ideas concerning how all the molecules link together to cause pathology and clinical disease requires a system in which all the body functions are interacting and the complex pathology and clinical symptoms can be modulated.

Reduction

For the majority of our studies we have used software called G*Power 3.1 to calculate the minimum number of animals that are required to prove that the expected changes (usually 30-50% increase or decrease), for example in the

number of cells or the area of pathology, are not occurring due to chance (ie they are statistically significant with 95% certainty). These calculations tell us that we need to use group sizes of between 5 to 10 animals depending on the animal model being used and the main variable that we want to measure. Individual experiments that involve the objective quantitation of data will be analysed using a blinded assessment of outcome. Good laboratory practice will be followed at all times to ensure that detailed protocols are written in advance of the experiment and that data is correctly recorded and protected.

Refinement

The models we are using have been chosen on the basis that they reproduce as closely as possible stages of the human disease, both in terms of the mechanisms and the clinical disease course. Our extensive study of human post-mortem brain tissues has allowed us to design models that accurately reproduce as many features of the human disease as possible. This maximises the usefulness of the data when translating into clinically useful therapies. For example, damage to the grey matter of the brain, where all the neurons are located, is most likely to be responsible for the progressive neurological symptoms characteristic of chronic MS. However, the models currently available do not exhibit pathology in the cerebral cortex. Therefore, we have designed a model to mimic the pathology and mechanisms that we have observed in the human tissue. This will enable us to more accurately test our hypotheses. We have chosen to use rats and mice due to the similarities in the way that inflammation occurs in the brain and spinal cord in MS.

Animal suffering will be minimised by using strict humane endpoints and excellent clinical care during the disease process. This will include regular observation by trained investigators with a knowledge of the underlying disease process. Animals will be hand fed when necessary and provided with extra nesting materials, food and water in the cage when movement is restricted. Analgesia and anti-inflammatory drugs will be given where appropriate and we have determined that these can be given in an oral jelly form, which the animals like. Unexpected symptoms will be carefully evaluated and the appropriate response taken so that neither the experiment nor the welfare of the animal is compromised.

Project 26	**Mechanisms of synaptic transmission and plasticity in the central nervous system**
Key Words	Synaptic Transmission, Learning and Memory, Ageing, Neurodegeneration
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;

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

We wish to understand how the excitable cells within the brain communicate under normal circumstances and how their transmission properties change with learning. Using novel optical and electrical methods to assess activity in key areas of the brain associated with particular forms of learning, we will then examine how the properties of these cells change during the process of normal ageing and the onset of neurodegenerative conditions such as Alzheimer's disease

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)?

By understanding how the excitable cells within the brain communicate, learn and store information, we will be in a better position to understand what happens in the brain during normal healthy ageing and the onset and development of neurodegenerative conditions. We live in an era when the average age of the population is increasing but for many, old age is associated with a reduced quality of life. As our lifespan increases, we need to understand the processes and diseases that accompany ageing and find ways to combat the devastating effects that ageing can bring. One of the other goals of this research is to develop new methods for visualising cell-cell communication using newly developed sensors that can report electrical activity in the brain. We have also developed new high speed and high resolution optical recording equipment that allows changes in electrical activity to be assessed in minute detail. There are some commercial benefits associated with

these developments as they have significant potential for use within the scientific community.

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

We will use rats and mice. We expect to breed approximately 800 mice over a 5-year period. Depending on the strain, only 25-50% of these animals will be transgenically positive but many of the non-transgenic animals will be used in control experiments, thereby reducing the total numbers of animals used. We will use approximately 150 animals over 5 years for experiments involving injection of substances under anaesthesia with subsequent recovery. Approximately 600 animals will be used over 5 years for non-recovery experiments.

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?

The animals we intend to breed (800) do not have any adverse phenotype and so there are no expected adverse effects. We will, however, age some of these animals. Some of these may experience conditions associated with old age such as development of tumours. The incidence of this is very low and most animals are used for experiments well before they experience problems associated with age. One group of animals will undergo administration of substances under recovery anaesthetic. Low volumes of inactivated viruses may be injected into defined regions of the brain. Neither the surgery nor the injection should not cause any problems but it is possible that some animals may die as a result of the anaesthesia or as a result of the injection. This latter issue can be minimised by ensuring the volume of the injection is very carefully controlled.

Application of the 3Rs

Replacement

Some of the work that we wish to do will be carried out using cells derived from immortalised cell lines. However, whilst these experiments allow us to test aspects of our work, we need to understand how neurones within the central nervous system communicate in situ and so this requires the physical connections between the cells to be intact. Whilst there are a number of intermediate preparations such as acutely prepared brain slices that can be used to asses some aspects of neuronal signalling and communication, this can only be examined in an intact system. Computer models of neuronal signalling exist but these are not yet sufficiently advanced for these studies. It is likely that the results from these experiments will be useful for those developing computer models and so in the future, it will be possible to use them.

Reduction

Our program of work is designed to move from simple models using cell cultures, to brain slices derived acutely from rats or mice to in vivo recordings in anaesthetised

animals and finally awake animals. The experiments are designed so that we validate each step of the process in the simplest model possible, so that we limit the numbers and nature of the experiments as much as possible to reduce, refine and minimise animal suffering. We ensure that numbers are kept to a minimum by carrying out appropriate statistical analyses of our models prior to experimentation so that we are aware precisely how many animals are required to test any particular experimental hypothesis

Refinement

Rats and mice represent the lowest species of sentient animals suitable for this work. They are widely used for this type of work and so the results we produce will build upon a substantial body of information. We have planned the work to take advantage of simple models using immortalised cell lines where possible or primary cultures derived from animals killed using Schedule 1 methods. This allows us to evaluate which experiments are likely to be productive and so we can move the project forward into in vitro or in vivo experiments using animals. The techniques that we use, including viral delivery of genetically encoded sensors and/or optogenetic activators are now well established and produce results efficiently and effectively reducing the numbers of animals and the degree of suffering. These methods also avoid the need to produce transgenic animals and so for experiments where creating a new transgenic line of animals is not necessary, then the numbers of animals used are dramatically reduced.

Project 27	**The role of glia in nerve regeneration and tumourigenesis**	
Key Words (max. 5 words)	Nerves, cancer, regeneration, glia, microenvironment	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The work in this proposal addresses how glial cells become cancer cells. The tumourigenesis process appears to mimic the process that takes place in glial cells and the associated inflammatory and regenerative response following an injury to the nerve- and these tumours have been described as an unrepaired wound. We therefore want to understand the nerve regeneration process to help us to understand tumour development and other diseases of nervous tissue. Moreover, these regenerative processes appear to mimic the growth of nerves into growing tumours, which appear to be important for both tumour growth and tumour spread. We aim to understand these processes using a model of pancreatic tumour development, the majority of which spread into nearby nerves.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	The primary potential benefit relates to new knowledge about the mechanisms underlying regeneration of the peripheral nervous system, the development of tumours in this tissue and the unappreciated importance of innervation and PNI in	

project)?	the initiation and spread of many tumour types. Additional benefits will include increased understanding of the blood nerve barrier in health and disease. The findings will be published in academic journals and are likely to be of great interest to preclinical and clinical scientists with interests in nerve injury, neuropathies and cancer. The second potential benefit relates to the possibility of the identification of new molecular targets and! or devices, which may be of benefit for the treatment of these diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use a maximum of 4900 mice/year and 1000 rats/year
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?	Most of the experiments described in this application require the use of genetically-modified mice that express inducible forms of genes and are therefore mostly indistinguishable from any other mouse. Other mice express genetically labelled cells that also have no observable phenotype. For a majority of the experiments, the animals will be bred to produce cells or tissue for in vitro experiments. Some of the experiments will involve manipulation of genes in vivo that will result in tumour formation or neuropathies. Other experiments involve injections of tumour cells to assess tumourigenic potential and spread. These animals will be monitored regularly and any animals showing signs of distress will be humanely culled. Based on previous work, the likelihood of distress in the animals is considered low and the severity level is judged as moderate. Animals will not be allowed to develop tumours greater than 1500mm3 and the tumour mass will not exceed 10% of bodyweight. Some experiments require the damage of a nerve in mice/rats. These experiments will be carried out under general anaesthesia. The recovering animal. will be medicated for pain and closely monitored. Based on previous work the suffering is minimal as the animals are able to move around and feed normally. In the unlikely circumstance that any

	animals show signs of infection or discomfort, they will be humanely culled. Some experiments involve in vivo imaging of cell migration. These animals will be anaesthetised and will be culled at the end of the imaging experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Complex processes such as nerve regeneration and tumourigenesis cannot be satisfactory modelled in vitro - therefore it is necessary to use animal models. We work in vitro with primary Schwann cells and can recapitulate some of the processes by which Schwann cells interact with other cell types. Moreover, we have in vitro assays for tumourigenesis. We have used both of these model systems to identify important signalling molecules involved in these processes and will continue to do so. However, many effects that we, and others, can measure in vitro are not reproduced in the in vivo environment. In particular, the role of the immune system and how cells behave in complex tissues cannot be reproduced in vitro. Therefore, it is always critical to test our models in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The proposed experimental designs and methods of analysis have been discussed with our experts in statistics to maximise the information obtained from the minimum resource. Randomisation and blinding methods will be used when appropriate. Our extensive use of in-vitro methods limits the number of animals required for the in-vivo stage. To minimise animal usage, prevent the unnecessary production of animals showing adverse effect and to ensure that animal breeding is inextricably linked to research requirement, we will: • Ensure high standards of animal care, welfare and utilise the most appropriate breeding methods. • Ensure that colony sizes are monitored and • adjusted within a formal forecasting system to meet the requirements of the research programme(s). Transgenic mouse lines that are not expected to be used in the next 6 months will be suppressed and

	embryos will be cryopreserved for future use • Ensure that breeding colonies are always kept to their minimum size so as not to over produce. • Ensure that Personal Licensees working on this project are appropriately trained and suitably competent to enable a high success rate to be achieved and thus minimise the number of animals used.
3. 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.	Generally, the anatomy, physiology and genetics of the mouse and rat are similar to humans, and thus they represent a powerful system to model human biology and disease. Rats and mice are the animals in which nerve structure and regeneration have been most studied and mice are the in vivo model of choice for tumourigenesis studies. The mouse is the only mammalian species for which transgenic techniques are widely available and is therefore the model of choice for genetic studies. It is widely accepted that mice NFl tumour models are a good model for the human disease. Moreover, the nerve regeneration process is similar in rodents and humans. Each protocol used is carefully designed to ensure physiological significance and to minimise suffering and where appropriate we are developing relevant in vitro models. The mice and rats are kept in purpose-built accommodation. The animals are mostly kept together for companionship and are given materials to encourage their natural behaviour. The animals are bred and cared for by a dedicated team of highly-skilled technicians. Animals are closely monitored for signs of ill-health or distress. The technicians have the expert assistance of two “named persons”, the named Person Responsible for Animal Care and Welfare and the Named Veterinary Surgeon. Some experiments require the damage of a nerve in mice/rats or injection of cells of substances into nerves or the pancreas. These experiments will be carried out under general anaesthesia. The recovering animal will be medicated for pain and closely monitored. Based on previous work the suffering is minimal as the animals are able to move around and feed normally. Any animals showing signs

	of infection or distress will be humanely culled. Some experiments require testing for the ability to form tumours in vivo. Animals will not be allowed to develop tumours greater than 1500mm3 and the tumour mass will not exceed 10% of bodyweight. Any animals showing signs of infection or discomfort will be humanely culled.

Project 28	**Impact of exercise and NAD levels on brain and fat tissue**	
Key Words (max. 5 words)	Voluntary exercise, healthy ageing, epigenetics, NMNAT1, stem cells	
Expected duration of the project (yrs)	1	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to understand at the molecular and cellular level how the levels of the coenzyme NAD (Nicotinamide adenine dinucleotide) and voluntary exercise affect brain and fat tissue in ageing animals. Using as a tool recently generated transgenic mice that express different levels of NAD, we will study in aged mice whether: A) the levels of NAD in the mice determine whether exercise will be beneficial or not; B) this mechanism involves changes in epigenetic markers and/or adult stem cell proliferation/differentiation, C) increasing NAD levels and/or exercise leads to browning of the white fat. Additionally we will also aim to identify blood biomarkers of healthy ageing (related to NAD metabolism and exercise).	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	This proposal will widen the understanding of how exercise and NAD affect the changes in epigenetic markers and adult stem cells that occur during normal ageing.	

project)?	These findings will serve as a springboard for future studies aiming to develop therapies boosting or mimicking the positive effects of exercise on healthy ageing and mental and physical health.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice totalling 70
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?	All our procedures are within the moderate severity and, based on previous experience, adverse effects are unlikely. Previous results from the laboratory where the transgenic mice were generated show that there are not major adverse effects in both transgenic lines when aged to 24 months (other than the known for normal ageing). The behavioural tests that we will perform are very mild. Based on previous experience in our laboratory and across the world we do not expect adverse effects from giving the mice voluntary access a running wheel (on the contrary, access to the wheel will enable the animal to reach the levels of physical activity it should have had in the wild, which are prevented by being housed in a cage). At the end of the study, animals will be euthanized humanely and their brains, organs and body fluids will be studied in vitro.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of living animals is essential as the overall objective of the research is to gain greater understanding of the impact of voluntary exercise on brain, body and behaviour. It is not possible to use human based studies, as it is not ethically feasible to obtain biopsies of tissues such as brain. We will use mice, as many cellular, molecular, physiological and behavioural mechanisms are similar between mice and humans. Moreover the gene.
2. Reduction Explain how you will assure	Making use of the available literature and pilot data we have determined the number of animals needed ensuring we keep the number of animals used to a

the use of minimum numbers of animals	minimum. An advantage of our experimental design is that we study the effects of exercise and/or NAD levels on several tissues from a single animal. Thus requiring only one group of animals, instead of several.
3. 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.	Replicating the effects of exercise across the body requires a mammal, and the rodent is the lowest sentient species suitable for this purpose. We use well established protocols to produce minimum discomfort to the animals. The welfare of each mouse will be monitored routinely. In the unlikely case of adverse effects linked to any of the experimental procedures will be controlled by expert personnel that will monitor the animals carefully.

Project 29	**Dorsal horn circuits in normal and pain states**	
Key Words (max. 5 words)	Interneurons; circuits; acute pain; chronic pain; mouse	
Expected duration of the project (yrs)	**5**	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Pain remains an important unmet clinical problem in both humans and animals. In most cases, acute, nociceptive pain (normal, “every-day” pain) can be managed, however, chronic pains (such as chronic visceral and neuropathic pain) are often severe, long-lasting and difficult to treat. The need for developing new therapies is therefore clear, but one barrier to preventing such advances is our lack of detailed understanding of the neuronal circuitry of the spinal dorsal horn. This project aims to identify circuits responsible for modulating sensory information entering the spinal cord. We also aim to determine how peripheral nerve injury impacts on these circuits and contribute to the development of chronic pain states.	
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)?	We have preliminary evidence that one of the populations of spinal cord neurons (interneurons) this project will study is important in directly modulating the passage of painful information into the central nervous system. If this inhibition is lost following peripheral nerve injury, it is likely that the behavioural consequence of this restructuring in neuronal circuitry will lead to the development of hyperalgesia, where painful stimuli are perceived more intensely (heightened or exaggerated responses to painful stimuli). By identifying the functional significance of these cells in health and disease, it should be possible to target them selectively for the	

	development of more effective analgesics.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to cover approximately 1000 animals under this licence, however, the vast majority of these will only be involved in breeding programmes to establish and maintain the various transgenic mouse lines needed for our experiments (Protocols 2 and 3).
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?	In many cases, we intend to set up experimental models of neuropathic pain by transecting, ligating or crushing peripheral nerves. These well-established surgical procedures will lead to the development of either tactile allodynia (where previously innocuous tactile stimuli are perceived as painful) and/or thermal hyperalgesia (heightened or exaggerated response to painful thermal stimulus) in experimental animals. Every effort will be taken to ensure that these animals do not show signs of excessive pain, of ill-health or deviate from normal/expected behaviours. In the highly unlikely event of this happening, the animals will be terminated using humane killing methods. If, as expected, the consequence of surgery leads to the desired behavioural output, the expected end-point for all experiments will be the induction of terminal anaesthesia and preparation of tissue for subsequent analysis. This will also be the expected end-point for all non-recovery experiments carried out under anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Studies designed to investigate the neuronal organisation and synaptic circuitry of the spinal cord, together with the changes that occur in chronic pain states, can only be carried out on animals. It is impossible to carry out studies of this type on cultured cells since these do not have the complex organisation of the intact spinal cord and it would not be possible to obtain suitable specimens from humans for these experiments either.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In all cases, the number of animals used will be the minimum required to provide statistically significant data. This will be determined by the use of power calculations where appropriate, and advice from experienced statisticians. For anatomical studies, we frequently use tissue from a single animal for more than one experiment, and any tissue that is not used immediately is stored frozen for subsequent use, thus reducing the numbers of animals needed for these studies. For similar studies to look at the expression

	of different neurochemical markers in both wild-type animals or in models of chronic pain (with accompanying behavioural testing analysis), data is typically obtained from experimental groups of 10-15 animals, to ensure that there is no inter-animal variability. For electrophysiological studies, experimental numbers will depend on obtaining a sample of cells that is sufficient to reliably characterise the physiological properties of the targeted population of interneuron, to label a large enough population to conduct all the necessary anatomical studies, and to determine the effects of peripheral nerve injury on both the anatomical and electrophysiological properties of these cells. This is likely to involve between 20 and 50 animals per experiment, and these may necessitate being carried out in naïve, transgenic and nerve injured groups. By using the transgenic mouse line where the expression of an intrinsic fluorescent marker(s) is/are driven under the influence of a promoter(s), we will be able to target the desired population of cells directly. This approach will reduce the overall number of animals required as the only other alternative for obtaining an adequate yield of cells to satisfy the project aims would be to employ a random sampling method in wild-type animals. Similar studies work with groups of between 20 and 50 animals for each experiment, with these numbers thought to be sufficient to yield a large enough population of cells for all subsequent experiments but also sufficient to identify variability between animals.
3. 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.	**Species.** The great majority of studies on neuronal organisation and circuitry in the spinal dorsal horn have been carried out in the rat. This species will therefore be used for many of the experiments in this project in order to allow direct comparisons to be made with previous work. We have shown that several populations of interneurons in the spinal dorsal horn can be recognised by their electrophysiological and morphological properties, although it is not possible to target these populations selectively in in vitro electrophysiological experiments using a random sampling method due to the cellular heterogeneity of this region. However, the increased availability of transgenic mouse lines, where fluorescent reporter molecules are expressed under the influence of specific promoters, allows neurochemically-defined populations of cells to be targeted specifically. We plan to use spinal cord sections from specially-derived transgenic mouse

	lines for our *in vitro* electrophysiological experiments. By adopting these targeted approaches instead of a blind (random sampling) approach in wild-type animals, we will maximise the number of cells we record from and label while also minimising the total number of animals used. Not only is this a refinement of this experimental approach, but it will also result in a reduction of total numbers of animals used. Models and Methods. Minimising animal suffering. We have optimised and refined the experimental protocols intended for use in this project, which will ensure that the number of animals used and the potential for any animal suffering is kept to an absolute minimum. Many of the procedures outlined will be terminal, however for non-terminal procedures, analgesics will be administered where appropriate to reduce potential suffering.

Project 30	**Assessment of vascular cognitive impairment in rodents.**	
Key Words (max. 5 words)	Dementia, cognition, vascular risk factors, neuroimaging	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim of this proposal is to establish a clinically relevant animal model of VCI that accurately reflects the neurovascular pathophysiology (white matter damage and microvessel pathology) as well as the clinical presentation of the disease (executive dysfunction and neuroimaging abnormalities). 1) To establish relevant animal models of VCI that accurately reflect the neurovascular pathophysiology (white matter damage and micro vessel pathology) and the clinical presentation of the disease (executive dysfunction and neuroimaging abnormalities). 2) To validate behavioural tests that enable accurate measuring of executive dysfunction in VCI. 3) To identify reliable neuroimaging biomarkers of VCI.	

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)?	The work proposed here will be presented at national and international meetings and published in scientific journals. This will have direct benefit for the students and fellows that will be trained to continue work in the highly relevant field of pre-clinical neurovascular disease. This will also have significant benefit to the scientific community, as the knowledge that will be produced will result in best practice recommendations regarding animal models of VCI models and this can reduce animal use in the future. Because the patient population is so heterogeneous, it is difficult to determine which vascular risk factors are playing the greatest role in disease progression. Studying each of these in turn in animal models can greatly assist with our understanding of VCI. This knowledge is the first step towards the development of treatment options. Currently, the lack of appropriate animal models prevents this from occurring. This project also has the ability to provide information that could result in significant benefit to patients and medical practitioners. If we can identify neuroimaging biomarkers of VCI, the same sequences are available on human scanners and could be used in patients to assist with early diagnosis.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 750 Rats 200 Over 5 years.
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 primary adverse effect will be that rodents will undergo a surgical procedure to induce a reduction to blood flow in the brain (hypoperfusion) or the corresponding sham procedure. This should result in some pain and discomfort from the surgical procedure that will be minimized by post-operative care and analgesia. Most animals recover from this quite well, and are expected to achieve a moderate level of suffering. There is the possibility that some animals will reach the substantial severity limit but these will be euthanized promptly and humanely. At the end of the study, all animals will be euthanized humanely.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Unfortunately, due to the nature of the project, there is no suitable alternative to animal experiments. VCI is a chronic, progressive, and heterogeneous condition with the primary symptom being a decline in cognitive function that is thought to be due to white matter damage. We wish to assess complex behaviours, and to find neuroimaging correlates of VCI in the white matter. This can only be accomplished using animals, and not in cultured cells or slice preparations that lack these features. Patients with VCI likely have several vascular risk factors, and animal models allow the opportunity to isolate the contribution of each factor towards the development of VCI. We have considered mathematical modelling as an alternative to animals, and while we can not yet replace animals, the neuroimaging data collected can be used to generate variables for mathematical modelling techniques. This will be performed in collaboration with the Mathematical Sciences Department.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to minimize the number of animals that will be used we will employ Good Experimental Practice to design our experiments. An important aspect of this includes using the available literature and pilot data to perform A Priori Power Calculations. This is a statistical technique that takes into account variations in experimental groups and allows estimation of the number of individuals required in order to find something meaningful. An advantage of using neuroimaging is that it allows us to monitor changes in the brains of a group of animals over time. Via this strategy, we do not need additional groups to perform invasive assessment of tissue at each of the timepoints of interest. Nevertheless, this means the potential for distress to the animals that are used is slightly greater due to the repeated anaesthetic regimes required to perform the imaging experiment.
3. Refinement Explain the choice of species and why the animal model(s)	The mouse is the lowest possible species that would be considered suitable as it still has complex behaviours and white matter. Furthermore,

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.	development of VCI models in the mouse offers the advantage that further investigation into mechanisms of disease progression can be accomplished with transgenic technology. However, for some experiments, the rat is preferred due to the ability to implement certain neuroimaging techniques, and the existence of strains with genetic susceptibility to hypertension. The most well characterized animal model of VCI is hypoperfusion (a reduction in blood flow to the brain). This can be achieved by ligating one carotid artery, for example, in situations where it is necessary to have an internal control (the other brain hemisphere will have normal blood flow). However, ligating one carotid artery does not result in cognitive deficits or white matter damage, therefore, the most refined method to produce both of these effects in the rats is to ligate both carotid arteries. Mice have impaired collateral blood flow in the brain, therefore, occluding both carotid arteries would result in death. Thus, the most refined method for mice is to make the arteries stenotic (narrow) using microcoils. Hypoperfusion in general (via any of the above methods, depending on the purpose of the experiment) is considered refined as most animals generally do not experience lasting pain or distress. We anticipate the rodents will tolerate induction of vascular risk factors (such as high blood pressure) as they are unlikely to be aware of their condition. In cases of all surgical procedures, the best and most up to date aseptic surgical methods will be used as well as appropriate use of pain relief under the guidance of the veterinary surgeon. All animals will be monitored to observe any signs of ill health. All of the behavioural tests and neuroimaging sequences were selected to provide the most accurate information compared to the clinical condition, but will be performed and timed in a manner that is the least distressing to the animals, for example, minimizing the number of tests and anaesthetics.

<table>
<tr><td>Project 31</td><td colspan="2">Neural mechanisms of pain and homeostasis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Nociception, Neuroscience, Autonomic Nervous System, Behaviour</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="9">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>✓</td><td>Basic research</td></tr>
<tr><td>✓</td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
<tr><td></td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
<tr><td></td><td>Preservation of species</td></tr>
<tr><td></td><td>Higher education or training</td></tr>
<tr><td></td><td>Forensic enquiries</td></tr>
<tr><td>✓</td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">Chronic pain is an increasing problem in society and was identified by the Chief Medical Officer as a key priority for both the NHS and for researchers in academia / industry as the existing treatments are inadequate. This reflects a lack of understanding of the mechanisms controlling pain which has resulted in a shortage of new treatments reaching the clinic.

We still do not fully understand how neuronal activity relates to pain perception and how the sensory input is processed to produce changes in behaviour. A further major unknown is why acute injury, normally associated with useful protective pain, can turn into chronic pain outlasting the normal healing period. We have recently found evidence that this is because of a failure of the body’s own pain control systems.

This project will study how the brain (and particular groups of brain cells in the brainstem) controls the pain response to sensory stimuli and how this fails in</td></tr>
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	chronic pain. We will also conduct linked investigations examining the influence of sensory processing on the ability of the same and similar brainstem circuits to control organ function (specifically of the cardiovascular system and bladder) in health and in models of disease. We have developed a number of experimental approaches that enable us to activate or inhibit the function of these brain circuits to examine their role at a cellular and behavioural level, both in health and in models of chronic pain. This programme of work aims to address this problem by a) investigating the neuronal mechanisms controlling i) pain and ii) autonomic function; b) identifying new targets for potential treatments, and c) defining the consequences of pain on mood and decision-making.
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)?	The results from these studies will provide us with important information relating to the way the brain controls pain and how this is altered in chronic pain. We aim to identify why the pain regulation systems fail to compensate for chronic pain. Similarly we will investigate why neural control of the bladder is changed in disease states. In so doing we anticipate that we will uncover new targets for treatment. In addition some of our approaches to engineer and control brain activity patterns may in the long term be adopted for treatment. As such, it may help in the development of therapies, that have can offer benefit to people and animals who are suffering from chronic pain or bladder diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	It is expected that the studies will use 680 mice and 610 rats per year for the five year duration of the project.
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	The expected level of severity for most of the animals in the protocols is of a moderate level. In order to control the activity of specific groups of brain cells animals will have micro-injections of vectors to the brain or spinal cord. This requires recovery surgery

happen to the animals at the end?	and a small hole to access the brain tissue and some will have implanted optical fibres or electrodes. The animals recover rapidly after such a procedure and have no lasting discomfort or disability. The pain models are internationally validated and involve the induction of localised sensitivity of a hindpaw. The bladder studies will model inflammation and spinal cord injury. The animal will be mobile, will eat and drink normally and remain in good condition. The increased sensitivity is often only exhibited when stimuli are applied and animals will be closely monitored to ensure their welfare. Many of the studies will be performed on tissues and organs from the animals or through recordings under terminal anaesthesia to minimise harms. Animals will be humanely culled within 8 weeks at the end of the studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This neural circuits controlling pain and regulating the function of the viscera are within the brain, brainstem and spinal cord and are connected to sensory fibres in the periphery. There is currently no way to study pain related behaviour and central neural control of the viscera in detail without using animals. In the proposed studies we will monitor the activity in these regulatory circuits in health and in disease models and will test novel therapeutic interventions. These integrated neuronal responses cannot be adequately modelled *in silico* nor is it possible to do these studies in cell lines at this point given the complexity of the circuitry and the many neurobiological unknowns. While we plan to conduct many aspects of these studies *in vitro* (in brain slices) to identify cellular mechanisms and to test the effect of interventions and in also in terminally anaesthetised animals to study reflex responses there is a need to conduct some tests in behaving animals to study their integrated responses. We will seek to do linked experiments in human subject using psychophysical tests and imaging studies for example to translate the findings across species.

	However, these approaches do not currently have the resolution to allow precise investigations of cellular mechanisms and molecular interventions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For each of the proposed studies we have defined humane end points and will use *a priori* power calculations to define the numbers of animals needed for each experiment. Whenever possible (and particularly for definitive experiments) experimenters will be blinded to treatment allocations and animals will be randomly assigned (by blocking where appropriate) to study groups.
3. 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.	Rodents have been chosen for these projects as there is substantial information about their nervous systems, they are employed for similar studies in many other laboratories worldwide and the models are analogous to human conditions. They constitute the least sentient species within which such pain related behaviours can be modelled. Additionally the use of GM-mouse (and rat) strains will allow precise molecular and circuit targeting of interventions. We will use validated and internationally accepted models including for chronic pain and visceral disease by causing local inflammation or by inducing nerve injury. For some animals we will assay their cognitive and behavioural responses. In these cases the period of sensitisation will be kept to the minimum that is therapeutically meaningful while also ensuring that longitudinal observations yield the maximal amount of useful data in the minimum number of animals.

Project 32	**Mechanisms of acute and chronic pain**	
Key Words (max. 5 words)	Inflammation, arthritis, neuropathy, diabetes,	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals.
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our overall aims are to increase mechanistic understanding of how chronic pain develops, to improve identification and development of novel analgesic treatments. Our specific scientific objectives are: 1. To identify the mechanisms through which networks of neurons in the brain and spinal cord can either enhance or block pain, and how this can affect different types of pain, such as fast sharp pain, or slow aching pain, differently. 2. To identify whether new ways of controlling which proteins are found in specific cells, can either protect the cells from damage, and/or prevent inflammation or pain. 3. To understand how drugs that interfere with protein expression in this way exert their effects, and to develop new drugs that might be useful new analgesics. 4. To identify the ways in which the blood supply to the spinal cord affects the function of spinal neurons; to determine whether blood vessels affect pain in different ways in arthritis and neuropathy in	

	experimental animals.
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)?	Potential benefits are: 1. The generation of new essential knowledge on neuronal and vascular mechanisms contributing to pain, and the changes associated with both acute and chronic pain. The chief beneficiaries of this knowledge will be the basic science, clinical science and veterinary science research communities. 2. The application of knowledge to drive both future mechanistic research and drug discovery strategies. This is worthwhile because it is known that similar work in the academic setting has resulted in novel analgesics that are currently near-market. 3. The work we are doing in this area may influence strategies for novel drug design in industry (medicinal chemistry) in addition to identifying possible new targets for drug discovery. The pharmaceutical industry may also benefit from this work through re-purposing or re-formulation of existing drugs for additional uses. 4. Our work will ultimately benefit patients with chronic pain. We are working towards development of new treatments for pain. We have patents on new analgesic drugs and have established a company through which we will develop these drugs. The work on this project will inform that development, potentially resulting in a new analgesic neuroprotective drug class. 5. Veterinary surgeons have more limited variety of drugs available to them than doctors, and so new analgesic drug classes could also contribute to new veterinary treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	Rat 2355 Mouse 850 5 years.

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?	This project will look at the ways in which the nervous system changes in response to pain, and how we might be able to stop such changes. As such we use animal models of arthritis and nerve injury (neuropathy) in rodents. We examine the ways in which the nervous system is activated to generate the perception of pain. In order to do this, we need to study the animals when they are awake – pain is an experience and so to understand this, we need to study the ways animals behave. All animals that have either arthritis or neuropathy (where possible limited to one limb) will experience mild to moderate pain, the study of which is the aim of the project. The overall experience of the animals will also include some or all of the effects of the additional experimental techniques that we use that may contribute to pain distress or lasting harm, such as: distress caused by behavioural testing for alteration in nociception; more severe inflammation than predicted/planned; infection as a result of setting up the model, or problems with anaesthesia used when generating the models. These are all very rare occurrences. If animals become unwell, have more severe reactions to the model, displayed e.g. by not using one limb for walking, or lose weight to any great extent, then they will be humanely killed. The expected level of severity is moderate based on our experience of using these models and procedures. All animals will be humanely killed at the end of procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use non-animal alternatives, such as cultured primary cells or cell lines, to test hypotheses, where such alternatives are available and can give us relevant information. We are developing new assays of this type, and using such assays to look at the changes in neurons following nerve damage, and different culture systems to look at how immune, cartilage and other cells in joints behave in arthritis. Unfortunately such alternatives cannot yet tell us how the nerves, blood vessels and other cells in the body

	interact with each other, so these assays cannot tell us everything we need to understand how pain develops, and how it affects the whole body. The development of inflammation and pain depends on multiple different systems in the body. It involves the blood vessels, the immune system, and the nervous system. Within the nervous system, the response to pain is controlled by interactions between neurons in multiple brain areas, the spinal cord and the areas of the body that are damaged. These complex interactions cannot be yet modelled in culture or by computers.
2. Reduction Explain how you will assure the use of minimum numbers of animals	1. We use current technological advances, such as in data capture software, that increase ability to collect data from multiple recordings in the same animal, and experimental designs that include repeated measurements in the same animals (where possible), to ensure that each experiment generates the maximum amount of data from each animal. 2. We use statistical modelling in order to determine the sample sizes needed for experiments before beginning, and in all our applications for funding to support this work. Numbers of animals and experimental designs are thus rigorously peer reviewed prior to any experiments. A priori calculations are based on either our previous data in the same models, or on published work from others working in similar areas, and are used to determine numbers needed. 3. We use historical or literature controls, such as previous sham control groups in which we know (such as vehicles or sham interventions have no observable effects) for example for comparison with experiments using novel pain killers, to ensure that we do not expose additional control animals to painful conditions unnecessarily (such as injections).
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	Rodents are the least sentient species in which studies of this nature are performed. They are capable of decision-making, which is completely necessary in assessment of pain. These attributes

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	are not apparent in species such as amphibia, and fish. Rats and mice have been extensively used in studies such as these, and so there is a great deal of published literature against which results can be compared. The models that we use have been extensively refined (often by us) over the years. As most of the work concentrates on the early changes that lead to chronic pain, the majority of studies do not last a long time, usually less than 1 month. Arthritis/inflammation/injury models are limited to a single joint/limb, and the mildest form of intervention suitable for the experimental aims is used. Models of nerve damage most commonly involve superficial nerves, with minimal necessary surgery. Diabetes can be associated with significant morbidity in humans, but in mouse and rat models, particularly when the animals can be treated with insulin, the disease is fairly mild over 8-12 weeks. Chemotherapy for cancer in humans also causes nerve damage, as it does in rats and mice, and can also cause effects in other tissues. Chemotherapy models are all relatively new compared with other pain models; we keep abreast of published refinements, and implement these when appropriate for experimental aims.

Project 33	**Understanding Cortical Plasticity in Health and Disease**	
Key Words (max. 5 words)	Memory, schizophrenia, parietal cortex, prefrontal cortex, mouse	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our aim is to understand cortical plasticity sufficiently to manipulate it safely for therapeutic benefit.	
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)?	The future benefits of our research are that we will gain an understanding of how synaptic plasticity works in the cerebral cortex, how it goes wrong in disease conditions and thereby learn how it can be manipulated for therapeutic benefit in neurological and mental health conditions such as schizophrenia, Alzheimer's, stroke and mental retardation.	
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate using approximately 1,000 mice per year, plus a further 1,600 in breeding colonies.	
In the context of what you propose to do to the animals,	Some of the protocols do not have adverse effects such as those for breeding and maintenance or some	

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	of the techniques such as behavioral testing. The protocols that involve surgery are conducted under anaesthesia and could potentially result in infection. The expected level of severity of the adverse effect might rise to moderate. The animals will be killed humanely at the end of the protocol or if the severity limit is exceeded despite remedial action.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are studying the biology of learning, memory and adaptation to sensory experiences. Only whole animals have these biological processes and therefore non-animal alternatives are not possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the appropriate numbers of animals to achieve statistical significance. Where possible we will use longitudinal studies and within animal controls to achieve as much efficiency as possible from the statistical design.
3. 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 the lowest sentient laboratory species with a cerebral cortex akin to humans. In addition, it is one of the most commonly genetically modified mammalian species, thereby allowing researchers to understand genetic models of human disease. Measures are taken to keep the duration brief and to administer anaesthetics where pain might otherwise occur.

Project 34	**Triggering and propagation of neurodegeneration**	
Key Words (max. 5 words)	Neurodegeneration; Parkinson's disease; ALS; synuclein; RNA-binding proteins	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Exact molecular and cellular mechanisms that trigger clinical manifestation and progression of neurodegenerative diseases are still elusive. Understanding of these mechanisms is paramount for designing novel therapeutic approaches to combat these currently incurable diseases. This project is aimed to reveal mechanisms of triggering and spreading pathology typical to Parkinson's disease, amyotrophic lateral sclerosis and related disorders.	
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)?	We believe that implementation of the project will substantially improve our understanding of the pathogenesis of certain neurodegenerative conditions. It is feasible that we will reveal new potential therapeutic targets and window of therapeutic intervention.	
What species and approximate numbers of	Mice, not more than 16000	

animals do you expect to use over what period of time?	
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?	Most of genetic alterations that will be studied in the project cause mild or moderate changes to mouse physiology that do not instigate significant problems to the health of juvenile or adult animals. However mice expressing certain combinations of proteins, some ageing animals or animals treated with specific (e.g. neurotoxic) agents might develop adverse phenotype (e.g. muscle weakness, coordination loss) and in these cases we will implement specific protocols to minimise animal suffering. Animal behaviour will be assessed in various tests, most of these tests mimic natural behaviour. However, some testing protocols include animal restraint, food deprivation or light electrical footshock to facilitate learning; these treatments will cause only transient discomfort and no harm to animal health. We do not expect the level of severity to be above moderate for any animal procedures that going to be used in the project. At the end of experiments all animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Detailed studies of such complex biological process as neurodegeneration require systemic approaches, e.g. should take into consideration interactions between neuronal, endocrine and environmental factors. This could be achieved only in the context of the whole organism. Although we widely use various in vitro and cell culture methods to investigate some aspects of the neurodegenerative process, at some stage creation and comprehensive studying of animal models become an inevitable requirement of further progress towards clinical applications.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We constantly improve the design of our experiments as well as our experimental techniques, which lead to reduction of the number of animals required for achieving goals of our studies. Animals may be continually used to achieve different experimental endpoints, for example tested for different types of behaviour and in some cases, used as breeders after

	assessing in tests that mimic natural animal behaviour. Whenever feasible we share animals from our breeding programme with other researchers that use them in their experiments. We stop breeding animal colonies that are not needed for current studies but use embryo freezing to preserve them for future use. We also deposit new mouse strains in international transgenic animal strain banks from where they may be distributed for use by other researchers.
3. 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.	In the project we will use the type of models of pathological processes that require production of new animal lines by gene targeting. Because this technique is still not reliably developed for any other vertebrate species, mice are the only practical choice for our studies. Some newly produced or to-be-produced mouse lines represent the most advanced approach for in vivo studies of a protein function and pathological processes associated with its malfunction. We always try to improve our techniques to reduce severity of procedures. We widely use analgesics before, during and after surgical procedures. Wherever possible we substitute procedures for ones less damaging for animal health, i.e. minimise the size and source of samples used for animal identification purposes. We always keep all our animals in enriched environment (“mouse homes”, materials for building nests, chewing materials, etc.). Most of our mutant mice display very subtle differences from wild type animals and do not require any special treatments for adverse effects. However, in those cases when the development of moderate severity phenotype is expected as the result of genetic modification, i.e. FUS, TDP-43, gamma-synuclein transgenic mice, or pharmacological treatment, i.e. MPTP, methamphetamine, we closely monitor animals health and follow established protocols to prevent unnecessary animal suffering.

Project 35	**Cortico-thalamo-cortical interactions in primate cognition**	
Key Words (max. 5 words)	Cortex, Thalamus, Learning, Memory, Decision-making	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals.
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Brain studies related to the sensory systems show that interactions between the thalamus and cortex provide on-going, dynamic feedforward and feedback lines of communication. My research focuses on understand how and when cortico-thalamo-cortical interactions linked to the dorsal thalamus (in particular the mediodorsal thalamus) are important for cognitive functions. My causal evidence in monkeys and rats indicates mediodorsal thalamus and cortex interact together during daily learning and adaptive decision-making. This evidence shows that these interactions are dynamic, with thalamus and cortex being necessary partners in processing signals that are important for cognitive functions, as previously observed in similar cortico-thalamo-cortical systems linked to visual, auditory and somatosensory functions. However, as yet we do not understand how the thalamus and cortex interact together and what messages are relayed between these structures for	

	successful cognitive functions to occur. This research will address these questions.
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)?	Neuroimaging and neuropathology studies show that links between the cortex and dorsal thalamus are altered in the human population with schizophrenia, dementia, Parkinson's disease, major depression and other mood disorders. My work on the mediodorsal thalamus and cortex in non-human primates is particularly relevant as this animal model provides invaluable insights related to understanding the causal neuronal influences leading to the cognitive symptoms associated with some of these neuropsychiatric disorders and diseases. Cognitive symptoms linked to many of these diseases may be attributed to marked changes in interactions between the dorsal thalamus and cortex. Furthering research in animals, in addition to studies being conducted in patients and healthy controls, is an extremely fruitful way to gain greater knowledge and advance our understanding about the neural mechanisms underlying how the brain supports successful cognitive processes and identifying the underlying mechanisms associated with cognitive impairments.
What species and approximate numbers of animals do you expect to use over what period of time?	Rhesus macaques – up to 18
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?	All monkeys will undergo magnetic resonance scanning to image their brains. Some animals will receive neurosurgeries to inject neuroanatomical tracers into targeted brain regions to determine their interconnected brain structures and relevant neuronal populations. Some other animals will learn cognitive tasks using computers and touchscreen software. They will then receive permanent implants fixed onto their skulls under general anaesthesia to gain access to the brain for recording electrical impulses from brain neurons while they perform these cognitive tasks. In addition,

	these animals may receive discrete manipulations to specific brain structures to assess how changes occur to cognitive functions and neuronal recordings. Some other animals will also learn cognitive tasks and, via their implanted devices attached to their skulls, they will receive injections of neurochemicals to temporarily disrupt or stimulate neural activity within discrete targeted brain regions. Neuroimaging after these perturbations will show the consequences at the whole brain level of network analysis. These studies will establish the causal influence of these brain structures while performing cognitive tasks. We will train the animals learning the cognitive tasks to tolerate head fixation during these testing and recording sessions. We are extremely skilled at all of the neurosurgery, electrophysiological recordings, neuroimaging, neuropharmacology, primate training and cognitive testing techniques. The expected adverse effects associated with neurosurgery, electrophysiology recordings, temporary neuronal inactivation, MRI scanning and cognitive testing have all been carefully documented. There may be acute consequences for the monkeys associated with performing any surgery (e.g. limb weakness and pain) that are all alleviated with appropriate medications and resolve within expected timeframes for healing. There are no long-term effects associated with surgery, only subtle changes in cognitive abilities that are detected via different recording methods when the monkeys perform the cognitive tasks. We work in close contact with the NVS and NACWO to continually assess the impact of the procedures on each individual animal. Any signs of pain, suffering, distress or lasting harm will be carefully monitored by our very experienced team, led by the NVS and NAWCO, and relieved by appropriately administered drugs. The level of severity is severe. The animals are all perfused at the end of the experiments so that we are able to assess how changes in their brain at the behavioural level

	correspond to changes in the brain at the neuronal level using *ex vivo* detailed histological studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal usage is necessary because invasive in vivo methods are currently the only way to study actual real-time brain mechanisms and establish their causal influence. Macaque monkeys provide the 'gold standard' for anatomical tracing with their six-layered cerebral cortex partnered to the mammalian thalamus. The macaque monkey is the most appropriate species for this project because macaques are capable of performing complex cognitive tasks and their brains show a much greater degree of homology with the human brain than other available mammalian species.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A key issue in reducing the number of monkeys used is outstanding animal care and support, best practice procedures and attention to the needs of the individual animal. Our university has invested significantly in staff training for excellence and critical support to ensure that primate care and welfare is superb. In addition, I have the necessary expertise and an excellent track record in experimental design of cognitive studies involving animal models. Further, I have established collaborations with other leading international neuroscientists who have extensive expertise in neuroanatomy using different mammalian species, electrophysiology, and magnetic resonance imaging analyses in non-human primates. These collaborations will add value by complementing my expertise, and ensure the success of these critical studies in non-human primates.
3. 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	Macaque monkeys are the most appropriate species as detailed above. Animals will be housed socially with environmental enrichment in state-of-the-art modern facilities. Their welfare will be optimally supported throughout the project with our research team working under the guidance of the NACWO. We will continue to keep abreast about the latest research and development of further refinements within the field of primate neuroscience. For example,

(harms) to the animals.	will continue to use and also develop the most refined monkey training methods. Further refinements in the use of head restraints and techniques for head implant surgeries will continue to be pursued. The use of the most refined neuronal recording devices and chronic deep brain stimulation methods employed in human neurosurgery will also be investigated for their suitability to target the mediodorsal thalamus in this project. The use of MRI combined with stereotaxic co-ordinates and visual guidance will increase the likelihood that the tracer injections are inserted into the deeper layers of cortex and mediodorsal thalamus.

Project 36	**Using transplanted neural tissue to understand neurological disease**	
Key Words (max. 5 words)	Neuron, development, brain	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Neurological disorders such as schizophrenia and autism are a major clinical burden and lack adequate treatment options. This is in part due to the lack of appropriate model systems to study and research these conditions. We have developed a system of neural tissue development in a petri dish that can be used to study these types of disorders. However, this system fails to model many later aspects of disease progression because of a lack of blood supply in the dish. Therefore, we will perform transplantations into mice in order to lead to formation of blood vessels and thereby allow us to investigate processes involved in neurological disease progression, and potential therapeutic avenues. Because we will transplant a different species' tissue into a mouse, we require immunodeficient mice to prevent host rejection of the tissue.	
What are the potential benefits likely to derive from	This approach has the potential to shed light on new therapeutic avenues for the treatment of neurological	

this project (how science could be advanced or humans or animals could benefit from the project)?	diseases such as autism, schizophrenia and neurodegenerative disease.
What species and approximate numbers of animals do you expect to use over what period of time?	We will perform studies in standard laboratory mouse strains and expect to require up to a maximum of 500 mice in 5 years.
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?	Some animals will undergo a surgery to implant neural tissues into a site prone to vascularisation, specifically, under a membrane of the kidney. This surgery will last no longer than 20 minutes and should not cause any lasting distress to the animals, since the animals' organs should not be disrupted in the process.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are investigating other vascularisation options in the dish that do not require animals. However, without a blood substitute or heart to pump the blood, we still lack the ability to sufficiently provide nutrients to the neural tissues. Therefore, until such options are available, we will make use of animals and hope to identify new ways to further maintain the tissues in the dish in the future.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Because we are analysing the tissues themselves, we can implant multiple tissues into the animal and therefore do not require large numbers of mutant animals.
3. 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.	Unfortunately, initial transplantations in chick have been unsuccessful, necessitating the use of mice which will allow for the needed longer-term experiments. We have chosen an approach that has the highest likelihood of vascularisation but minimal effect on the animal, since we will transplant into only one kidney of the adult mouse, to limit the overall effects on the animal. Genetically modified mice that are immunocompromised will be used to minimise the possibility of rejection of transplanted orqanoids.

Project 37	**Experimental Molecular Medicines for Restorative Neuroimmunology**	
Key Words (max. 5 words)	Molecular therapies, inflammation, brain, stem cells, brain repair	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Treatment options for patients with central nervous system (CNS) disease are currently limited, and many CNS disorders still do not have a cure. Despite years of research in the field of CNS injury, this persistent lack of therapeutic options has prompted the scientific community to re-evaluate the mechanisms underlying the pathophysiology of these diseases. In the past few years, it has become increasingly clear that many of the events that characterize CNS injury are profoundly intertwined with the activation of the immune system, which guides most of the degenerative and reparative processes. The new direction of research (both in Cambridge and worldwide) is to treat CNS damage (either acute or chronic neurodegenerative) by optimally engaging and modifying these immune responses (immunopathobiology). Our project's aim is to understand the mechanisms underlying immune system activation in CNS disorders, in order to enable the identification and	

	validation of new drug targets, with the ultimate goal of developing novel therapies.
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)?	Treatments for CNS damage are currently limited, and once the clinical deficits are established, it is almost impossible to revert them. This leads to massive costs for the long term care of patients and a gigantic burden for their families and the NHS. Within this project we purposely focused our attention on the three major CNS disorders that are characterized by the highest prevalence, the uppermost degree of long term disability and the lack of any real restorative treatment. Spinal cord injuries (SCI) — It is estimated that almost 40,000 people in the UK are living with SCI, with approximately 1,000 new cases each year. Most of the injured people are young adults between 20 and 35 years old and, although modern medicine has allowed them a normal life expectancy, the lack of any regenerative therapy results in massive costs related to long-term medical support (the cost to the nation is estimated at £1 billion per annum). Multiple sclerosis (MS) - The onset of MS is typically between the ages of 20 and 40 (female:male ratio is 3:1), and it is calculated that in the UK alone (which is a high rate country for MS) more than 100,000 people are afflicted by this disease. As a matter of fact, MS is one of the most common causes of chronic neurological disability of the early-to-middle adult life, and it is calculated that the associated costs (direct and indirect) in the UK are about £1.5 billion per annum. Current available therapies for MS contribute to these expenses (being very expensive), but in the end they are only partially effective to limit the inevitably progression of disease. Ischemic stroke - Stroke constitutes a major health problem in UK, being the fourth largest cause of death (after heart disease and cancer), the second cause of dementia and the primary cause of long-term disability in the UK. In figures, stroke kills twice as many women as breast cancer and more men than prostate and testicular cancer combined a year. The economic costs

	of stroke in the UK are about £9 billion per annum, and while there is only one specific treatment for acute stroke (thrombolysis, which is applicable in only 5-10% of stroke patients), there are no effective treatments to revert established stroke deficits. Within this project we are proposing an original approach to these diseases that focuses on the study of the contribute of the immune system in mediating CNS damage and halting recovery. By studying the pathways involved in inflammatory-mediated CNS damage, our aim is to develop highly innovative therapeutics to help overcome limited efficacy of existing approaches. Despite the severity of the protocols herein proposed, our approach will bring to massive benefits for the NHS (reduced costs), the patients and their families (better quality of life). Moreover, the use of our basic science approach will help the scientific community to advance its knowledge on the biology of CNS diseases, as well as to develop novel methods, technologies, and protocols.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice: 9,400 in 5 years Rats: 1,950 in 5 years
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Since no experimental treatment could be ever tested in human patients without extensive and appropriate validation in relevant animal disease models, concepts developed in tissue culture have to be tested and refined in vivo where the complex environment of the adult nervous system is present, and where functional recovery can be measured. Both mice and rats are widely used research because their genetic, biological and behavioural characteristics closely resemble those of humans, thus allowing the study of many of the pathologies (or part of these) seen in patients. In particular, the possibility to use transgenic mice in which the expression of specific genes can be monitored and/or altered allows the study of target

	proteins and pathways involved in CNS injuries and repair. Moreover, immune-compromised mice are extensively used to host and test the safety of allogeneic transplants Within this license we propose the use of different animal models to mimic three major human diseases (namely SCI, MS and ischemic stroke). However, before proceeding with animal work, all our new interventions are tested and refined in tissue culture. In case of cellular therapies and cell-derivatives (e.g. EV5), we routinely test measures that include the estimation of growth rate, differentiation ability, and negative screenings for pathogens (e.g. mycoplasma). For all candidate nanotherapeutics and AMID, the immunogenicity of the preparation will be tested in vitro first on relevant cell lines. Finally, all these interventions are tested for efficacy in vitro on microglia, macrophages, astrocytes and neurons prior to in vivo testing. To further ensure the significance of the results observed in vitro, all the experiments are run in multiple technical and biological replicates.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In our studies we always try to minimise the number of animals used while ensuring the achievement of sufficient data to properly answer a specific research question. The efficacy of the proposed therapeutic will be evaluated via a combination of behaviour, imaging, neurophysiology and conventional pathology approaches. The use of these combined readouts is part of our reduction approach as it allows to gain serial measurements from the same animal, avoiding the need of further experiments, with the final aim of maximizing the amount of data that can be obtained from a single animal without compromising animal welfare. For this reason, we have adopted the use of non- terminal anaesthesia (where possible) to guarantee proper biological fluid sampling, electrophysiological monitoring, and imaging of the same animal over time. This will provide better information about the progression of the disease/therapy while reducing the number of animals used. In our lab we have been extensively using Sd, EAE and MCAO models in rodents (approximately 60

	published studies over the last 10 years) and we are therefore able to ensure a high rate of reproducibility (consistency of immunopathology, neuropathology, lesion size, and behaviour/disability and outcomes). The animal group size for our experiments is based on our previous experience and statistical power calculations, so that the number of animals is sufficient to achieve statistically significant results. However, pilot studies will always be performed for new treatments (e.g., to assess feasibility, monitor potential side effects, and refine the main outcome measures of the experimental paradigm), and the number of animals to include in these pilot studies will be kept to a minimum (usually 5 per experimental treatment group).
3. 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.	Both mice and rats are widely used in research because their genetic, biological and behaviour characteristics closely resemble those of humans. However, in some cases there may be some important differences between mice and rats. For this reason and in accordance with the specific experimental purpose, in the under this PPL we will be using either mice or rats. Modelling SCI: SCI is a complex pathophysiology characterized by different aspects, such as acute and delayed inflammation, cell death, demyelination and astrogliosis. Traumatic SCI represents the most common cause of injury in humans (49% incidence). Thus, the most appropriate model that mimics the human condition is the contusion Sd. There are two main reasons that support our choice of this model: (i) it mimics both anatomically and pathophysiologically the human development of this pathology. (ii) Contusion SCI selectively targets the region of the dorsal spinal cord hosting the corticospinal tract. Rodents, compared to the other species, allow the generation of a reproducible injury model, one of the crucial points for screening therapeutic approaches and treatments. This model has the advantage of allowing researchers to couple CNS pathobiology (e.g.

	white matter damage, inflammatory response, and cell engraftment in case of transplant studies) with neurophysiological outcomes. Moreover, compared to other less severe models of CNS damage used to study nerve regeneration (e.g. optic nerve transection) this model permits the measurement of quantifiable behavioural deficits which are pivotal to monitoring the efficacy of our interventions. Modelling MS: to efficiently model MS (and its inflammatory driven pathobiology), we are currently adopting the EAE model in rodents. It is important to say that this model is not MS, but it recapitulates most of the mechanisms leading to MS. EAE is the prototype for T-cell-mediated autoimmune diseases and it is induced by immunizing the host with myelin specific antigens. This generates a peripheral immune response which ultimately leads to immune cell infiltration in the CNS with consequent damage. The EAE model has the advantage of allowing researchers to study neuroimmune interactions alongside with behavioural deficits that can closely resemble both the first (relapsing remitting) and second (progressive) phase of MS. This is important if we compare EAE with other less severe models of CNS demyelination (e.g. cuprizone induced, lysolecithin or ethidium bromide injections), which are characterized by a toxic damage to the myelin with little (or no) behavioural outcome. Modelling ischemic stroke: In order to model ischemic stroke, and the complex ischemic cascade, in vivo non-reductionist approaches are needed. We are currently adopting the MCAO rodent model to mimic major ischemic events. This model has the major advantage of being highly reproducible (compared to the cardioembolic model of stroke), of best mimicking the pathophysiology of the ischemic disease (contrary to the phototrombotic model), and having observable behavioural deficits. Refinement procedures include maintaining the mouse/rat internal temperature between 36.0°C and 37.5°C with a feedback heating system, as well as measuring the local cerebral blood flow with laser Doppler flowmetry. Both these approaches help reducing lesion variability and

	interpreting results. Upon disease induction and treatment animals will be observed closely and, at the first sign of distress (e.g. animal failing to feed, drink, drop in the body weight) or pain/inflammation, the NVS will be contacted for a further advice. If animals show unexpected signs, or existing adverse effects will not respond to the treatments suggested by the NVS, animals will be killed.

Project 38	**Mechanisms of brain function and dysfunction**	
Key Words (max. 5 words)	Neurons, Glia, Receptors, Glutamate, Dopamine	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	One of the main neurochemicals in the brain is glutamate, which has important functions but can also cause nerve cells to die. My overall aim is to increase our understanding of how glutamate affects brain neurons via their protein receptors, how these receptors are regulated, and the consequences of receptor regulation for brain health versus disease. Key questions we aim to address are: How are glutamate receptors regulated by processes inside the cell, and those occurring within the brain environment? Do these processes influence the health of nerve cells? Do environmental toxins, inflammation or low oxygen levels regulate glutamate activity and so cause greater harm to nerve cells?	
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)?	The new information that our studies will provide will benefit research on brain function by: 1. Aiding our understanding of fundamental brain physiology. 2. Increasing knowledge about how brain	

	environment influences glutamate activity. 3. Shedding light on glutamate receptor activity in unhealthy brain environments. In the longer term, this may aid identification of novel therapeutic targets in diseases of the nervous system, for example: 4. Drug targets for reducing neurodegeneration. 5. Drug targets in stroke and other brain disorders resulting from low oxygen levels.
What species and approximate numbers of animals do you expect to use over what period of time?	In 5 years: Mice (<3500) Rats (<500).
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?	Most of our experiments involve removing brain tissue: the animals are terminally anaesthetised and then killed whilst still under anaesthetic so they do not experience adverse effects. We also breed mice with genetic mutations, for example mice in which the gene for a specific protein of interest is removed, or mice in which specific cells express a protein that allows to us to identify them. The genetic alterations that will be made to the mice we use are not expected to have an impact on their health and wellbeing but are important to our work because they allow us to identify the role of these specific proteins, or the properties of these specific cells.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our research relates to the function and dysfunction of the mammalian nervous system. We carry out experiments using brain tissue taken from rats and mice. The brain tissue allows us to study nerve cells and their interactions. Brain areas of interest can be isolated and controlled and it is possible to measure the activity of individual cells. Severity is lower than in living animal models. Computer models do not yet contain sufficiently detailed or accurate information to

	be a valid alternative.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We interact with the technicians overseeing the husbandry of our mice to keep the numbers at an optimum level. Within the lab, brain tissue is shared among laboratory personnel; for example, where possible we co-ordinate the timing of our experimental work such that, if one brain region will yield more tissue than one person can use, more than one person is ready and available to use it- this can avoid a second brain being required for that day. For data analysis we use statistical methods (that have been subject to peer-review) to ensure that we use the minimum number of animals necessary to enable rigorous statistical analysis.
3. 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.	Rodent brains have very similar anatomy, physiology and receptor systems in the brain regions we study to those in humans. The use of genetically modified mice has improved the efficiency of our experiments (for example, they allow us to identify specific types of brain cells more accurately). There is no mammalian animal species of lesser sentience suitable to address the proposed aims. Our rats and mice are housed in individually ventilated cages to protect their health status and have environmental enrichment. All laboratory personnel who work with animals are appropriately trained and hold Home Office Personal Licences.

Project 39	**Drug Development in Tuberous Sclerosis Complex**	
Key Words (max. 5 words)	Tuberous sclerosis, drug development, seizures, tumours, cognition	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Tuberous sclerosis complex (TSC) is a genetic disorder caused by mutation of one of two genes: Tscl and Tsc2. TSC is a prevalent disorder affecting one in every 6000 newborns, and is characterised by the growth of tumours throughout the organs of the body, epilepsy, mental retardation and autism. There is no cure for TSC and current treatments are limited to removal of tumours, minimising complications that arise from tumours, and relief of symptoms- mainly antiepileptic medications to treat the seizures suffered by patients with TSC. We will establish and maintain a colony of mutant TSC rats, the Eker rat, to support the development of new drugs for the treatment of this condition. We will assess whether our drugs are beneficial for the treatment of seizures in TSC, reduce the number of tumours and whether they can alleviate the cognitive deficits and autistic traits that are commonly seen. If these drugs show beneficial effects then we will use tissue from these and additional rats for in vitro experiments to	

	determine the mechanism(s) by which our drugs exert their effects. Objective 1: Determine the anti-epileptic efficacy of a given study drug using behavioural monitoring methods. Objective 2: Determine the neuropsychological tolerability profile of a given test compound using cognitive, emotional and motor behavioural tasks Objective 3: Determine the anti-tumoural actions of a given study drug using ex vivo methods in Tsc tissue Objective 4: Determine the underlying mechanism of action by which the study drug exerts its effects using in vitro methods in Tsc2 tissue.
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)?	Knowing whether our drugs work in this rat model of TSC will help regulators, industry, clinicians and patients benefit by informing and, if results are positive, speeding up the process of clinical licensing of these drugs. Knowing how these drugs work will help us better understand why the disease occurs and will allow us to develop better medicines to treat the disorder.
What species and approximate numbers of animals do you expect to use over what period of time?	Tsc2 mutant (Eker) rats; —2500 over 5 years
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?	Pairs of animals containing the Tsc2 mutation will be bred together. 33% of animals will exhibit no disease symptoms, 66% of animals will exhibit tumours and mild cognitive impairments. Therefore, this work is considered to be of severe severity. We will need to take tissue samples from these animals in order to determine which have the disease and which do not. This can cause some pain but will be minimised by pre-treatment with a local anaesthetic. We will assess the anti-epileptic activity of our study drugs in these animals. In order to do this, we will use animal models that induce either a single seizure or full-blown recurrent epilepsy. Seizures can cause

	suffering and may be fatal. However, whilst these symptoms can appear distressing, seizures are preceded by a loss of conscious awareness by the animal such that it is unaware of the induced state (as is the case with human epilepsy patients). We will also treat animals with our study drugs over a number of months to assess any changes in tumour growth and/or cognitive and autistic traits. Whilst these drugs have been well tolerated in our long experience we cannot rule out rare reactions in a tiny proportion of animals. All animals will be killed at the end of the study unless asymptomatic animals are transferred to other authorised users to establish colonies for their own research.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	TSC is a disorder that affects the whole body (brain and periphery) producing tumours, epilepsy, cognitive deficits and autism. The range of effects produced by TSC means that it is too complex for current in vitro approaches to properly model it. Regulatory requirements for new drugs demand testing in whole animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals will be minimised via the following: i) Experimental group sizes will be determined by statistical power calculations. ii) A professional statistician will be consulted, when required, to ensure that our statistical designs are appropriate and that the number of animals required is minimised. iii) We will not breed more animals than we will need for our experiments. iv) Animals, or animal tissue, may be used for multiple experiments, if appropriate. v) Use established protocols to maintain high data quality and reduce variability in experimental results

	meaning fewer animals needed for our experiments.
3. 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.	There are a number of different models of TSC, however none of them exactly mimic the human form of the disease. For this program of work we have chosen to utilise the Eker rat, the most widely used animal model to study TSC and the model that exhibits the greatest number of TSC symptoms of interest. Similarly to human TSC, the Eker rat shows similar cognitive and autistic-like behaviour and exhibits tumours (primarily in liver, spleen and kidneys), The Eker rat does not have spontaneous seizures (unlike the human form of the disease), it does show greater susceptibility for seizures following a chemical insult. We will only breed sufficient animals to meet our planned experimental needs and we will use animals as early as it is scientifically possible for our experiments to minimise the time during which tumours manifest. Although we plan to utilise our animals early in their life cycle (6 months max), previously it has been shown that tumour burden itself is not lethal and Eker rats have been shown to survive to —24 months old despite developing kidney tumours by 4 months old. We have extensive experience (>8 years) of using whole animal models of epilepsy and seizure, including working with two severe severity. Our group is, therefore, one of the best placed in the UK to ensure that the welfare of these animals is maintained to the highest standard possible given their disease status.

Project 40	**Neuron-glial-pericyte-vascular interactions**	
Key Words (max. 5 words)	neuron, astrocyte, oligodendrocyte, microglia, pericyte	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Nervous system function depends on nerve cells, but also on supporting “glial” cells and on an adequate energy supply provided in the blood. The project will study how nerve cells interact with glial cells and blood vessels, which is poorly understood but plays an important role in the development, normal function and pathology of the nervous system. For the nervous system to develop and function normally, nerve cells must interact with glial cells. These include astrocytes (which control the composition of the space around nerve cells), oligodendrocytes (which speed nerve cell impulses), and microglia (which are immune system cells protecting the brain). Nerve cells also communicate with muscle cells controlling the blood flow to the nervous tissue. Interactions between these cell types involve signalling using neurotransmitters and other molecules, but the details are obscure. Disorders of these interactions are thought to occur in diseases such as stroke, multiple sclerosis, spinal cord injury, cerebral palsy, Alzheimer’s disease and HIV infection,	

	and so have an enormous human and economic cost.
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)?	We hope to advance our understanding of the following issues. (I) How blood flow in the brain is controlled. The brain depends crucially on its energy supply, which is disrupted in diseases like stroke and Alzheimer's disease. By understanding the signals which regulate blood flow we may be able to correct disorders in which there is too little flow. (2) How myelin forms around axons and how it is disrupted in diseases such as multiple sclerosis, stroke and cerebral palsy. Loss of myelin causes physical and mental impairment. By understanding how it forms and is damaged we may be able to treat disease of myelin loss better. (3) The function of immune cells in the brain. These cells are important in diseases like Alzheimer's disease, HIV infection and stroke, and they also regulate the development of the brain, but we know little about how their activity is controlled. By improving this knowledge we would open up novel therapeutic targets for treating neurological diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats: about 250 Mice: about 4000
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?	Most experiments have a severity of mild or less. A small number of experiments are classified as moderate because the animals are given substances that might cause them harm; it is essential to give the animals these substances (e.g. a drug called tamoxifen which is used to treat breast cancer in humans, or a higher fat diet) in order to carry out research which will provide information that may in the long term help the development of therapeutic drugs. Usually animals will either be killed at the start of an experiment and their tissue will be used for experiments, or they will be anaesthetised and studied before killing them with an overdose of anaesthesia. In a small number of experiments they will be given substances for a few days before the experiments occur.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Because the work studies interactions between different cell types it can oniy be done on tissue from real animals — there is no adequate cultured cell line replacement, partly because cells change the proteins they make once they are put in culture. Wherever possible we employ computer modelling if it can replace animal experiments, and we are also starting to use live human tissue to check that our animal work is relevant to humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We ensure that the minimum number of animals is used by sharing tissue from each animal between different researchers and, when possible, by designing experiments to use the minimum number of animals sufficient to achieve a desired level of statistical significance in the results. Using transgenic technology to express coloured dyes in particular cell types for identification also reduces the number of animals that we need to use for experiments.
3. 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.	Rodents have been chosen for this work as the lowest species which mimic the human nervous system well enough for our work to be relevant to human disease, We minimise suffering by either killing animals humanely and then taking tissue from the dead animal, or by anaesthetising them and killing them after the experiment is complete but while they are still under anaesthesia. Changing the proteins made in the animals' cells using transgenic technology is not harmful to the animals for the experiments we propose. Although the animals used must regrettably be killed, we envisage no other significant adverse consequences for them during the procedures used.

Project 41	**Synapses and plasticity in health and disease**	
Key Words (max. 5 words)	Alzheimer's disease; learning & memory; diet; improved mouse models;	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	There is a great need for improved models of Alzheimer's disease. The models currently available do not model the complete syndrome, offering good models for the hallmarks of the disease: rising amyloid beta protein and plaque deposition; or tau tangles and cell death; but failing to make the link between the two. Some people have combined the current genetic models to make mice with plaques, tangles and cell death, however this bypasses the link as genes that cause tau tangles are never found in Alzheimer's disease. We therefore propose to combine risk factors for Alzheimer's disease, such as high-fat diet or other genetic factors, such as APOE or TREM2, with the amyloid models to hopefully produce a model of the disease that presents the full symptoms of the disease.	
What are the potential benefits likely to derive from this project (how science could be advanced or	The absences of a complete model has hindered the translation of results from the bench-side to the clinic. Having such a complete model will offer better tools in which to develop and test drugs.	

humans or animals could benefit from the project)?	At the same time we will learn new information from the control groups, for example the impacts of high- fat diet or dietary restriction on the cellular mechanisms that underlie learning and memory.
What species and approximate numbers of animals do you expect to use over what period of time?	Genetically modified mice (and some rats). In total, over the 5 years, we expect to use 5200 mice in developing and maintaining mouse colonies and performing the experiments, and 600 rats.
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?	Initial experiments to develop a mouse that shows the full plethora of symptoms of Alzheimer's disease will reach a moderate level of severity and they will develop cell death in the brain. However, once established, the experiments will typically be aimed at earlier stages, prior to the onset of cell death, where we will target the events leading to cell death.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are currently performing preliminary experiments to take advantage of two recent developments in cell culturing. The first is induced pluripotent stem cells (iPS cells), which are derived from skin biopsies from patients (and healthy controls) that are reverted to stem cells and then differentiated into neurones that carry the full genetic background of the human from which the biopsy was initially taken. The second development is that of 3 dimensional cultures, which use a gel matrix into which neurones are seeded. The neurones can then make connections in a structure more akin to the brain than the single plane traditional cultures are restricted to. Our aim is to combine these two advances in order to develop 3D iPS cell cultures. While 3D iPS cells cultures obviously provide an excellent model for disease, being human neurones carrying a complete Alzheimer's disease genome, they still can not replace a whole animal approach as the cultures are limited in the range of types of neurones and glia. They also lack sensory inputs and effects of, for example, hormones and diet.
2. Reduction Explain how you will assure	We maximise the data obtained from each animal by optimising experimental design including performing

the use of minimum numbers of animals	multiple types of experiments both within our own lab and with our collaborators. For example, from one brain, we can perform live recordings on tissue from one half of the brain and freeze the other half for genetic and molecular biology testing. By performing multiple analyses on tissue from one animal, we increase the statistical power and interpretation of the data as direct correlations can be made. Furthermore, we have an agreement with ShARMUK, which is a tissue bank from aged animals for use by other experimenters. Therefore, other tissues can be harvested from each animal. In order to ensure suitable numbers of animals are used, power analyses are performed using data from pilot or previous similar studies, thus ensuring the minimum number of animals are used to give us data of which we can be confident.
3. 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.	Thirdly, they are easily housed in animal units and do not require much specialised care beyond food, bedding and minimal enrichment within the cage. As we are trying to model diseases that result in cell death in the brain, some of our mice will develop a harmful phenotype. However, upon the first appearance of clear symptoms of cell death, animals will be killed humanely for use in experiments. Generally, however, most experiments will be aimed at the stages leading up to cell death and therefore most animals will never reach this stage. Where surgeries are employed, every care will be made to minimise pain and distress. Anaesthetics will be used during the sterile procedures and analciesia provided pen- and post-operatively. The choice of using rodents has manifold advantages. Firstly, they are mammals and therefore share many of the features of humans (although consideration of their differences should be made). Secondly, they prove very amenable to genetic modification, making expression of genes associated

	with dementia more practicable than many other mammals.

Project 42	Genome involvement in brain function, disease and development.	
Key Words (max. 5 words)	Disease, genetics, gene regulation, transgenic, mouse	
Expected duration of the project (yrs.)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	What are the mechanisms that switch genes on and off? How can these switches differ between people and how can these differences make people sick? We can show that the DNA, that separates the genes in the human genomes, contains short areas that act as switches that turn genes on in the right places in the body and brain, at the right time and in the right amounts. Finding these switches has previously been very difficult as they are small, unpredictable in structure (as opposed to genes which have predictable structure) and are often very far away from the genes they control. The breakthrough came with the sequencing of the human genome and the genomes of several other species including rodents and chickens. Using computers we saw that within genomes there were short stretches of DNA that have been even better conserved than the genes themselves, an	

	indication of their importance during 300 million years of evolution. For the first time this provided us with a way to rapidly identify potential switch sequences that could be isolated using standard genetic engineering methods Very recently we have acquired a new technology called genome editing (AKA CAS9/CRISPr technology) that allows us to quickly and accurately edit these switches in mice in a way that uses far fewer animals than previous now redundant methods. Genome editing allows us to delete these gene switches or to reproduce sequence changes within them that are associated with human disease. Furthermore, we have started to learn how to affect the activities of these switches by giving different potential medicines, some with psychoactive properties to GM rodents. Critically, genetic differences within these switches often alter their ability to respond to drugs thus altering the expression of the genes that they regulate. Because many people respond differently to different drugs, and experience unacceptable side effects, recognition of the role of genetic differences in genomic switch sequences will be essential in the future development of personalised medicine. This will be achieved by providing drug companies and doctors with biomarkers to recognise those who would most benefit from specific drugs. We will also examine how the genetic changes within these switches alter food intake, weight and alcohol consumption. different well characterised drugs known to affect the activity of signal transduction systems, will also be used to identify the signalling systems that modulate the activity of these switches in order to identify the pathways most affected by genetic changes. Most excitingly are the findings that these switch sequences act as the major functional target of epigenetic mechanisms that are influenced by environmental factors such as early life stress. We believe that the novel use of genome editing to further the study of these switches, and the effects of

	genetic and epigenetic changes on their activity, will allow us to personalise drug treatments by predicting who would benefit most based on their DNA sequence. We will also be able to eventually predict susceptibility to obesity, or addiction and provide new ways to treat them. (465)
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)?	Diseases such as obesity and addiction cause untold misery in Western societies whilst greatly affecting their economies and ability to compete globally. The experiments outlined in this licence will permit a greater understanding of the environmental and genetic factors that increase susceptibility to these diseases and will provide novel pathways to allowing their prediction and for the development of personalised treatment. (59)
What species and approximate numbers of animals do you expect to use over what period of time?	I estimate that up to 4200 rats and mice will be used during the 5 years that this licence is active most of which will be used in the generation, breeding and maintenance of the genetically modified lines to be used. (47)
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?	None of the DNA sequences or targets of genome editing under study to be used to make GA animals encode protein, or proteins essential to survival, so it is unlikely that they will have any effects on the animals. The possibility of deleterious effects as a result of random insertion into the genome can be negated by maintenance of line as heterozygotes. Surgical procedures- vasectomy and embryo implantation are required in order to generate new GA lines, but pain will be well controlled and recovery to normal behaviour is rapid. In feeding tests animals will not be allowed to experience ill health as a result of excessive obesity. Also, in early life stress tests measures such as only removing part of the litter from the mother at any one time and transferring pups in the presence of bedding will greatly reduce the chances of maternal stress. In the absence of maternal care pups will be kept warm using a heated pad to reduce the chance of hypothermia.) After stressor the majority (80%) of animals will be killed to recover DNA and RNA for further analysis. up to 20%

	of animals may be retained for further feeding/alcohol intake or drug administration tests. During this time animal will be continuously monitored for signs of distress (huddling, hunching, irregular or rapid breathing, shivering, freezing behaviour, lack of appetite in excess of what is expected, reduction in body weight) by either the licencee or trained technical staff. If such behaviour is noted NVS advice will be sought and, if required, the animal will be killed by a schedule 1 method. After these behavioural tests all animals will be killed by schedule 1 or non-schedule 1 methods
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Most of the immortalised cells used in tissue culture are derived from cancerous tumors and bare little or no resemblance to the cells found in the human body in terms of their behaviour, their ability to communicate with other cells or the way they express the information contained in their DNA. This poses a problem as many of the processes that make us human will affect biological systems that depend on a number of different and highly specific cell types which must communicate with each other in very specific ways in order that the information in their DNA is expressed in the correct manner. Primary cells are derived from living brain tissue and therefore represent a more representative neuronal cell type than can be derived from cancer cells. We have devised methods of introducing DNA constructs (transfection) into these cells that allow us to understand the effects of single nucleotide polymorphisms and DNA methylation on the gene regulatory elements that keep us alive and healthy. Transfection of these primary brain cells has removed the the need to generate transgenic animals by as much as 80%
2. Reduction Explain how you will assure the use of minimum numbers of animals	The main method we will use to reduce the number of animals used will be the introduction of CRISPr technologies to generate our GM animals. Cas9/gRNA targeting of specific loci within the mouse genome is at least an order of magnitude more

	efficient that “traditional” embryonic stem cell targeting that can use hundreds of mice for the generation of one knockout mouse line and could take 2 years. With CRISPr CAS9/gRNA technology we have been able to generate 12 homozygous knockout mouse lines in 6 weeks by only using 6 superovulated donor female mice and 4 female host mice. We will also use statistical analysis of the number of animals to be used to ensure that a minimum number will be used for each experiment. We will use statistical analysis techniques recommended in consultation with a professional statistician to ensure that as few animals as possible will be used but sufficient to ensure statistical significance of data derived from our experiments. We will also maintain lines for the minimum length of time after which lines will be used to produce, eggs, sperm or embryos which will be stored as frozen. Once successfully frozen, lines of breeding animals will be terminated. We will also endeavour to devise experiments involving the use of primary cell lines which do not involve the generation of transgenic lines or the use of live animals. (131)
3. 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 the most suitable model as it is the least sentient small mammal whose genome can be readily manipulated whilst the majority of its anatomical and physiological features are shared by humans. Anaesthesia and analgesia methods will be used in surgical procedures and in all other regulated procedures with the exception of the those in which the administration of these substances would result in more distress and suffering than the distress and suffering likely to be caused by carrying out the regulated procedure without the use of these substances All surgical procedure will be carried out in accordance with the minimum Home Office standards.

	We have chosen early life stress test as it represents a proven non–invasive methods to alter the methylation status of the genome. Animals will be closely monitored and the NVS consulted as deemed appropriate.

Project 43	**Regeneration and cancer in the nervous system**	
Key Words (max. 5 words)	Neural stem cells, glioblastoma, tissue environment, injury, repair	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objectives of the project are to identify the molecules and cellular processes that underlie regeneration and cancer in the adult nervous system. In particular, we wish to understand how different cell types cooperate to drive repair in peripheral nerves and how neural stem cells are regulated and respond to brain injury or neurodegeneration. We also want to understand how glioblastoma, the most common and malignant type of brain tumour, invades the normal brain.	
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)?	Nervous system pathologies such as stroke, traumatic brain and nerve injuries and neurodegenerative diseases are extremely common and represent an enormous socioeconomic burden. For example in the UK, injuries to the PNS occur in 3-5% of all trauma patients (Pfister et al., 2011), stroke occurs 152,000 people a year (one every 3 minute 27 seconds, Stroke statistics, 2016) and neurodegenerative disease affects 850,000 people per year (MRC website). Together,	

	stroke and neurodegenerative disease alone have a health and social care cost of around £35 billion per year. Tragically, there are currently no effective treatment options for any of these devastating diseases. Although some basal repair does occur in both peripheral nerves and in the brain following damage or disease, in most cases this is not sufficient to fully restore function. By understanding the intrinsic regenerative processes that occur naturally in nervous tissue, we should be able to identify therapeutic strategies to harness and improve these processes for nerve and brain repair. Therefore, this work has the potential benefit to identify novel therapies for nervous system pathology, including traumatic nerve/brain injury, stroke and neurodegenerative disease. Over 10,000 cases of brain cancer are diagnosed each year in the UK (CRUK website), with glioblastoma being the most common. Glioblastoma is one of the most lethal and malignant types of human cancers with a median survival of <1.5 years following aggressive therapy. one of the main causes of therapy failure is that the tumour cells spread extensively into the normal brain tissue. This renders complete surgical removal of the cancer impossible, leading to tumour regrowth from the remaining tumour cells and rapid death of the patients. Therefore, understanding the mechanisms by which tumour cells spread within the brain, will identify potential new therapeutic targets for improving clinical outcome of this devastating disease by preventing tumour regrowth.
What species and approximate numbers of animals do you expect to use over what period of time?	These studies will use mice and we estimate that we will use 7450 animals/year.
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	All of the protocols proposed carry a mild or moderate severity and are well-established experimental models. We will closely monitor the animals for the appearance of any adverse effects and any animals that display undue suffering or distress will be removed from the experiment and humanely killed. All animals at the end

to the animals at the end?	will be culled by Schedule I or non-schedule I methods under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The vast majority of our molecular studies will first be conducted in cells freshly isolated from mice and rats euthanized by schedule I methods. These freshly isolated cells are crucial for our studies because all available long- term cell lines have undergone non-physiological changes as a result of extended culture. Using these culture models, we have previously identified important molecules involved in nerve and brain repair, and in the spread of glioblastoma and will continue to do so. However, many effects that take place in vivo cannot be satisfactorily reproduced in vitro. For example, in vitro systems cannot adequately model the inflammation or immune reaction that occurs after an injury or the interactions of tumour or stem cells with their surrounding brain tissue. Therefore, in vivo experiments are necessary where suitable culture systems are not available. However, wherever possible, we will first use in vitro systems to identify important molecules involved in these processes and then carry out in vivo experiments as a final step to test our in vitro findings.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of mice to be used are based upon our calculations, previous experience and the literature and will be the minimum numbers of mice required to achieve the objectives of this license and obtain significant and accurate results. Our breeding programme and experimental designs will be streamlined to obtain the maximum amount of data from a single animal. For example, through our use of in vivo imaging, multiple data points can be collected over time from each animal.
3. 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	For all the in vivo studies we will use adult mice because the tissue organization and biological principles underlying nerve and brain repair, and brain cancer are similar between mice and humans. Mice are also the mammalian species in which nerve and brain structure, regeneration and cancer have been

general measures you will take to minimise welfare costs (harms) to the animals.	most extensively studied, thereby reducing the number of animals required for characterisation. In addition, it is possible to remove or introduce genes in specific cell types and/or at particular times in the mouse and a large number of genetically modified strains are already available. Thus, genetically modified mice are the most effective means of testing the role of genes of interest in nervous system regeneration and cancer and, as such, are the most refined model organism for our studies. To minimise harm to animals we will carefully monitor all mice to identify at an early stage any potential welfare costs and allow us to intervene promptly to minimize side effects or suffering.

Project 44	**Establishing connectivity in the brain**	
Key Words (max. 5 words)	Brain development, neurodevelopmental disorders, vision	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We are interested in how the brain develops and in particular how connections between neurons are formed correctly during this fundamental process in neuroscience. Some molecules that are important for individual steps, such as the outgrowth of a cell, the guidance of its processes or the recognition of other cells to make appropriate connections, have been described. However, most of the mechanisms and molecules are yet to be identified. Over the last 10 years, my laboratory has made significant advances mainly focussing on the visual system as it has been shown to be an excellent model to study these general principles of building a brain. Our objectives are: 1. To identify specific types of neurons and analyse their connectivity and functionality 2. To identify the molecular mechanisms that lead to the formation of such cell types 3. To identify the molecular mechanisms that lead to the appropriate neural connectivity in	

	the brain 4. To identify how mutations in genes associated with neurodevelopmental disorders alter circuit development and function
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 project will increase our knowledge how a normal brain develops and how signals coming from the outside (such a information we see with our eyes) are processed in order to enable us to recognise the world around us. In addition, understanding the assembly of a healthy brain is essential to identify the possible underlying causes of neurological disorders, such as Autism Spectrum Disorder (ASD), Bipolar Disorder, Schizophrenia or Epilepsy. Finally, the knowledge created within this program of work will also be crucial in order to develop therapeutic strategies for brain repair, for example after trauma or disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Over five years: Mice: Up to 9000 adults, most of the will be used for breeding to generate experimental animals. Up to 1500 embryos, the majority of which will be used prior to the developmental stage at which they become protected by the act. Chick: Up to 1000 embryos, all used in procedures prior to the developmental stage at which they become protected by the act. Zebrafish: Up to 10,500 Adults, 10,000 of which are used solely for the production of embryos, which will be used in experiments. Up to 25000 larvae, the majority of which will be used prior to the developmental stage at which they become protected by the act.

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?	Mouse A significant part of our research is done using tissues samples from animals, taken after death, to identify genes in a particular tissue during development. Some of the mice we use for tissue collection will have a genetic alteration, which may affect nerve cell development. Therefore we will need to maintain colonies of mice bearing appropriate genetic alterations to provide tissue for laboratory studies. The genetic alterations will cause minimal effects in animals; in fact it is usually impossible to distinguish these mice from normal mice, except after examining a small sample of tissue from which the genes can be extracted. A limited number of studies will use living animals. Here we will analyse the role of molecules in the correct formation of a particular area in the brain, and establishing connections between brain areas. There are two main protocols, with moderate severity: (1) For visualisations, we will inject small amounts of fluorescent dyes into brain cells of anaesthetised animals and later analyse their structure in post-mortem animals under the microscope. (2) For a different group of mice we will inject very small amounts of DNA into embryos and then put them back to the mother for further development. These animals will then either be killed before birth or left to term and killed at a postnatal stage. Complications during these experiments (for example the surgery or the injections) are very rare (<1%). Good aseptic surgical technique and our long-standing experience conducting such experiments will help reducing adverse effect to a minimum. Zebrafish For the vast majority of zebrafish they will live a normal life within the animal facility with no adverse effects and be used for breeding until they are humanely killed around 18 months of age. The majority of experiments will be performed on zebrafish larvae prior to the developmental stage at which they become protected by the act.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our studies focus on development of the brain and the establishment of the connections between cells. Furthermore, we plan to assess consequences of possible wiring defects in behavioural assays. In order to address these questions the hugely complex neural architecture of the brain must be intact. Tissue cultures systems do not exist that recapitulate this functional architecture. Thus, alternatives not using animals are not available. These processes can only be studied in the living organism. Therefore the use of animals for in vivo work is absolutely necessary as there are no satisfactory in vitro substitutes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our work usually involves the analysis of a particular phenotype under the microscope. If possible we will have two different persons carrying out the counting/grading/observing of the phenotype, and performing the procedures. This “blinding” towards the genotype or group of animals approach will be important for a non-biased statistical value of the analysis. The use of significance tests will reduce the numbers of animals tested. For some of the experiments we will use untreated areas of the brain as an internal control, therefore reducing overall animal numbers.
3. 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.	About half of the proposed animal experiments will use mice, the simplest available mammalian model to study the establishment of connectivity in the brain. Importantly, over the last few years, we have established a system that allows us to investigate some of our questions in the zebrafish larva. Many fish will be used prior to the stages specified by the Act. Our focus on the zebrafish as a model organism arose to comply with 3Rs by using the approach necessitating the fewest animals past mid-gestation and reducing to the minimum animal suffering.

Project 45	**Genetically defined neuronal networks regulating sleep, wake and circadian rhythms**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Yes	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The alternation between sleep and wake presents many similarities with the natural daily allocation of behaviours known as circadian rhythms. While daily rhythms are regulated by a biological clock, which is anatomically well defined, the mechanism by which the brain controls the transition from wake to sleep, and when sleeping, through dream and non-dream sleep, remains largely unknown. What is so far clear, is that whilst few, rather similar neurons in the brain can set the timing of the body clock, sleep/wake regulation requires the interaction of many different neurons that are found at different locations within the brain. The objective of our research is to map new neurons that are important to induce sleep or the transition between a dream-type of sleep to a deep dream-less sleep. By conducting this work, we hope to contribute new knowledge regarding the potential pharmacological targets and cellular substrates for the treatment of sleep disorders.	

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 research aims to increase our knowledge of how the brain controls behaviours. The question of why do we sleep has yet to find a precise answer. The development of novel technologies to manipulate the function of few, well defined neurons within the brain and measure the resulting changes in an animal behaviour now makes answering that fundamental question possible. The benefits of our work consist in providing the scientific community with an accurate anatomical map of the brain networks that are important to generate sleep and to suppress it when waking. This information can then be used by other scientists to test novel compounds that improve the life quality of people suffering from sleep disorders. Our work will also be instrumental in understanding why several brain disorders, including schizophrenia, Alzheimer, Parkinson are accompanied by drastic changes in the sleep/wake pattern.
What species and approximate numbers of animals do you expect to use over what period of time?	The project is based on the use of genetically modified laboratory mice that are born and bred in a laboratory environment. This presents the advantage of being able to target experimentally only a defined neuronal type, leaving all other nearby brain structures intact. Over the duration of the project (5 years) we estimate to use 7000 mice. More than half of these animals will not be undergoing any other procedure than mating and generating an offspring. Approximately 2500 mice will be used in experiments that require surgical intervention to either inject specific compounds in the brain or to implant recording devices. All surgical steps are performed under total anaesthesia with pre and post-operative analgesic therapy. It is in fact very important for this study that the data obtained is a true reflection of the brain control of sleep and not caused by discomfort or pain experienced by the mouse.
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	The types of surgeries that will be performed are known to lead to rapid and uneventful recovery and will not exceed a moderate level of discomfort. Complications may arise from infection at the site of surgery and mice will be monitored daily to ensure that immediate action is such cases is taken. As the ultimate goal is to be able to alter neuronal function to

to the animals at the end?	change sleep/wake patterns, it is likely that some mice will experience loss of sleep, or increased sleep- these alterations in behaviour are not expected to cause more than a mild discomfort. Once a mouse has undergone a surgical procedure and the study to measure altered behaviours is completed, the animal is humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex neuroanatomy and timely integration of different neuronal signals with different properties that is required to induce sleep and drive its progression makes it impossible to conduct this study in anything other than an intact animal model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Research involving animals is extremely costly, hence beyond the more basic human grounds, financial considerations too drive the careful estimate of the minimum number of mice that is necessary to perform a measure that has statistical significance and is hence a reliable source of information for future studies that will not need to replicate the animal work. Power calculations in consultation with a statistician have determined the number of animals required in this study.
3. 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.	Research on sleep and wake has its ground in important discoveries made in the I 960s, which were obtained using larger mammals, including cats. Since then the development of mouse genetics has, on one side made it possible to use a simpler animal system (mouse) and on the other also made it possible to dramatically refine the type of surgical manipulations. Whereas , previous work in cats may have involved large portions of the brain being removed, it is now possible to selectively target a handful of neurons in genetically altered animals such as mice without any macroscopic damage to the brain. Because mice are now the model system of choice for biological research, good protocols and procedures are in place such that any associated suffering is minimized.

Project Title 46	**Mechanisms underlying Huntington's disease**	
Key Words (max. 5 words)	Behaviour, sleep, circadian rhythms, EEG	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The main objective of this project is to better understand the mechanisms underlying Huntington's disease (HD). At present, there is no treatment or cure for HD; and relief from only some of the symptoms. HD is caused by a single gene, but the symptoms are complex, and include deterioration of motor function, learning and memory, and emotions. Understanding the processes that underlie the disease is key to developing treatments. Our approach has already been successful in identifying potential targets for treating HD. If we enjoy continued success with this project, we will advance understanding of HD, develop methods for analysing behaviour in other animal models of neurological diseases, and improve therapies used for treating HD. In particular, we aim to develop treatments for the emotional, learning and memory, sleep and rest/activity rhythm deficits that typify HD.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit	We will develop a range of tests that measure in mice those behaviours that are most relevant to the symptoms seen in HD patients. Our tests will help to identify those mouse models that are most relevant to different aspects of the human disease. We will gain a better understanding of how the mutant	

from the project)?	Huntington protein causes both behavioural problems and pathology. Our data will be useful for other researchers who do not have our mouse colonies, and for clinicians who may have to predict onset and severity of HD in their patients. We will learn how disrupted brain function affects behaviour in both normal and HD mice. Since abnormal sleep is a common features of HD, our data will be particularly useful for clinicians trying to treat behavioural symptoms that may be caused by sleep deficits in HD patients. Our work will produce a better understanding of how problems with the heart affects behaviour in both normal and HD mice. Since heart failure is a common feature of HD, our data will be particularly useful for clinicians trying to treat behavioural symptoms that may be caused by defective hearts in HD patients. We will gain a deeper understanding of the relationship between damage to the brain and other organs caused by HD, and abnormalities in behaviour. If pathologies in organs other than the brain contribute to the learning and memory problems, it may be possible to use existing treatments to treat patients (such as antihypertensive drugs to treat heart failure). We hope that the project will identify drugs or other treatments that delay the onset of, or improves the symptoms of, HD. Any treatment that improves the symptoms in an HD patient will be a major advance.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse. 36,150 over 5 years.
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	Approximately 50% of the mice will develop a form of Huntington's disease. Affected mice are expected to develop problems with memory and sleep, and to be less interested in other mice. 90% of affected mice will experience mild to moderate clinical signs, but will still be able function normally. The remaining 10% will experience a more severe form of the disease, exhibiting signs such as weight loss, impaired movement and reduced sociability,

at the end?	and will need greater care. HD does not involve pain, so it is highly unlikely that the animals experience pain. Most of the animals undergo non-stressful behavioural testing, where either the severity of their disease is measured, or the behavioural effects of drugs are assessed. Testing is not harmful to the mice, and in fact we have shown that a moderate level of behavioural testing is beneficial for the animals. Some mice will be housed individually, for example to measure their sleep patterns and activity rhythms. Where possible, these mice are returned to their home groups at the end of testing. Single housing does not appear to be harmful to the mice, as shown by the fact that the animals very rarely fight when re-grouped. Sleep restriction is carried out for short periods, after which the mice can sleep freely. Where drugs are given, we will use the least stressful route possible, ideally by adding the drugs to food or water. Where lesions are induced (under anaesthesia) in the brains of mice to mimic certain brain pathologies associated with Huntington's disease, such lesions are occasionally associated with adverse events such as epileptic fits, but these will be transient and closely monitored. We have strict criteria for grading fits to ensure that these are not permitted to continue any longer than absolutely necessary. Surgical implantation of minipumps (for drug administration), electrodes, cannulae or catheters will be carried out under general anaesthesia and in aseptic conditions. Animals are routinely given painkillers for as long as necessary, and are closely monitored for any ill-effects. Where drugs are given by injection, distress to the animal is minimised by careful handling and rigorous training of personnel in injection techniques, such that injections cause no more than temporary discomfort. Imaging (e.g. MRI) is non-invasive and does no harm. The mice will be anaesthetised throughout scanning, and will not have a repeat scan until they fully recovered from the previous anaesthetic session. Placement of catheters for injecting contrast agents or drugs will at most cause only mild bruising. All mice will be humanely killed at the end of experiments, and their tissues taken for histological analysis.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is necessary to use animals for this project because HD is primarily a disease of the brain. HD affects complex behaviours such as movement, emotion and learning and memory. There are no computer, cell culture or invertebrate models that can mimic emotional or cognitive disturbance. Rodents are the lowest vertebrates in which cognitive and emotional correlates of human behaviour can be measured..
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use the smallest number of animals needed for good statistics. This is usually 8-12 animals per group. Power calculations are performed whenever we start a new procedure to ensure that the minimum number of animals is used that will give us a statistically relevant result. The majority of our behavioural tests are designed so that the same animals can be tested repeatedly as they age. This enables us to accurately monitor how the disease progresses, and also reduces the numbers of animals necessary overall. Tissues will be taken from all animals for histology, to avoid the need for additional groups. Wherever possible, we will make our data available to other experimenters (as we have done in the past with our MRI brain data), eliminating the need for them to obtain their own data from additional mice. We aim not to breed an animal unless it can be used in an experiment that will generate valuable data.
3. 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.	In terms of sentience, mice are the 'lowest' species in which complex learning and emotional behaviours can be studied. The animals that we will use have a gene that causes them to develop either the clinical signs seen in human HD, or the pathology, or both. The mice appear to be very abnormal, because their motor deficits (muscle weakness and loss of coordination) cause them to move in a slow and shaky manner. However, these mice are not in pain, and they are responsive to food, rewards, and novel objects, even at the end of their lives. They remain alert, motivated and curious and are able to perform learning tasks if their physical strength allows it. Animals are usually bred in our laboratory. Strenuous efforts are made to ensure that the animals are kept in the best possible conditions. Most live in enriched cage environments, and special attention is paid to the consequences their disabilities may have on their daily lives.

	The major adverse effect is the consequence of the disease itself. For example, the muscular strength of HD mice deteriorates over time, as is seen in HD patients. They become less active, and develop a characteristic hunching, as is seen in HD patients. We pay particular attention to ensuring that the impact their disabilities have on their life is minimised. We provide easily accessible food and lowered water spouts to mice with movement problems. Adverse effects of our experiments vary depending on the kind of test we do. Most of our work is non-invasive behavioural testing. Stress is minimised, and in fact we have shown that a moderate level of behavioural testing is beneficial for the animals.

<table>
<tr><td>Project 47</td><td colspan="2">Signalling and Neurodegeneration</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Parkinson’s disease, Alzheimer’s disease, disease mechanism, finding new targets for treatment</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="2"><table>
<tr><td>Basic research</td><td>x</td></tr>
<tr><td>Translational and applied research</td><td>x</td></tr>
<tr><td>Regulatory use and routine production</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td></tr>
<tr><td>Preservation of species</td><td></td></tr>
<tr><td>Higher education or training</td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">The motor symptoms of Parkinson’s disease and dementia in Alzheimer’s disease result from progressive neuronal death in multiple brain areas. While some symptoms can be treated for a time for example in Parkinson’s disease with dopamine replacement therapies, there is currently no known cure for any of these neurodegenerative diseases. We found that genetic changes in the above diseases cause changes in the way cells signal in cellular pathways. Our project aims to understand the role of genetic changes in cell signalling pathways using modern molecular, biochemistry and cell culture techniques and animal models that have alterations in cell signalling pathways or Parkinson’s disease or Alzheimer’s disease genes. Our aims are:

i) Define the importance of genetic alterations in the</td></tr>
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	above diseases in cell signalling using mutants in cell culture models. ii) Define the importance of genetic alterations in the above diseases in cell signalling using brain tissue form genetically altered mice. iii) Examine cell signalling in live genetically altered mice using modern imaging techniques. This project will evaluate changes in gene and cell signalling expression in different brain areas in mice. None of the animals involved in the study has any gross abnormalities. The animals will be euthanized in a humane way. Their tissue will be used for staining, to determine levels of protein expression in different brain areas, interactions between proteins, the generation of nerve cell cultures and live imaging. We conducted and will conduct as many experiments as possible in cell cultures not requiring the euthanasia of any animals. Nonetheless we believe that the interplay between proteins in cell signalling pathways is influenced by their natural environment and it is important to investigate proteins in this context. Therefore we will euthanize as few animals as possible but enough to ensure solid statistical analysis to test our hypothesis developed previously using in vitro assays.
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 reveal new insights into how defective genes cause neurodegenerative diseases and has the potential to uncover important new roles of these genes in cell signalling with importance for neurodegeneration. Our goal is to understand how defects in genes cause neurodegeneration, providing key information that underpins the development of new drug treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to a maximum of 10000 mice in five years for the maintenance of colonies and gaining experimental results.
In the context of what you propose to do to the animals, what are the	The animals are not expected to show any adverse effects beyond moderate discomfort including pain from needles used for injection and recovery from

expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	anaesthesia. In the event of animals developing any problems including difficulties with moving around, feeding, weight loss or social interactions they will be culled humanely. All animals will be culled humanely at the end of the experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Experiments will whenever possible be conducted in cell lines to inform experiments investigating the role of cell signalling in animal models. Nonetheless, a key question is what happens when signalling is changed in living beings? Cell lines cannot address changes in a complex context such as the mammalian brain. For example connections from different brain areas and interaction with immune cells do not take place. In addition, changes occurring during development or in different brain regions over time cannot be addressed. As Parkinson's disease and Alzheimer's disease are late on-set slow progressive disorder changes need to be observed over longer period of time, months rather than days or weeks as possible in cell culture.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have taken precautions to reduce the number of animals required to the minimum required for reproducibility and to obtain quantitative data from our analyses. Our initial experiments obtained on tissue suggests that a number of approximately five animals per group is sufficient to show significant differences in cell signalling in comparison to control animals with $p < 0.05$. Unnecessary production or import of genetically altered animals will be avoided. The strain used for generating a new colony will be carefully considered to avoid producing unwanted mice. Animals will only be bred if a user requirement has been established, and the breeding programme will be subject to regular review to optimally meet anticipated demand. Spare animals will be made available for use on other scientific projects. Breeding will be optimised, wherever possible, to produce only the genotype required.

3. 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.	Mice are universally used for work involving genetic alterations and have provided ground-breaking results regarding the causes and possible new treatments for neurodegenerative diseases. In our experiments we use animals that have similar genetic defects to patients with familial forms of Parkinson's disease and Alzheimer's disease to have a better model for the human conditions than would be provided by unmodified mice. Published guidelines for best practice will be followed. Animals will be monitored for food intake, coat grooming behaviour, reduced movement and social interaction. Their weight will also be monitored on a daily basis if any concern for their wellbeing arises. If the animals lose weight rapidly, food supplements will be provided and if appropriate the animals will be encouraged to engage with their environment. If these measures do not succeed the animals will be sacrificed before their welfare is compromised.

Project 48	**Information encoding for memory storage and retrieval in mammalian brain circuits**	
Key Words (max. 5 words)	Brain, memory, perception, Alzheimer's Disease	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals.
Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objectives of this project are (i) to understand how information is stored in memory, consolidated and recalled by brain circuits; (ii) to understand how this is disturbed in neurodegenerative disease; and (iii), to use this knowledge to develop new therapies for neurodegeneration and dementia.	
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)?	Understanding how memory works is one of the grand challenges faced by neuroscience; in order to do this, it is vital that we understand how information is represented and processed by brain circuits for memory storage. By specifically examining how this is affected in neurodegenerative disease, we will potentially be able to advance the state of medical intervention for dementia.	
What types and approximate numbers of animals do you expect to use and over what period of time?	We will use mostly mice (up to 5000) and some rats (up to 500) over the 5 year course of the licence.	

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?	We do not expect the vast majority of our animals to undergo anything beyond moderate suffering. Animals will usually undergo a surgical procedure (always under general anaesthesia) and will be closely monitored and provided with pain relief before and after surgery. Animals will have restricted access to water, and may experience thirst. Thereafter, animals will learn to perform simple tasks for reward, that are mentally stimulating. Rarely, in less than 5% of cases, complications can occur including weight loss and failure to recover from anaesthesia. However, animals will be constantly and closely supervised by trained individuals. If any animal is deemed to be suffering, they will be humanely killed.
Application of the 3Rs	
Replacement State why you need to use animals and why you cannot use non-protected animal alternatives	The project involves investigation of the neural mechanisms underlying memory, which requires recording signals from real brains. We do make use of computer modelling, however this requires animal experiments to constrain parameters and validate predictions. We make use of an *in vitro* brain slice preparation to replace *in vivo* work wherever possible.
Reduction Explain how you will ensure the use of minimum numbers of animals	We use computer modelling to analyse our data and make precise predictions. We use principles of experimental design and analysis to get meaningful data from minimal numbers of animals. We minimise animal numbers by recording the activity of large numbers of brain cells at the same time, thus reducing the overall number of animals required.

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.	Mice are a very good animal model, because their brains are similar to human brains, and because we can use genetic technologies to mimic human diseases and probe circuit mechanisms. We at all times strive to minimise harm to animals, performing experiments under terminal anaesthesia except where it is necessary to simultaneously measure brain activity and behaviour. Animals are constantly and closely supervised by trained individuals and advice sought from the veterinary team if there is any cause for concern. If any animal is deemed to be suffering beyond the limits specific in the license, it will be humanely killed. Overall, we use anaesthesia, analgesia, and humane endpoints to limit suffering.

Project 49	**Pathogenesis and treatment of Huntingdon's Diesease**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Yes	Basic research
	Yes	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to (1) understand the mechanism that cause Huntington's disease (HD) and (2) to use mouse models to assess new treatments for HD in order to advise which ones should be tested in clinical trials.	
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)?	HD is a devastating disease of the nervous system. On average, symptoms start at around 40 years of age, but this can vary widely from early childhood to very old age. Affected individuals develop psychiatric illnesses, an inability to control their movements, difficulty in reasoning and solving problems and lose weight. In the latter stages, HD patients are often bedridden and unable to talk or swallow (being fed via a feeding tube). The disease progresses for 15-20 years until death. Children of affected individuals have a 50% chance of inheriting the faulty gene and have often witnessed the disease in relatives. There are no treatments that will delay or slow down the course of the disease and most of the symptoms do not have effective medicines.	

	We know that our mice are good models of HD. Over the past 20 years, they have taught us about important aspects of the disease. We have shall use HD mouse models to determine what might be the best type of therapy for treating HD and to test the effects of new drugs that might prevent the onset of symptoms or slow disease progression. We expect our studies will provide important information on the mechanisms that cause HD and on the types of treatment that should be developed for testing in clinical trials. 219
What species and approximate numbers of animals do you expect to use over what period of time?	We shall use mouse models in this project. Over the course of five years we expect to use up to 38,000 mice.
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?	Our mouse models develop symptoms related to HD and therefore exhibit changes in their gait, develop cognitive decline and after a while, fail to gain weight. The level of severity associated with the symptoms of HD is moderate. We are interested in understanding the first changes in the brain that cause HD and therefore usually study the mice before symptoms start or when symptoms are mild. On occasions, when the later symptoms are studied, mice are culled before these symptoms could lead to suffering. Many of the animals in this project will be used for breeding. Genetically altered mice (which do not themselves exhibit symptoms), will be bred to HD mice to see if changes in the altered gene slow down the onset and I or the progression of the disease. We shall also test potential therapies in our HD mice. All of the mice used in this project will be culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In the development of treatments for HD, we need to study the earliest stages of the disease. As HD is mainly a disease of the brain, patient tissue is not available for this purpose.

	Our work is part of a large international research programme. The processes that we study often follow on from work in single cell or invertebrate HD models (flies and worms). However, there are close to 100 genes, which showed effects when manipulated in these simple systems and have been proposed as therapeutic targets for HD. In most cases these have turned out to be artefacts. Modelling a slowly progressive disease in these simple systems is very artificial and cannot reproduce the complexity of the mammalian brain. Clinical trials for HD are extremely expensive because in order to have a good chance of detecting a beneficial effect, several hundred patients must be studied over a period of two to three years. Therefore, it is not possible to test the potential treatment suggested by work with the simple systems directly in clinical trials. We use HD mouse models to provide an important intermediary resource to test potential therapies and prioritise those that should be developed for clinical trials.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our experimental design is based upon on extensive knowledge of HD mouse models. Through our experience in planning these studies and consultation with statistical experts we use the minimum number of animals required to obtain scientifically valid and robust results. We adhere to the following guidelines: i) We are aware of the minimum number of animals required to obtain valid results in our tests. We design our studies to limit the number of experiments required. ii) We are testing approaches that could, in some cases, replace complex breeding programmes thereby reducing the number of mice required. iii) Breeding mice can produce more animals than are needed. We take tissues from these additional mice and maintain an HD model tissue bank, allowing us to provide the HD scientific community with a tissue resource obviating the need for some labs to keep HD

	mice. iv) We cryopreserve all of our mouse lines, so that we do not need to breed live mice unnecessarily. v) We have developed tests that can be performed on tissues at early stages of disease and need less mice than the tests that measure neurological symptoms.
3. 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.	Mice have been chosen because as a 'lower' mammal, they have a nervous system of comparable complexity to man to allow studies of neurodegenerative conditions. HD mouse models have been extensively compared with HD patients and are good models of the human disease. The mouse is also very versatile because of the availability of other genetically altered mice with which the HD models can be bred. We have developed tests that can be performed on mouse tissues to inform us as to whether the disease has started, and if so, at what stage it has reached. These tests can be used before the neurological symptoms can be seen and need less mice than measurements of in gait or cognition. We now use these tests as the first assessment of whether a treatment, or breeding to another genetically altered mouse line, has had a beneficial effect. If we do need to keep a mouse once it has developed neurological symptoms, we apply a set of criteria to ensure that suffering is kept to a minimum.

Project 50	**Investigating and modulating cortical network activity**
Key Words	Dementia, Epilepsy, Neurons, Brain Circuits
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;

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

The work outlined in this project aims to assess network activity in four main diseases (schizophrenia, dementia, drug addiction and epilepsy) in which abnormalities in circuits of neurons (brain cells) in the brain may be a key feature of the disease. Interestingly, these diverse conditions are linked by changes in the excitatory/inhibitory balance in the brains chemicals. There is evidence in all these conditions that neurons may have excess activity or hyperexcitability. The evidence also suggests that an impaired function of a specific type of brain cell (interneuron) may also be a common feature in these conditions. This project aims to understand how these interneurons control the normal level of activity in the brain and what may have gone wrong in different disease states.

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)?

Understanding how these different patterns of network activity in the brain are generated, i.e. which neurons (brain cells) are involved in what patterns of activity is essential information. Only by understanding the basic mechanisms that generate this activity, can new drugs or therapeutic approaches be developed that can intervene when the normal network fails to function, as occurs in many brain diseases. Data generated will be published to which will be beneficial to clinicians as well as the scientific community.

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

Rats (~2000) and mice ~4000 - including breeding of transgenic lines over 5 years.

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?

All protocols used in this project are classified as non-recovery, mild or moderate. Some animals may have epileptic seizures that can be severe so all animals will be very carefully monitored and any animal that is having severe seizures will be humanely killed. Some animals with have a chronic treatment to model a disease e.g. cocaine to model the changes in the brain related to human drug abuse but this causes only changes in cognitive function and no reported distress to the animals. All other procedures including testing cognition, or drug administration have been widely used and are not associated with any adverse effects. All animals will be inspected regularly and any abnormal signs or symptoms will be discussed with the veterinarian. Animals will also have chronic electrodes implanted into the brain that enable us to monitor activity while the animal moves freely during both the sleep and wake state. These electrodes can remain in place for months without any adverse effects on the animal's health.

Application of the 3Rs

Replacement

Why animals? Brain network activity is generated by large populations of brain cells and can therefore only be studied in living brain tissue. While EEG recordings can be made from the scalp in human studies it is only possible with animals to record from specific brain structures and from individual brain cells.

Are there alternatives? Data from these experimental studies will be used in computer modelling studies where additional ideas and hypothesis can be tested without the use of animals. Although it is possible in some circumstances to obtain epileptic tissue from patients undergoing surgery for intractable epilepsy this tissue is from patients with many different types of epilepsy, who may have had many years of medication, and the tissue is taken from different brain areas. While this tissue from patients is very important to study it cannot be used to inform us about the causes of epilepsy or be used to assess new interventions.

Reduction

The number of animals used in these studies can be minimised in a number of different ways. Animals will be used in several procedures. For example, an animal may undergo behavioural assessment before and after a drug treatment and then be

used to generate brain slices. The use of multi-channel recording systems also increases the yield of neurons obtained from each animal. In addition, following recordings tissue can be subsequently fixed at the end of the experiment and used in the morphological studies.

Post mortem slice work will be used in preference to *in vivo* experiments where possible, to minimise the potential for animal suffering.

The numbers of animals chosen for each experimental protocol are based on many years of experience of similar experiments in which we know the numbers required to reach meaningful statistical conclusions for the different experimental assessments planned in this proposal.

Refinement

Choice of species: Rats and mice will be used as these animals have been widely studied and much information concerning neural anatomy and physiology has already been established. Both species generate cortical brain activity that is similar to that observed in humans. Established protocols have been widely reported in the literature, and used for several years, to model cocaine addiction, schizophrenia and epilepsy in rodents and genetic models also exist for Lewy body disease and schizophrenia. The models of cocaine addiction and schizophrenia cause cognitive changes in the animal but do not cause any other long-term effects. The mouse model of Lewy body dementia does develop motor dysfunction in old age but animals in this project are used prior to the onset of any movement abnormalities.

Minimising suffering: The different animal models chosen are based on a considerable literature in which similar models have been used and many basic properties of the models are well established. When animals undergo several procedures, this will only be done where the benefit of acquiring improved data by continuing to use the same animals outweighs any additional cumulative welfare cost due to use in multiple procedures. Where possible, non-recovery protocols will be used to minimise the number of conscious animals undergoing procedures.

Severity: All protocols used in this project are classified as non-recovery or moderate.

Project 51	**The function of the neural circuitry of the olfactory bulb**
Key Words	Olfactory bulb, smell, neural circuit
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:

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

The objective of this project is to understand how the circuitry of the olfactory bulb functions, this is still an outstanding question. The insights gained from this project will have relevance to understanding the brain in general.

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)?

The task faced by the olfactory system is one of pattern recognition; the olfactory system has to distinguish between and recognise a multitude of different activity patterns generated by odours binding to subsets of olfactory receptors. Pattern recognition is an increasingly relevant field of artificial intelligence so learning how biology has solved this problem will have general interest. Furthermore, the olfactory system may be a useful model for studying neurodegenerative disease to which it seems particularly susceptible, with olfactory deficits appearing long before any other symptoms in both mice and humans.

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

Genetically modified mice ~250 mice will be used. Around 1000-2000 mice will need to be bred to achieve sufficient numbers with the correct genotype.

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?

The expected level of severity is non-recovery, as all experimental procedures will be carried out under terminal anaesthesia. To sustain the transgenic line of mice a few

mice <10 may need to undergo vasectomies that is deemed moderately severe, postoperative analgesia will be supplied where necessary. All animals not required for experiments will be culled humanely with a schedule 1 method

Application of the 3Rs

Replacement

There are no adequate models or alternative means to understand how the brain works other than by studying it. How the brain processes information is largely due to how neurons are wired together, this wiring cannot be achieved in neuronal cultures.

Reduction

Our aim is to reduce the number of animal experiments whenever possible. Computer models to describe the function of the olfactory bulb will be constructed from the data collected from animals; this will then limit further use of animals to refining the details of the model. Furthermore, I will reduce the variation in measurements by gaining precise control of stimuli using mice that express an optically activated switch in defined neurons. This allows precise control of stimuli reducing the variance in measurements, which in turn will reduce the number of required mice.

Refinement

These studies aim to characterise the properties of the neural circuitry of the olfactory bulb with the hope of a better understanding of olfaction in humans. The organisation of the mammalian olfactory bulb is distinctly different to invertebrates or even other vertebrates. In humans neurodegenerative disease impacts on olfactory function at a very early stage, this is mirrored in mouse models of neurodegenerative diseases such as Alzheimer’s. Therefore, mice are an excellent model to study the function of the olfactory bulb and will lay the groundwork for future studies of dysfunction in the olfactory system at the early stages of neurodegenerative disease.

The genetic amenability of mice also means that optogenetic tools are available to study circuit function. This project relies on one such line of mice, which express an optical switch in all olfactory sensory neurons.

Project 52	**Information processing in neural networks**
Key Words	Brain, Neural Code, Multisensory, Perception, Memory
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;

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

Although we know approximately how sensory input is routed through different brain areas, each extracting distinct aspects of the percept, the neuronal “language” used to represent and store sensory experiences remains elusive.

We wish to study how activity in large networks of interconnected nerve cells represents sensory stimuli, particularly touch. We also wish to find out how these neural activity patterns are relayed between different brain areas to combine sensory input from different modalities, such as touch and vision, into a unified percept of an object. Finally, we also wish to study how these neural activity patterns change during learning and allow us bringing back to mind perceptions of previous experiences.

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)?

Improving our understanding of the neural basis for sensory perception is in the first instance a matter of considerable, fundamental scientific interest. In the longer term, the insights gained from this project should allow us to identify new therapeutic targets for mental illness that affects sensory processing (e.g. schizophrenia or ADHD) and storage of sensory input into memory (e.g. Alzheimer’s disease and dementia).

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

Over the five-year period of the project, we will expect to use 3100 mice in procedures other than simple breeding and maintenance. We may breed and/or maintain up to 5000 mice, some of which will be the same ones as used in the other procedures of the project.

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?

Part of the project will involve the generation of genetically altered mice to allow us to investigate the functions of particular molecules and cell-types in sensory processing. These animals are expected to be not fundamentally different in the way they behave from wild-type controls and thus expected levels of severity will be mild. In some animals, we will need to trim some of the whiskers of the animal to be able to relate neural signals to touch sensation in specific whiskers. This has no effect on animal well-being. In some animals it will be necessary to inject substances into the brain to deduce anatomical structures and function. This will be carried out under general anaesthesia, in aseptic conditions, with most animals being humanely killed before regaining consciousness and only some animals being recovered with appropriate post-operative care and only causing moderate amounts of discomfort to the animals in the study. The behavioural tasks we will use to record conscious, sensory perceptions are painless. In some cases, it will be necessary to motivate the animals to perform these tasks by rationing their food or water during testing. This may result in a temporary weight loss, but this will always be monitored carefully and extra food or water provided if this occurs. The availability of modern techniques for monitoring or altering neural activity in particular regions of the brain make it possible to carry out almost all of this work in a manner that should cause only moderate amounts of discomfort to the animals in the study. For example, surgical operations for implantation of ultrafine microelectrodes or for inserting genes into the brain will be carried out under general anaesthesia, in aseptic conditions, and with appropriate post-operative care. The adverse effects that may occur following surgery include transient pain and bleeding, but their incidence is likely to be very low. Chronic implants for recording neural activity or for delivering flashes of light for the purpose of altering that activity are small and light-weight, and do not materially affect the animal’s quality of life. A relatively small percentage of animals (about 12%) will be used in tests where the head needs to be fixed to enable stable recordings of brain activity. In these tests, the animal is supported on a moveable platform that allows the animal to perform behavioural tasks, for example, navigating through a virtual maze projected onto screens. This method is now very established and well tolerated by mice displaying the same behaviour as when walking freely and should cause only moderate amounts of discomfort to the animals in the study. Animals will be killed humanely at the end of the experiment.

Application of the 3Rs

Replacement

Our project investigates the neural basis for sensory perception. Currently, this can only be studied by using the brains of animals or humans, as our understanding of brain function is too rudimentary to generate realistic mathematical models for

testing. Brain imaging measures in humans lack the sensitivity to observe changes in the properties of individual, identified brain cells in response to sensory stimuli. Moreover, a key aim of this project is to try manipulate brain activity with single-cell resolution using non-invasive optical stimulation, which is not available in humans. Additionally, we aim to relate brain cell activity to the underlying neural circuitry at a microscopic level. This requires the use of post-mortem histological measurements, which would not be ethical or practical to carry out in humans.

Reduction

Calculations are carried out to determine the necessary number of animals for each experiment, ensuring significance of our results but also minimising the number of animals used. We are additionally able to keep animal numbers to a minimum by using cutting edge methods that yield large amounts of data and experimental designs that allow multiple measurements to be made from each animal.

Refinement

Mice will be used because they are the lowest vertebrates with a sensory system that is comparable to that in humans.

Animal welfare costs will be minimised by carrying our procedures in state-of-the-art facilities and using best practice methods. Breeding and colony maintenance, including genetically altered mice, will follow the Home Office assessment framework for efficient breeding and maintenance. We will only use genetically altered mice that exhibit a mild phenotype (e.g. with no effects on feeding or welfare) or no measurable behavioural phenotype (e.g. mice producing a fluorescent marker in certain brain cells). Surgical operations are carried out very carefully under anaesthesia and aseptic conditions, and the animals are given painkillers and will be closely monitored until they have fully recovered.

Sometimes it will be necessary to regulate the food or water intake in mice in order to motivate them to perform behavioural tasks for a food or water reward. We have very strict guidelines in place to mitigate any harm from this food or water regulation, as well as for the behavioural tasks used.

The use of state-of-the-art methods, such as optogenetics and recording/manipulation of brain activity in behaving animals aimed at reducing the impact on animal welfare, while, at the same time, increasing the amount of scientific insight that can be obtained from each experiment. The earliest endpoints consistent with the scientific aims are applied.

The data obtained from these experiments will be used to refine computer models of the brain that will help to guide subsequent experiments and contribute to a reduction in the number of animals needed.

Project 53	**Preclinical research of novel pain therapeutics**
Key Words	Chronic pain, Analgesia, Hyperalgesia, Allodynia, Therapeutics
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	(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);

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

Our principal objective is to develop new therapeutic strategies to treat chronic pain. To achieve this we will identify nervous system mechanisms contributing to chronic pain and test novel therapeutics targeting these mechanisms. The sites and mechanisms of action of substances and medical devices known to provide pain relief in humans, whose mechanism of action is currently unknown, will also be identified and this knowledge used to develop new, improved therapeutic approaches to treat chronic pain.

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)?

Our studies will provide new insights into the treatment of pain and will provide further stimulus in the scientific community opening new lines of investigation for the development of new and improved analgesic therapies. The availability of new classes of compounds, targeted towards novel mechanisms involved in the generation and maintenance of pain will provide relief from suffering and a better quality of life for millions of sufferers worldwide.

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

In total, we expect to use approximately 6000 adult Rats, 2500 adult Mice and 250 adult Guinea-Pigs over a period of 5 years

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?

Under a typical experimental setup, for example, nerve ligation followed by behavioural testing, animals will only experience mild-moderate discomfort, associated with exposure to the pain models. This discomfort is typically transient in nature, with animals returning to baseline behavioural status on return to their home cages, as indicated by return of social interactions and normal physiological function. This suggests that the majority of animals (98%) will not experience any adverse effects that significantly impact on quality of life. A minority (no more than 1%) may show moderate discomfort, for example a slight limp or lack of use of affected limb for a few days following surgery. Should more severe effects be observed, and at the end of the experiment, animals will be immediately killed using approved procedures.

Application of the 3Rs

Replacement

Pain is complex, arising from integration within a fully functioning nervous system that can only be investigated in models with intact circuitry comparable to that involved in human pain perception. Thus no suitable alternatives exist to address underlying mechanisms contributing to pathophysiology.

Once molecular targets/pathways are identified, detailed mechanistic studies will use *in vitro* systems.

Data generated is subject to mathematical/systems biology approaches to develop predictive, computer-based models of gene and neural networks for future replacement and refinement of experiments.

Wherever possible *in vitro* techniques will be employed including: cultured and/or dissociated neurons, isolated brain and spinal cord slice preparations.

Ex-vivo tissues from animal models will also be utilised wherever possible.

Wherever possible we will supply tissues to other researchers and collaborators to maximise model use

Reduction

We have vast experience of performing these experiments over approximately 25 years and using this experience coupled with mathematical calculations, we have

accurately predicted the number of animals that will be required for each experiment to provide confidence in results generated. Biostatisticians are consulted as and when deemed necessary to confirm the use of minimum numbers of animals.

Animal number is also minimised by ensuring tissue is analysed in multiple ways (e.g. tissue used in one specific experiment will later be used for other studies)

Refinement

All models utilised in these experiments are the ‘gold-standard’ models for neuropathic pain. Since no model encompasses all symptoms/elements of the disorder the use of multiple models is a requirement for a complete understanding of the biological mechanism that lead to the diseased state of the disorder.

The use of post-operative analgesia is incompatible with the procedures as the aim of the project is to investigate pain. A mini-project performed under our previous project licence performed upon recommendation of the home office inspector showed that analgesic use prior to surgery in the animals significantly altered the output of the experiments, with animals showing reduced central pain sensitisation and increased variability post-surgery. Thus, to refine and reduce usage in these models, analgesic use post-operatively is contraindicated.

Rats and mice provide the basic model and are the lowest vertebrate groups on which well characterised, minimal severity models of pain have been developed and characterised anatomically, neurophysiologically and behaviourally. Guinea pigs have certain attributes more comparable to man where the involvement of these faculties is known, guinea-pig would be the first-choice model.

To minimise suffering and maximise output we will adhere to our existing (and where appropriate, develop) standard operating procedures for all surgical and behavioural protocols. In all models, animal health and welfare is carefully monitored and documented using standard criteria based on adverse effects. Wherever possible cultured tissue and donated human post-mortem tissue is used. Animal husbandry procedures are constantly reviewed and improved to maintain and enhance quality of life through-out the project.

Furthermore, regular contact with collaborators, pharmaceutical/biotechnology partners, statisticians, the establishment named veterinary surgeon and animal welfare officer will ensure refinement of existing and novel approaches.

Project 54	**SUMOylation accelerates supply-rate depression**
Key Words	Epilepsy, SUMOylation, Synapsins, Levetiracetam
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;

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

In the brains of healthy people, intense electrical activity doesn't lead to seizures, indicating the presence of an inborn protective anti-epileptic mechanism. Therefore, a fundamental, but understudied question is why more people don't suffer from epilepsy, or put another way, what keeps most people epilepsy free? This is important because finding ways to switch on or enhance this innate protective pathway could provide powerful tools for reducing or preventing seizures in people with epilepsy. Supply-rate depression (SRD) has recently been identified as one such potential defensive mechanism. The new data suggest that SRD is regulated by a process called SUMOylation, which alters the functions of specific proteins at synapses, the points of communication between nerve cells. Our hypothesis is that manipulating the SUMOylation of these proteins could protect against seizure activity by reducing the aberrant transmission of electrical signals between neurones, while leaving the transmission of normal signals unaffected. The project aims toward understanding the molecular mechanisms underlying these processes and translating these discoveries as a focus for the design of new targeted therapies for epilepsy.

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)?

Many people with epilepsy do not respond well to therapy with the currently available antiepileptics. This is because current medicines target processes and structures that normally occur in nerve cells. Thus, they inevitably disturb normal function, creating significant adverse effects. Developing new drugs that target only those

processes that occur in the brain during the seizures (such as SRD) would keep the normal functions intact, while having a beneficial therapeutic effect. It has been shown that modification of proteins involved in communication between brain cells targets one such epilepsy-induced process - SRD. Understanding how SRD may be modulated to be initiated earlier will help to find the way to prevent the seizures from developing. This will further help with devising a new strategy in fight against epilepsy and its devastating consequences.

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

Mouse, 1600.

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?

The animals specifically designed for this project will be genetically modified, but this should cause no adverse effect by itself. The commercially available animals (synapsin I and synapsin II knockouts) generally exhibit spontaneous seizures, but will be used for experiments before the age at which this starts occurring. Any animal experiencing an unexpected adverse effect will be treated as advised by the NVS or will be killed humanely.

Application of the 3Rs

Replacement

Currently, there is no satisfactory alternative model for investigation of the mechanisms of synaptic changes in epilepsy models that does not require the use of brain tissue acutely removed from animals.

The project is intended to result in development of the new transgenic mouse strains. We will make extensive use of the transgenic mouse strains engineered to evaluate the role of SUMOylation on neurotransmitter release. Therefore, this requires maintaining viable breeding colonies.

Reduction

Using the preliminary data, we have used validated statistical procedures to calculate the minimal number of animals necessary to produce meaningful data, without compromising the scientific validity of the study. In addition, I will share the tissues with other groups to ensure that neuronal and non-neuronal tissue from the animals is used to the fullest extent possible.

Refinement

We chose mice as the species widely used in transgenic animal design, while also simultaneously validated as the species of choice by current scientific literature.

Further, there is a wealth of correlative studies between mouse and human which indicate that the results gained by the animal use are translatable.

All of the procedures I propose: a) are validated in current scientific literature b) will be performed according to the relevant legislature and c) will be performed by trained staff.

Mice will be monitored on a daily basis and for any animal that shows signs of adverse or unexpected responses, depending on the severity, either the advice will be sought from the local NACWO and/or NVS or the mouse will be culled immediately to limit any additional discomfort.

Project 55	**Mechanisms of brain plasticity in health and disease**
Key Words	neuron, plasticity, learning, memory
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;

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

My aims are twofold. Firstly, I want to understand the mechanisms underlying experience-dependent plasticity, learning and memory in the brain. Secondly, I seek to use this knowledge to obtain a deeper understanding of the mechanisms underlying neurological and psychiatric diseases with a view to developing new or improved therapies.

The objectives of this project are to:

1. determine which cellular plasticity mechanisms are used during brain plasticity induced by experience, learning and memory.

2. establish whether the mechanisms underlying brain plasticity change with aging.

3. address whether brain plasticity may be accelerated or retarded by interventions, e.g. pharmacologically, or with dietary restriction.

4. study how disruption of plasticity mechanisms or abnormal plasticity contributes to impaired brain function or behaviour in animal models of neurological or psychiatric disease.

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)?

The benefits of the work described in this project licence application are increased understanding of how plasticity occurs in the normal brain and how plasticity may be harnessed to treat brain diseases. We will develop a clearer idea of why learning becomes harder in the aging brain. Knowledge of the neural basis for learning and memory in the healthy and aging brain will help us to understand how learning and memory can fail, for instance in dementia. If we understand better how memory fails and cognition is disrupted, then we will be in a stronger position to develop new treatments to prevent them from occurring.

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

I anticipate that we will use approximately 4500 rats and mice in total. The precise breakdown of rats and mice varies with the balance of experiments performed.

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?

We are interested in how animals learn and form memories. This requires that our animals are healthy and are free or pain, distress and suffering throughout experiments. The majority of procedures that we perform are non-invasive and may simply involve behavioural testing of the animals. However, we may administer substances to animals to modify learning by routes that include: orally, under the skin, into muscle or into the tummy. A subset of animals may have molecules that affect learning and memory injected into their brain. Alternatively, non-replicating virus particles may be injected to cause brain cells to make the molecules. These animals will be managed carefully to minimize adverse effects, for instance, by giving analgesia to prevent pain. In the unlikely event that an animal shows signs of suffering, we will kill the animal humanely and notify the Named Veterinary Surgeon. All animals will be killed humanely at the end of experiments because we want to study the brain tissue in the laboratory. This analysis will be performed in concert with neuroimaging and behavioural data.

Application of the 3Rs

Replacement

The goal of my experiments is to understand the cellular mechanisms that enable the brain to adapt to experience, to learn and to form memories. These are all features of an intact organism. My experiments require that there is a sensory input

to the brain and that this sensory input can be modified. The presence of learning and memory is assayed by a change in behaviour. This means that experiments have to be performed using animals. Hence, my experiments can not be carried out on reduced preparations such as cell cultures. Similarly, I can not obtain the data that I need from computer modelling.

Reduction

Experiments are designed, whenever possible, to have an internal control. In the behavioural experiments, animals are tested on more than one behavioural task to reduce the total number of animals studied. We aim to share brain tissue between researchers when possible. Our data are analysed with advanced statistical techniques to obtain the maximum amount of information.

I have used computer modelling to make predictions about the cellular effects of plasticity. This means that our experiments can be targeted more directly, which facilitates experimental design and reduces the number of animals required.

Refinement

The rodent somatosensory system is extremely well characterized and has been extensively used to study cortical plasticity. The sensory input from the rodents' whiskers can be modified by innocuous whisker trimming protocols. Importantly, rodents exhibit learning and memory on whisker-based behavioural tasks. Furthermore, the rodent somatosensory system is a good model for comparison with human studies because sensory testing indicates that rats' whiskers are as sensitive as human hands. We aim to use positive reinforcement for during training on behavioural tasks rather than water restriction. If positive reinforcement fails and water restriction is necessary, we will use the shortest period of restriction possible that results in learning.

Project 56	**Neurogliaform cells in health and disease**
Key Words	interneuron, neocortex, neural circuits, neurogliaform cells, synaptic neurotransmission
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;

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

Nerve cells in the brains form complex networks, mainly consisting of excitatory and inhibitory neurons. It is essential to gain a detailed characterization of the participating neuronal cell types and their connections to understand the principles of information processing in the brain. One class of GABAergic inhibitory interneurons is called neurogliaform cells and our research unravels the importance of this peculiar type of neuron in health and disease by using transgenic mouse/rat lines in combination with sophisticated methods such as electrophysiology and optogenetics.

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)?

Neurons in the brain could broadly be subdivided in two major groups: excitatory principal neurons and inhibitory GABAergic interneurons. Importantly, many disorders such as epilepsy and Alzheimer’s disease are characterized by a dysbalance between excitation and inhibition. Understanding of how excitatory and inhibitory neurons form synaptic connections between each other is therefore vital for our understanding of how the brain works and of how neural circuits are altered in disease. Our project will significantly contribute to advancing our knowledge in this area of neuroscience research, potentially benefiting patients in the distant future.

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

For this project we will be using 500 rats and 2000 mice.

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?

The majority of the animals are genetically modified and will experience sub-threshold to mild discomfort. All animals will be humanely killed at the end of the procedure for further investigation.

Application of the 3Rs

Replacement

There are no feasible alternatives that would entirely replace the use of a living animal in order to conduct neural circuit research assessing how neurons communicate with each other. Neurons in a dish form more or less random connections. These connections are neither related to behaviour nor sensory processing.

Reduction

The proposed experiments will be carefully designed using rigorous statistical approaches. Advices from a statistician will be sought whenever necessary. In addition, using advanced technologies, the number of animals to be used will be minimised by increasing data quality and quantity

Refinement

Rats and mice were chosen because the existing literature describes relevant methods and techniques in rats and because of the availability of genetic alterations in mice. We will minimise welfare costs to the animals by the use of experienced personnel and proven techniques and by use of aseptic technique

Project 57	**Mechanisms controlling vertebrate development.**
Key Words	Neuronal Development, Axon guidance, Vision
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;

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

This work will provide information on the mechanisms that direct the development of the nervous system, particularly the formation of functional connections between nerve cells. Neuronal connections are established during embryonic and early postnatal development by the growth of neuronal processes along highly reproducible pathways to link up with appropriate target cells. Failure of these connections to form normally can result in neurological deficits and have been implicated in the development of relatively common psychiatric disorders such as schizophrenia. However, our knowledge of the mechanisms controlling the formation of normal brain wiring patterns is only rudimentary and further studies are required. Many molecules that direct the outgrowth of neuronal processes are also essential for the development of other organ systems, such as limbs and the vasculature, and this will also be investigated.

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 advance our understanding of the mechanisms underlying the formation of functional brain wiring patterns and help us understand why, in some instances, brain development goes wrong. This knowledge will also be important for formulating strategies aimed at inducing functional regeneration of damaged connections in the adult nervous system following injury or disease. Understanding the process that regulate normal embryonic development also is essential if the causes of birth defects, many degenerative disorders and cancers are to be determined.

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

The planned programme of work involves the use of mice that have been genetically modified to lack specific molecules. Breeding strategies will be designed to minimise the number of animals needed to maintain the colonies whilst producing sufficient numbers for analysis. Based on the numbers required to enable biological replicates of phenotypic analyses, power calculations, the breeding strategy used to prevent birth of homozygotes that display neonatal lethality and the need to replace culled pregnant females, we estimate we will need to breed around 1000 mice (genetically modified and wild type littermates) over 5 years.

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?

The only procedure that will be performed on conscious animals is breeding and maintenance of genetically modified animals. The genetically modified mice will be of mild severity and are expected to behave and breed normally. In cases where the genetic mutation is deleterious, we will use breeding strategies that minimise the birth of affected animals. Breeding strategies also will be designed to minimise the number of animals needed to maintain the colonies whilst producing sufficient numbers for analysis. All analyses will be performed using fixed tissue obtained from animals culled using an approved method or using tissue culture approaches.

Application of the 3Rs

Replacement

Whilst expression analyses and tissue culture approaches provide an invaluable means for identifying potential molecules of interest, these approaches alone cannot reveal the precise function and requirement of specific signalling pathways within the complex environment of the intact embryo. Computer modelling also cannot be used to address this issue due to the current lack of understanding of the multiple complex interactions involved. The use of animals in these studies therefore is unavoidable.

Reduction

Where possible, we obtain fixed tissue from researchers maintaining already strains of the relevant lines of mice in order to reduce the numbers of animals used and need for transfer of live animals between institutions. Breeding will be kept at the minimum required to generate sufficient animals of the appropriate genotype and to allow repeats of each experiment for statistical comparison.

Refinement

The use of mice in these studies provides a number of advantages. Firstly, the planned programme of work builds on a significant body of previous work on the

mechanisms directing neuronal development in mice. Secondly, mouse models with precisely engineered mutations in many molecules of interest are readily available. Thirdly, since the ultimate goal is to generate information relevant to humans, the use of a mammalian model is beneficial. Live animals are only used for breeding which is unlikely to cause any pain or suffering. Breeding strategies are used that minimise the birth of lethal mutants and postnatal animals from lethal mutant strains will only be used when scientifically justified and no alternative approach is possible. Animals are cared for by competent and highly experienced animal care staff.

Project 58	**Organisation and function in cerebellar and pre-cerebellar circuits**
Key Words	Cerebellum, movement, learning, Neurophysiology, motor control
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;

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

These experiments ask how our ability to make accurate coordinated movements is supported by the cerebellum, a major structure within the brain. Damage to the cerebellum leaves movement uncoordinated and clumsy.

Frequently patients with damage in the cerebellum are viewed by the general public as if they were intoxicated, they move inaccurately, jerkily and generally lack precise control. One of the ways in which the cerebellum clearly contributes to motor skills is by learning through experience, and this is a central theme in research work on how the cerebellum works.

We know a great deal about the structure of the cerebellum which has been studied for more than 100 years. Unlike most other parts of the brain it has a high degree of organisation and a small number of clearly different types of nerve cell, which make it relatively simple in comparison to other parts of the brain. This organisation has led to people making comparisons between the structure of the cerebellum and the structure of electronic circuits or computers. By analogy, work in this project seeks to understand how these elements contribute to the function of the whole through experimentation.

The experiments address two issues, the first a general question about how the information available to the cerebellum is organised in its input. This comes from sensory systems that tell us about the outside world, but also from parts of the brain that generate movements. How these two sorts of information interact is a key question. The second is a question about learning: a great deal of previous experimental work suggests that the cerebellum contributes to a simple form of learning by association. In this project experiments will be done that ask whether the cerebellum supports more complicated forms of learning.

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)?

These experiments ask how our ability to make accurate coordinated movements is supported by the cerebellum, a major structure within the brain. Damage to the cerebellum leaves movement uncoordinated and clumsy. Frequently patients with damage in the cerebellum are viewed by the general public as if they were intoxicated, they move inaccurately, jerkily and generally lack precise control. One of the ways in which the cerebellum clearly contributes to motor skills is by learning through experience, and this is a central theme in research work on how the cerebellum works. We know a great deal about the structure of the cerebellum which has been studied for more than 100 years. Unlike most other parts of the brain it has a high degree of organisation and a small number of clearly different types of nerve cell, which make it relatively simple in comparison to other parts of the brain. This organisation has led to people making comparisons between the structure of the cerebellum and the structure of electronic circuits or computers. By analogy, work in this project seeks to understand how these elements contribute to the function of the whole through experimentation. The experiments address two issues, the first a general question about how the information available to the cerebellum is organised in its input. This comes from sensory systems that tell us about the outside world, but also from parts of the brain that generate movements. How these two sorts of information interact is a key question. The second is a question about learning: a great deal of previous experimental work suggests that the cerebellum contributes to a simple form of learning by association. In this project experiments will be done that ask whether the cerebellum supports more complicated forms of learning.

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

Over the life of the project (five years) I expect to use approximately 200 rats in experiments that seek to understand how information is processed in pathways that feed information into the cerebellum. I also expect over the lifetime of the project to use approximately 60 rabbits: 20 in experiments the determine the abilities of rabbits to learn complex patterns of stimuli and approximately 40 rabbits in experiments that seek to understand how the cerebellum contributes to this learning.

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 the experiments done in rats, the objective is to understand how sensory information and information from other parts of the brain are combined before being used within the cerebellum, or are combined in the cerebellum itself. Given that the cerebellum is highly conserved across different species, findings in rats are very relevant to other mammalian species. These experiments will all be done under

terminal general anaesthesia (with no recovery). The experiments involve the insertion of fine metal wire electrodes which detect the electrical signals from individual neurons of different identified types. The project asks what sorts of signals these neurons are affected by, and therefore what sort of information they carry to the cerebellum. These animals are anaesthetised at the very beginning of the experiment and are killed whilst under anaesthesia, so they never regain consciousness. The experiments done in rabbits examine how the cerebellum learns and will be done in parallel with experiments in humans that are also taking place in my laboratory. In these experiments we combine sensory stimuli with an air puff to the eye, and subjects (humans and animals) express learned behaviour in very much the same way. However, we cannot intervene or record activity in the human cerebellum, which would involve invasive procedures that are not ethically acceptable. The experience of the animals is similar to the experience humans have during glaucoma tests that are part of the standard opticians eye tests most of us have regularly (at least when over 50). In some of the experiments in rabbits we will intervene to alter brain function by delivering small amounts of drugs which modify the signalling between nerve cells to a very small region of the cerebellum. Alternatively, we may put small recording electrodes into the central nervous system to record the signals that neurons generate which underlie the learning process. These animals will undergo surgery, and are expected to make a full recovery. Similar approaches are now being applied to human patients undergoing neurosurgery (e.g. Nature 442, is this the Bionic man?) and are not associated with adverse consequences or lasting harm. All animals will be humanely killed at the end of the experiments. This is to allow verification of the sites of interventions, to confirm the appropriate locations of the recording and stimulating electrodes and cannulae.

Application of the 3Rs

Replacement

While we know a lot about individual cells in the brain, the questions addressed in this project relate to how different parts of the brain communicate with each other. If these questions are going to be addressed, they can only be addressed using animals with the relevant parts of the nervous system intact.

For the recordings made in rats, I know of no alternatives to invasive experimental studies to obtain specific information addressed in this project. Much work forming the background to the project has been done in reduced systems (brain slices taken from rodent cerebellum), but as making the slices involves disruption of the input and output connections between cells, this type of study cannot address the questions asked here about connections between cells and the information they carry.

In the case of the learning experiments proposed in this project, these occur in parallel with and are informed by experiments in my laboratory done with humans.

The rabbit experiments will be done only when we have confirmed that the same form of learning occurs in humans. The questions we will address using animals relate to processes within the brain: we cannot interfere with or monitor those processes in the human brain for ethical reasons. Experimental studies on animals are essential if we are to know the mechanisms underlying these processes.

Reduction

We minimise the number of animals used by recording from and testing multiple neurons simultaneously, reducing the need for multiple experiments. We maximise the yield of data so as to obtain large amounts of data from individual animals. Our analysis continually monitors the statistical reliability of our findings, so that we can stop with the minimal number of experiments that give us significant findings.

Refinement

For questions about connectivity in the cerebellum, rats are the species of choice:[the cerebellar circuitry is highly conserved across mammals, so findings generally apply across different species. Similar pre-cerebellar nuclei have been described in rodents and in primates. Rats have a cerebellum that has parallel organisation to the human cerebellum yet have a cerebellum large enough to perform electrophysiology in with a high yield: the mouse brainstem and cerebellum is small and this limits both the yield of data per animal, and access to manipulate different CNS pathways. The brainstem pathways are also better understood in rats.

For the behavioural neurophysiology rabbits are an ideal species, particularly since the background information we have on eyeblink learning is primarily from this species. One refinement we apply in my experiments is that we are studying this species without restraint during the recordings ie the animals are freely moving and wear a head cap which enables recordings to be taken whilst an animal moves around unrestricted],ie the animals are freely moving and wear a head cap which enables recordings to be taken whilst an animal moves around unrestricted, which is much less stressful than the conventional approach involving complete immobilisation, which has been used previously in this form of experiment. The behavioural recordings are made under direct observation of the experimenters at all times, ensuring that we do not stress the animals.

Project 59	**Interactions Between the Developing Visual and Nervous System**
Key Words	eye development, brain development, eye disease, sensory guided behaviour
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;
Yes	(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);

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

We are investigating how, from a single fertilised egg, cells organise to form organs such as the eye, or how nerve cells connect to form the central nervous system. We would like to understand how the brain receives information from its environment, and, ultimately, how it processes this information to evoke behavioural responses.

The visual system of the fish we use is a simplified version of that found in mammals, and so can be used to understand the fundamental processes without the need to use a mammalian model. Furthermore, in the fish, the entire brain continues to grow, even in adults, and allows us to study the maintenance of stem cell niches.

A better understanding of the underlying defects leading to human congenital eye diseases as well as the role of stem cell populations in human degenerative pathological conditions is of clinical importance.

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)?

Several human congenital eye diseases (such as microphthalmia, anophthalmia and coloboma) arise from abnormal cell specification or cell behaviour at early stages of eye development. Our research will contribute knowledge of genes that are candidates for human congenital eye diseases, a better understanding of the processes that these genes control, and will attempt to model human eye diseases.

As such, our research is of relevance to and has the potential to have impact upon i) clinical research scientists, who will use our data to screen cohorts of patients for mutations; ii) pharmaceutical industry researchers who could further develop our novel disease models and transgenic animals for drug screens. In parallel, we will investigate the mechanisms underlying the specification, function and survival of stem cell in the eye and brain which will allow us insight into which processes are affected in certain developmental defects or degenerative diseases affecting the eye. Lastly, we would like to understand how the neuronal circuitry of the brain, especially circuitry involved in visual responses, is functionally related to the behaviour of an animal. To this end we are using behavioural tests that allow us to observe how small fish interact with each other and how differences in the sensory input and neuronal connectivity pattern is reflected in a difference in interactive/social behaviour. The intended research will improve our understanding of growth of neural circuitry in growing animals and the molecular mechanisms that govern stem cell behaviour.

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

We are using a small tropical fish. Over the course of 5 years, we will use about 20,000 fish for breeding. An additional 12,000 fish will be used in research experiments, of these the majority (about 10,000) will be simply observed (filmed) as they make choices e.g. to swim either towards another group of fish or avoid them.

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?

For >95% of all used fish, the maximum severity level is undetectable or, on rare occasions, mild and will consist largely of breeding or testing the swimming behaviour of very young fish (larva) and we do not expect there to be any adverse effects. In a very small number of animals (less than 150 fish a year) it is possible that there is a temporary moderate discomfort. Should we notice any undue distress behaviour, the affected animal will be immediately anaesthetised and culled using humane euthanasia methods in accordance with guidelines. All animals will ultimately be culled in the same way.

Application of the 3Rs

Replacement

To study highly complex structures such as the nervous system, where a wide variety of different developmental processes must be co-ordinated to ensure function and survival, animal model systems are currently the only feasible approach and fish are proving to be very useful for studying developmental processes in the whole animal.

Reduction

We mainly use fixed tissue from embryonic stages before the nervous system is mature and endeavour to combine different molecular and histological techniques on the same fixed specimen to gain a maximum of information. For our work using larvae, we carefully plan our experiments and use the minimum number of animals needed to obtain a result with statistical significance. Where appropriate, we will collaborate with a statistician to plan experimental design and discuss results.

Wherever possible, we will use custom-made software for efficient data extraction and analysis. We also collaborate and coordinate with other groups to avoid duplication.

Refinement

The fish species used is the simplest vertebrate model system that can be used to achieve our research goals. Its optical clarity allows visualisation of cell behaviour as well as whole animal behaviour in the intact unharmed animal using a microscope or camera. In contrast to rodents, we obtain large numbers of eggs and without invasive intervention.

We do not know much about pain perception in fish, but it is likely to be similar to higher vertebrates such as mammals. To minimise suffering, most of our experiments are performed after anaesthesia and fixation at embryonic stages before the nervous system is fully functional and pain perception is limited or absent.

The recent advancements in research technology and microscopy (e.g. the use of transgenic fish which allow us to study specific types of cells under sophisticated microscopes) have resulted in a significant reduction in the number of animals used. In addition, in almost all experiments fish are allowed to swim naturally. We continuously monitor an animal during testing and any animal showing abnormal behaviour or abnormal distress, for example in an altered swimming behaviour, body position or positioning the tank, will be culled by a Schedule 1 method.

Project 60	**Role of Gut-Brain axis on brain and behaviour**
Key Words	gut-brain, casein, early-life
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	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

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

1. To determine the mechanism underlining the negative impact of prolonged exposure of milk casein at early developmental age on emotional behaviour (mood). More specifically we aim to determine if gut microbiota and hence gut-brain axis regulation are involved in the underlining mechanism.
2. To specifically determine the influence of bioactive breakdown products of casein, b-casomorphines, exposure at early developmental age on emotional behaviour (mood)

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)?

The planed work in this project will help re-examine the question in humans “is post-weaning consumption of milk actually beneficial for emotional regulation?”. We expect the results from the proposed work will be of interest and of benefit to the nutrition, neuroscience, psychiatry, microbiology, paediatric and agricultural community. In particular it will be of interest to the dairy industry. It may also drive policy in term of advising on nutritional content and length of breast feeding and formula milk exposure. Given that early life exposure to nutrients is known to have have long term effects on health, we believe that this proposed work would be highly beneficial and impactful to the community on the whole.

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

We will use rats. We expect to use 110 rats

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?

We have found that milk casein when consumed by weaned infant rats induces depression and alter brain receptors and gut bacteria. We believe that gut bacteria may be responsible for causing this depression. We plan to see if the effect of milk casein remains or not if we knockout the gut microbiota by treating weaned rats with antibiotics. If gut microbiota are involved, then we would expect to see a reversal of this depression state following milk casein exposure. We are also planning to investigate if exposure of A2 milk, which does not produce the bioactive molecule which we believe to cause depression, in weaned infant rats induces less “depression” than rats exposed to A1 milk which can produce the bioactive molecule b-casomorphins. To measure depression the rats will be left to swim in a beaker for 6 mins. This causes a slight distress to the rats which is why we used “moderate” as level of severity. Rats will be killed at the end of the treatment but brain, gut content and urine will be extracted for analysis of brain, gut bacteria content and metabolic profile of the urine.

Application of the 3Rs

Replacement

The behaviours proposed cannot be studied in *in vitro* assays and we are not aware of any computer programme which could simulate them. Epidemiological studies in offspring of breastfeeding mothers are planned as part of the PhD project, however, due to ethical reasons it is impossible to directly explore the mechanism underlining the effect of prolonged maternal milk exposure on psychological state in humans.

Reduction

The between subject variability for the behavioural outcomes on the animal model proposed is low, which results in the study being highly powered with small sample size. From each animal, we will obtain behavioural, neurochemical, metabolomic, endocrinological, histological and microbiological data. Correlation between these factors will be analysed by factorial design in order to maximise the amount of data obtained with a high degree of precision, without the need to use more animals.

Refinement

Taking into account that the protocols involve treating rats with antibiotics and exposing them to forced swim test (FST) which could cause some distress, we estimate the severity index to be moderate. The animals will not be allowed to swim to exhaustion. We will reduce the level of distress following the FST by immediately drying the animal in a towel and place it in a heated chamber so that the rats recover

quickly. Throughout the FST the well being will be recorded and body temperature will be monitored.

Project 61	**Understanding brain function and dysfunction using stroke models.**
Key Words	Brain, stroke, blood flow, ageing, MRI
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;

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

Overall, this project is aimed at understanding how brain function is altered, or becomes dysfunctional, as a consequence of vascular disease and the ageing process. In terms of vascular disease we are primarily interested in ischemic stroke which occurs as a consequence of a blockage in a blood vessel within the brain. Stroke is a significant cause of mortality and functional disability with limited treatment options available. Ageing is a significant risk factor for stroke and impacts upon the damage produced due to changes within the vascular system during ageing. In the current project, we aim to further understanding of the molecular mechanisms involved in the damage after stroke and aim to identify potential therapeutic targets that may then undergo further clinical investigation.

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)?

The primary aim of this work is the advancement of scientific knowledge. Studies are designed (e.g. using the NC3Rs experimental design assistance) and performed (e.g. in accordance with ARRIVE guidelines) in a rigorous manner. Information obtained is disseminated within the scientific field via presentations at conferences and peer-reviewed publications. Secondly we aim indirectly to contribute to the treatment of ill health. Through this advancement of scientific knowledge we aim to contribute to the treatment of ill health by identifying novel treatment strategies that warrant clinical investigation. In addition, we aim to constantly refine the use of experimental stroke models and improve animal welfare.

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

This work will be carried out in both mice and rats. We predict using approximately 400 animals over a 5 year period. We will be using animals from young adults to older adults.

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?

Work under this authority forms part of larger multi-disciplinary research programme which utilises a variety of in vitro, in vivo, imaging and epidemiological approaches. Some animals will undergo minimal procedures, such as injection of a compound of investigation, before being terminated in order to examine the effects of such a compound on brain function. For animal undergoing experimental stroke it is necessary to create an area of tissue damage which produces pathological and functional deficits. Such damage is expected to result in moderate degrees of: paralysis of the contralateral face and limbs, sensory loss in the contralateral face and limbs, hemispatial neglect, hemiparesis and hemiplegia. Affected animals require fluid and food supplementation. All animals post-stroke are carefully and regularly monitored. Post-operative monitoring sheets are completed and kept with the animals ensuring their progress can be identified by any member of technical staff. Our previous data indicates that ~40% of animals are classed as severe, 50% classed as moderate and 10% classed as mild. Weight loss has a significant effect on recovery following MCAO and we implement a number of measures to promote feeding and drinking following MCAO. We also apply a local anaesthetic/analgesic regime to all animals undergoing experimental stroke. A large number of MRI studies will be done under terminal anaesthesia. However, for those that involve imaging with recovery no adverse effects are anticipated. MRI imaging is carried out by skilled technicians who have spent a considerable amount of time refining the methods used during the MRI imaging procedure.

Application of the 3Rs

Replacement

The overall aim of this work is to investigate how brain function is altered during ageing and vascular disease. Animals are required in order to dynamically study the relationship between vascular supply and neuronal function. In vitro experiments are a complimentary aspect of the overall research programme but cannot replace the use of in vivo animals for studying dynamic changes in blood flow (e.g. via MRI scanning) and the impact of vascular disease i.e. stroke.

Reduction

The minimum number of animals required to achieve the scientific objectives will be accomplished through good study design largely using the NC3Rs Experimental Design Assistant. Statistical ‘in-house’ support is available as well as consulting

clinical statisticians through established collaborations with clinical stroke physicians. All Personal Licensees (and dedicated animal care staff) working on this project will be appropriately trained and suitably competent to ensure a high success rate is achieved with the minimum number of animals used. For studies investigating potential therapeutic strategies following stroke in vivo experiments will only be conducted once satisfactory outcomes have been obtained using in vitro (e.g. cell cultures) and/or ex vivo (e.g. brain slices) approaches.

Refinement

In order to mimic clinical stroke lesions, we require an experimental species high in the evolutionary tree (i.e. mammalian). Rodents are a clear and desirable choice, being mammalian species that are widely-used in scientific research due to their relatively-low neurophysiological sentience. Their cerebral vasculature is relatively well-known and is in many ways similar to the human. The Middle Cerebral Artery Occlusion model is the primary model used in experimental stroke research meaning data obtained here is highly relevant/applicable to the majority of those working within the experimental stroke field.

Project 62	**Mechanisms underlying neural development**
Key Words	Brain, Stem cells, Neurogenesis, Gliogenesis, Transcription factors
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;

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

Our general aim in this project is to gain knowledge of how the brain is built, and why its development is disrupted in patients with a neurodevelopment disorder and suffering from intellectual disability.

Our specific objectives are:

1. To understand how the different kinds of cells found in the brain are produced during embryonic development, by identifying proteins important in the generation of these cells and elucidating how these proteins function.

2. To introduce in mice, genetic mutations that are found in patients with neurodevelopmental disorders and understand by studying these disease models why these mutations disrupt brain development.

3. To study the divisions of stem cells present in particular locations in the brain of mice and understand how the frequency at which these cells divide is controlled by other brain cells.

4. To identify proteins that determine how each neuronal cell acquire its unique features, including how it connects to other neuronal cells in the mouse brain and thereby participate to mouse behaviour.

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)?

There are several potential benefits to our research. Firstly, the human brain is extremely complex and studying how the brain of a related organism, the mouse, develops will help better understand the organisation of the adult brain. Secondly, mutations in genes that participate to the development of the brain are a frequent cause of mental retardation. Our research will help understand how brain

development is affected in these patients. Thirdly, stem cells present in the brain of adult humans and mice stop progressively to divide as the organism ages, with negative effects on learning and memory. Our research will help understand the causes of this intellectual decline. Fourthly, a particularly vulnerable group of neurons regulating voluntary movement and motivation is and is defective or destroyed in several pathologies, including Parkinson's disease, Schizophrenia and Attention Deficit Hyperactivity Disorder. Our studies on the generation and maintenance of these neurons will shed light on the aetiology of these disorders.

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

We will only use mice in this project, including genetically altered mice that carry mutations in genes involved in brain development, and normal mice where we will disrupt brain development transiently by introducing or suppressing genes specifically in some brain cells. We request authority to use 35,000 mice within this licence.

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?

Prior to animal work, we collect information from published studies and we carry out biochemical and cell culture experiments which identify candidate genes that are essential for the formation and function of the nervous system. This information is then used to generate transgenic animals in which the expression of selected genes is modified. Our main experimental approach involves histological and microscopic analysis of post-mortem tissues and physiological analysis of organs isolated from transgenic animals. Therefore, in the majority of cases, there will be no further interventions than those required for breeding and genotyping. We will also administer substances (e.g. tamoxifen or doxycycline) that induce/repress/modify gene expression to perform genetic alterations at particular stages of development and in specific tissues. These substances will be administered by the most appropriate route selected for the minimal invasiveness compatible with efficient delivery. In less than 10% of cases we may do further analysis, in particular observing and imaging behaviours. In small numbers of cases, we will also manipulate developing foetuses in the mother's womb. In a very small proportion of animals, <1%, we will produce injuries to specific regions of the brain to study how the brain reacts and attempts to repair itself. For the large majority of this work the only procedures will be those involved in breeding and maintenance of genetically altered lines of mice. It is expected that all but a few animals will experience no more than mild adverse effects.

Application of the 3Rs

Replacement

To define the mechanisms by which genes and cells control the development and function of the nervous system that are relevant to the whole intact animal and are therefore meaningful for human clinical studies, it is necessary to perform animal experiments. In particular, the effect of different genes and proteins and their interaction with each other is influenced by nutrients, oxygen, circulating hormones and other aspects of the complex physiological environment inside the body. It is not yet possible to recapitulate all of these parameters in vitro.

Nevertheless, our current and future research makes extensive use of cell cultures as alternatives to animals. Stem cell cultures will be used for many of our studies to study how genes and proteins function. The relevance of these studies will be confirmed using similar approaches, but in much fewer cases, with cells obtained from animals.

Before embarking on any animal experiments, we will collect as much evidence as possible from our own experiments and by surveying the literature, to determine whether a candidate genetic or environmental manipulation has a reasonable chance of success.

Reduction

Where possible, genetically altered lines will be maintained in a homozygous state, thereby obviating the generation of a large excess offspring with inappropriate genotypes. In other cases, homozygotes will be generated from heterozygote intercrosses, with littermates genotyped as heterozygous or wild type used as age-matched controls. Most (~80-90%) of the experimental work will be ex vivo, and we expect that 5-6 animals per treatment group will usually be sufficient to obtain robust results. For most of the quantitative experiments, design will be based on ARRIVE guidelines and sample sizes will be set using power analysis, generally using a significance level of 5% and a power of 80%. Otherwise, we will use the minimum number of animals to provide an adequate description, generally on the basis of previous practical experience (our own or from the literature).

This programme of work will make optimal use of several tissues, fluids and cell types per individual mouse. We will aim to collect organ samples from multiple body sites and to provide other affected tissues to appropriate scientists, so that they do not have to breed mice specifically for their experiments. This integrative approach will maximise the information obtained from the minimum resources. Cryopreservation of gametes, embryos, tissues and cells is routine and will ensure that the minimum number of mice is bred.

Refinement

We are using refined genetically altered mouse models, employing conditional and inducible technology where appropriate. To minimise stress during breeding and maintenance, we will follow best practice guidelines and follow local refinements of

husbandry such as cage enrichment and sufficient amounts of nesting material. On receipt or generation of a new line, we will minimize suffering by ensuring increased observation and monitoring until a detailed phenotypic analysis for each line is accomplished. If any welfare implications are identified, they will be acted upon and refinements considered in consultation with the NVS and NACWO.

The majority (~95%) of animals produced under the breeding protocol are not expected to exhibit phenotypes beyond a mild classification, but a small proportion may exhibit a moderate phenotype - particularly if they are modelling a human disease. However, it is not possible in all cases (such as newly generated lines) to predict fully the nature or severity of any potential defect and for that reason the limit has been set at moderate. For all types of mice, however, there will be careful monitoring of strain characteristics and the information will be collated and regularly reviewed to ensure that phenotypes to not exceed their usual features.

For all manipulations we will adhere to local or national guidelines that aim to minimize suffering. Most of the work as well as the administrations of gene inducers/repressors or other agents are standard and previous refinements from our own experience and from the literature will be used. If however, there is insufficient information available, new manipulations will be pre-screened in small-scale pilot studies to obtain indications e.g. of the minimum dose and exposure time that is likely to be effective, thereby minimising any potential suffering.

Unless otherwise specified, all surgical work in this project will be undertaken in accordance with the principles set out in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2010) or other such publication promoting best practice. Analgesia will be provided according to contemporary best practice and advice from the NVS/NACWO.

Project 63	**Neural circuit mechanisms for animal behaviour**
Key Words	Neuron, Brain, Cognition, Behaviour, Navigation
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;

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

Understanding the biological basis of human and animal behaviour will help to answer centuries old questions about the physical nature of mental processes and may be of general importance for human mental health and disorders of the nervous system, such as depression, anxiety, schizophrenia and memory loss. This project explores mechanisms and effects of computations carried out by neurons in the brain. The research will aim to elucidate the organization of neurons in circuits important for cognition, to identify and test mechanisms by which neural circuits carry out computations, and to investigate how neural computations contribute to behavior.

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)?

The primary benefit of the project will be new fundamental knowledge about how the cells in the carry out computations used to guide behaviour. We hope that advances made during the project will also form a basis for new rational approaches towards development of treatments for many brain disorders for which there are at present few effective treatments, for example schizophrenia, autism and depression.

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

The project will use either mice or rats. Over the five years of the project we anticipate using approximately 17,500 animals, with the majority used for breeding involving harmless genetic modifications.

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?

The project will primarily combine labelling and manipulation of neurons with recording of neural activity either in ex-vivo brain tissue, or in behaving animals.

Experiments with behaving animals will be necessary to investigate mechanisms used by the brain to control specific behaviours. For the majority of experiments the expected severity is classified as moderate. The expected adverse effects primarily relate to surgical procedures required to manipulate and observe brain activity. The maximum severity will also be moderate. At the end of experiments animals will be sacrificed by an approved humane method.

Application of the 3Rs

Replacement

The project seeks to understand how brains generate cognitive processes used to guide behaviours. .Cognitive processes require that brains combine complex sensory stimuli with information that is remembered. The outputs from cognitive processes are then observed as changes in behaviour. Cognitive processes rely on complex patterns of connections between nerve cells. A nerve cell often receives on the order of 10,000 connections, many of which originate from neurons that are a considerable distance away. Investigations of mechanisms that underlie cognitive functions require that disruption of this connectivity is minimal, and that sensory stimuli and motor outputs are in tact. Therefore while we use ex-vivo approaches and computational models as much as possible, the only way we can investigate how cognitive processes actually work is by doing experiments with live animals

Reduction

We will use the following strategies to minimise the number of animals used. 1. We will design our experiments so that hypothesised differences between a control and an experimental manipulation are large. We will use appropriate statistical strategies in determining the number of animals required to distinguish the hypothesised effect and in carrying out the analysis. 2. Where possible by use of experiments that do not use live animals, for example by using brain tissue obtained from animals that have been sacrificed humanely . 3. Where possible by use of computational models. 4. Through careful selection of breeding strategies.

Refinement

Mice and rats are capable of cognitive tasks, in particular spatial behaviours, that are similar to those carried out by humans. Many cognitive tasks involve anatomically similar brain areas and our working hypothesis is that the underling computations are similar. In modern biological research mice have been the species of choice for genetic manipulation and many sophisticated genetic tools are available. Rats, for which an increasing number of genetic tools are becoming available, are advantageous for some research questions primarily because of their larger size. Surgical procedures will be carried out with appropriate anaesthesia and analgesia

We will adopt a number of refinements that minimise welfare costs, which by increasing the precision of experimental tests and manipulations will lead to clearer and increasingly specific answers to the questions we aim to address. These will often involve adopting specific molecular and ex-vivo strategies for precisely targeting single nerve cells or populations of nerve cells instead of invasive and less specific in vivo methods. We will also adopt behavioural assays that give precise control of the experimental animal's sensory experience and clear read outs of cognitive outputs

Project 64	**Understanding and treating neurodegenerative disorders**
Key Words	Neurological diseases, Mechanisms of disease, New Therapies
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;
Yes	(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);

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

Diseases that affect the brain and the nervous system are a major problem in society. They can affect both children and adults and often have no available treatment leading to premature death.

The overarching aims of this project are twofold: (1) To understand the underlying mechanisms that lead to debilitating and lethal neurological diseases and (2) Use this information to design and evaluate new therapies that can be used in the clinic to treat patients.

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 project will help us to understand how neurological diseases that affect children and adults progress and how they cause the damage that leads to premature death. By using this information we can understand how best to intelligently design or apply

novel therapies and also gauge how effective the therapy is. These pre-clinical studies are invaluable in developing new treatments for these lethal neurological disorders. Without this pre-clinical data, it is almost impossible to move to clinical trials of new therapies. This project will have both societal and academic impact and a significant benefit to patients, their families and the healthcare system.

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

5500 mice and 200 rats over a 5 year period of time.

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?

The diseases that we are studying are severe neurological disorders that affect children or adults. Therefore, the mice or rats that we use to model these diseases may fail to thrive, lose weight and develop neurological symptoms over time e.g. tremor, reduced mobility or the onset of paralysis. Using such models we have defined humane endpoints at which animals will be culled to prevent suffering. This requires us to be vigilant and careful in monitoring these animals. The expected level of severity is moderate.

Application of the 3Rs

Replacement

The brain is a complicated organ made up of hundred of discrete regions and different cell types that interact with each other. For this reason, *in vitro* studies on cells are of limited use and animal models are the only way to fully study diseases of the brain and to evaluate the effects of novel therapies. Some neurological diseases are also accompanied by problems in other organs e.g the spleen or liver. So to fully understand the effect on the whole body, an animal model is required.

A major focus of this project is to conduct evaluations of novel therapies for potential application in the clinic. As part of this process, medical regulatory bodies require animal studies as part of the traditional approval path for use in humans.

However, wherever possible we always use *in vitro* models in this process. An example of this is that we test all therapeutic agents on cell lines first in order to validate their quality and purity before any introducing them into animal models. These cell studies will be done before and instead of *in vivo* studies. We continue to keep abreast of the latest developments and potential application of new technologies as potential replacements to certain aspects of animal work.

Reduction

Many of therapies we are evaluating are very efficient and mice remain fertile. This allows us in many cases to maintain a colony as ‘cured knockouts’ and reduce numbers required by up to four-fold.

We intend to use pharmacological or surgically induced mouse models of disease. This is used as an alternative to a transgenic model of the same disease and circumvents having to keep a breeding colony and hugely reduces numbers of mice required.

For most experiments, a pilot study using six animals per group is performed. Statistics are then used to test whether sufficient animals have been used per group. The experiment may then be repeated, or not, in order to provide sufficient power. The type of statistical test to be applied usually dictates the experimental plan and, where appropriate, larger experiments utilising the same control group are preferred to smaller experiments where a control group has to be included each time. The PI and PPL applicant is experienced in the use of statistics for *in vivo* analysis and we have access to a full time statistician.

Refinement

We have chosen rodents, mainly mice but also rats, as the majority of the neurological diseases that we are studying are inherited genetic disorders and mouse models are available for most of them. We often use an outbred CD1 background since this strain breeds well and rarely cannibalises litters, an important aspect of the fetal and neonatal work in this project.

Where possible, we attempt to prevent onset of disease, rather than treat the symptoms; this prevents undue suffering.

As discussed above, the therapies often permit correction of the mouse model of genetic disease, enabling us to breed the colony as cured knockouts. This allow a fourfold reduction in colony size and refines the experimental procedure. Since the genotype of cured knockouts is known, there is no need to perform an ear punch for PCR analysis of genotype. Furthermore, many of our experiments involve injection at birth or in utero i.e. before the genotype of the animal is determined. Previously, we had to inject all mice and only afterwards determine which of those were knockouts. Therefore, heterozygotes were injected un-necessarily.

We are also refining our behavioural analyses through introduction of filming so that data can be reviewed in slow motion. This is more accurate and more information can be gained from a single experiment rather than having to deal with this in real-time and miss vital measurement that may occur to quickly. We have found that this reduces repetition of the experiment, is less traumatic for animals because filming can be conducted from a distance and less handling is required. All filming is done subject to local biosecurity measures and password protected/encrypted. We are also introducing more automated software driven analyses where possible e.g. the

Catwalk system for gait analysis and tracking software for open field software. Again, this reduces the amount of handling that is required and reduces stress to animal. In turn, a more accurate readout of behaviour can be ascertained.

Project 65	**Molecular determinants of cortical development**
Key Words	cortex, development, neurogenesis, migration, circuits
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;

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

The objective of this project is to gain insight into the four different processes that are necessary for the formation of the cerebral cortex, to better understand how alterations in these processes lead to brain disorders in infants or later in life.

1) Neurogenesis: different types of neurons need to be produced in appropriate numbers. We want to study how neuronal progenitors regulate the production of neurons and balance it with their need for self-renewal at different stages during development.

2) Neuronal fate specification: the cortex contains many different types of neurons. We will study how the different neurons are specified and whether their environment plays a role in this specification process.

3) Migration: neurons need to move from their place of birth to their final position within the cortex. We will study the mechanisms that regulate this movement and, in particular, how one specific protein, Cdh2, fulfils different functions in neural progenitors and migrating neurons.

4) Circuit formation: neurons need to communicate with each other, but how they select their targets is still unknown. We will test adhesion proteins to check if they can provide a recognition code for neuronal interaction.

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)?

The research proposed for this project comprises basic neurobiology but it could also eventually be relevant from a clinical point of view in the future. The expected results

would translate into two potential benefits: - First, it will generate new knowledge about the function of several genes, involved in distinct processes during cortical development, contributing to our understanding of brain development and function. - Second, it will shed light on the pathophysiology of megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 3 (MPPH3), a human disorder caused by mutations in the CCND2 gene (one of the genes that we will study), and potentially of other disorders, providing information to affected families to help them understand the disease. A good understanding of the different molecules and processes involved in corticogenesis is also essential when new risks are identified that can severely affect the mental health of the population. An example is the current epidemic of Zika virus. This virus infects cortical progenitors very efficiently and has been linked to microcephaly, but new studies suggest that it might be causing further neurological problems that are only detected later in life.

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

Over the 5-year duration of the project, approximately 4500 adult and 10,000 foetal mice will be used.

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?

The main techniques to be applied to the animals in this project are: - breeding of genetically altered animals - administration of substances by injection or in the drinking water - tissue collection for identification and analysis purposes - in utero electroporation - behavioural analysis The adverse effects that we expect are: - transient pain from tissue collection for identification/analysis - post-operative discomfort - very rarely post-operative infections All of the above-mentioned effects are not very likely to occur and most of them are expected to be of mild severity. Surgery is considered to have a moderate severity limit, but refinement methods should minimize any discomfort or pain to the animal. All animals will be killed by humane methods or under general anaesthesia at the end of the experiments.

Application of the 3Rs

Replacement

The developing cerebral cortex is a very complex structure. Cells and signals from different origins converge there and it also has a very defined temporal and spatial architecture. At present, there are no in vitro models that can replicate these complex conditions. Also, to gain insight into the development of the human brain, a mammalian species needs to be used because lower vertebrates do not have a six-layered cortex.

Reduction

To ensure that animal numbers are kept to a minimum, there are three key aspects that will be considered:

1) Experimental design: experiments will be designed to provide the maximum amount of information with the minimum number of animals. Power analysis will be used. Experiments will be analysed before other experiments are planned.

2) Animal samples will be clearly labelled and properly stored to maximise its use through the course of the project. Careful planning and consideration of future experiments will ensure that surplus samples are processed in ways that allow future use.

3) Researchers will receive extensive training for the procedures where technical expertise significantly influences the success rate.

Refinement

Choice of species: For this project the chosen species is the mouse. Mice are the lowest vertebrate group that has a six-layered cortex and are close enough to humans to reveal principles of brain development. Because mice have been extensively used as model animals in this field, there is a wide body of research to build upon and findings can be related to previous results.

To maximize refinement, many of the techniques in this project are done ex vivo, on material obtained from animals killed by Schedule 1 methods or under terminal anaesthesia. In vivo methodologies will be used to address questions where the complex context of the developing cortex has to be preserved to obtain conclusive results. By affecting only a proportion of cells in the whole brain, in utero manipulations are more refined than conventional knockouts because the effect on the animal as a whole is reduced.

Surgery will be performed under general anaesthesia and under aseptic conditions to prevent any post-surgery infections. Analgesics will be administered as standard to minimise suffering, and animals will be monitored regularly to ensure their wellbeing. If the animals show signs of severe adverse effects, they will be humanely sacrificed to prevent prolonged suffering

Project 66	**Metabolic and cardiovascular regulation by GLP-1 and the autonomic nervous system**
Key Words	diabetes, obesity, neurophysiology
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;

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

Diabetes and obesity put a rapidly increasing burden on society, both in terms of reduced quality of life and ever-increasing financial cost. It is becoming increasingly clear that obesity, diabetes and also heart disease, are linked and that simply treating the individual symptoms might not be the best strategy for the patient. A promising drug that improves both diabetes and obesity is glucagon-like peptide-1 (GLP-1), a hormone produced both in gut and brain. Currently, we know little about how GLP-1 works in our brain. This understanding, however, might be needed to explain how GLP-1 causes weight loss and improves diabetes. In fact, a number of clinical studies have linked problems with the autonomic nervous system to an increased risk of diabetes. We will explore a) how GLP-1 produced in the brain controls the autonomic nervous system, and thereby food intake, blood pressure and blood sugar levels, and b) the importance of the parasympathetic vagal nerve cells, which are an important part of the autonomic nervous system, in the development (or prevention) of diabetes and obesity.

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 project is likely to increase understanding of how autonomic nervous system function in general, and GLP-1 in particular, affect blood sugar and cardiovascular control. Dependent on its results, this study might influence policy on the use of GLP-1 analogues as anti-diabetic and anti-obesity drug, and establish the contribution of autonomic nervous system dysregulation to diabetes and obesity, and thus open new treatment avenues.

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

This project will be carried out using rats and mice. We estimate to use up to 1100 rats and up to 3000 mice in procedures over the next 5 years.

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?

The surgery required to target the GLP-1 cells and vagal neurons in the brain is the most severe part of the procedure and carries similar risks as procedures in humans. The likelihood of surgical complications is very low (1%) and the animals recover well and fast. In general, the behaviour of post-surgical animals is undistinguishable from that of their untreated littermates by day two post surgery. The subsequent tests are designed to cause minimal distress to the animals, and in most cases the stress of being handled by a person and being removed from the home cage for the test is the largest concern. Each animal will also be subjected to several tests in order to keep overall numbers of animals required low.

Application of the 3Rs

Replacement

This work involves evaluation of the functional interaction between various organ systems (e.g. brain, digestive tract, heart). Such complex interaction cannot be reproduced with cell cultures and thus has to be performed using live subjects.

Reduction

Experiments in our laboratory are carefully designed and executed. This ensures that all data obtained is of highest quality. Appropriate statistical tests are used to keep sample sizes as low as possible. For most experiments this means that we can expect to obtain statistically significant results with a sample size of six animals per experimental group. Occasionally, these numbers may be increased to nine if the data suggest that this would increase the likelihood of obtaining statistically significant differences.

Also, as outlined above, individual animals will be subjected to more than one test each and after the *in vivo* program their tissue will be used for further *in vitro* analysis, thus reducing the overall number of animals required.

Refinement

This project requires the use of mammals, because the control of food intake and glucose homeostasis by the brain are too different from man in other vertebrates, or invertebrates. Since these functions are fulfilled mainly by ‘lower’ parts of the brain rather than cerebral cortex, rodents are seen as adequate model systems. Mouse and rat can be bred and maintained in a laboratory setting without too much impact on the welfare of the individual animal. In this case we have to use mice for the bulk of the work, because the genetic modification required for this project is only available in mice. Individual animals will receive gene therapy that will incorporate a ‘switch’ into their GLP-1 producing cells, GLP-1 responsive cells, or into their parasympathetic vagal neurons, that allows to ‘switch on or off’ these cells at will. The effect of such manipulation on food intake, body weight, blood glucose control and blood pressure or heart rate will then be measured. These measurements will be performed as much as possible with telemetry which reduces handling, and therefore stress, for the animals, leading to better data with smaller variations, and thus might also lead to a reduction of the number of animals needed.

Project 67	**Regulation of the circadian clock and sleep homeostasis**
Key Words	Sleep, Circadian, Clock, Neuroscience
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;
Yes	(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);

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

The overall goal of this project is to find out molecular, behavioural and physiological mechanisms by which the 24 hour body clock (“circadian clock”) and sleep are regulated. These fundamental functions impact on body function, including metabolism, control of cell division and immune function. In this respect, a further sub-goal will be to look at virus infection and the interactions of the clockwork and/or sleep with viral infection.

The key objectives are to:

1. Determine how established and novel genes that alter the body clock and/or sleep bring about their changes at the molecular, organ physiology, and behavioural levels.

2. Elucidate the brain pathways that mediate circadian rhythm and sleep physiology, and determine how manipulation of these can affect physiology at the molecular and behavioural levels.

3. Determine how the body clock and/or sleep affect the ability of viruses to infect the body, and investigate whether drug treatments targeting circadian clocks or sleep prevent (or minimise) this.

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)?

Elucidation of the cellular processes regulating the core clock cycle will both enhance understanding of basic biological processes that govern biological timing, and also reveal potential targets for therapeutic intervention and management of sleep and other clock-related disorders. For example, we have developed a number of drug compounds that are, in cell culture models, able to alter the parameters of the clock (e.g. speed it up or slow it down). On the path towards translating these basic findings to the clinic, testing the most promising of these compounds in rodent models will facilitate their future use, and also highlight any potential safety issues early in the development process. In aspects of the project related to sleep biology, we anticipate that key insights into the molecular architecture of sleep will inform us about potential pathways to target therapeutically. At present, medications that are used in sleep disorders such as insomnia effectively “shut off” brain activity, which does not mimic natural sleep, nor the benefits of it. Therefore, it we succeed, we may be able to find ways to enhance “normal” sleep, which could help 10% of the population who suffer from chronic insomnia. In addition, we will also focus on how the clock and sleep can “gate” pathological processes, such as virus replication, which could lead to new ways to block these processes with novel classes of anti-viral compounds, for both human and animal health benefits in the future.

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

Mouse – approx. 20000 over 5 years Rat – approx. 150 over 5 years

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?

The adverse effects for the majority of animals fall into the “mild” severity category – such as observation of 24 hour rhythmic behaviour by non-invasive means with perturbation of light-dark cycles that the animals experience, sleep deprivation/fragmentation studies. Some experiments will involve brain surgery to test the effect of drugs directly in the brain, or for special optical recording in animals during their waking and sleeping phases. These will be of moderate severity. For virus studies, the animals will likely reach moderate severity in a small percentage of

cases. In all experimental protocols, animals will be humanely killed at the end of the experiment.

Application of the 3Rs

Replacement

It is necessary to use animals for this work because we seek to understand the body clock and sleep, which are processes that only occur in the intact living organism. They are emergent properties of the intact brain and body and therefore cannot yet be adequately simulated with cell culture systems.

We have considered, and will use when possible, various *in vitro* cell culture based assays using mouse cell lines for circadian clock assays. We are also currently developing a “sleep in a dish” model using cultures of neuronal cell lines to switch on / off neuronal activity to try to develop alternatives to using whole animals. However, this might not be possible to achieve fully given the likely importance of brain nuclei connectivity, proteins and metabolites within living tissue that are important for sleep regulation.

Reduction

We have performed statistical analyses that enable us to estimate how many animals we need to use in order to obtain meaningful results from our experiments. This tells us that typically groups of eight animals are enough to see statistical differences between two conditions (e.g. treated vs. non-treated (control) animals).

In some cases, we will use novel methods such as a specialised piece of equipment to measure luminescence emitted from certain transgenic animals that we use to map body clock rhythms. Since we can track a single animal for many days and determine body clock parameters at the molecular level in high detail, this circumvents the need to use large numbers of animals for certain experiments. This will reduce the overall number of animals that are required to fulfil the project’s objectives.

Should we start to measure different end-points with which we are less familiar we would seek statistical advice to ensure group size and experimental designs are adequate for that situation.

Refinement

The work will be conducted predominantly in mice. A small number of rats may be used for comparative purposes, or for analysis of species-specific functions (e.g. sleep analyses) since the rat was the dominant model organism used for experimental sleep research until recently. Experimental rodents in general, and

mice in particular, are the subjects of choice for such work as they express excellent body clock and sleep rhythms and the nature of the brain pathways is very close to that of humans. Moreover, the genetic details of the body clock are closely aligned between mice and humans – for example the proteins are interchangeable in cell-based assays. Finally, the enormous recent growth in knowledge of the mouse genome and the ability to manipulate it are unsurpassed when seeking to examine the role of body clock and sleep processes in mammals.

Transgenic mice have become the leading vertebrate model to study gene action because of their relative ease of breeding and genetic manipulation: the rich diversity of genetically altered mice provides an unrivalled resource for understanding the molecular genetic basis to circadian physiology. Moreover, refinement of procedures is ever increasing due to the continuing development of mouse lines with, for example, tissue-specific and temporally selectively inducible mutations. Indeed, the recent introduction of CRISPR/Cas9 technology will minimise the number of breeding cycles required to generate homozygous transgenic animal. Moreover, it will allow us to generate more focussed knockout and knockin models using systems to specifically target a tissue (e.g. disrupt a gene in the brain only, rather than the whole body). Therefore, these manipulations will minimise animal suffering because only specific tissues will be targeted, rather than the whole organism, and in some cases only when an adult (minimising the overall welfare impact over the animals' lives). In turn, the results emerging from this project will increase this information, and have the potential to influence scientific progress, which will feedback to allow further refinement of methods to minimise harm.

For sleep studies, one principal measurement is the electroencephalogram (EEG), which involves the placement of electrodes on the brain surface. Although we cannot completely replace the need for EEGs in sleep studies, we have implemented video tracking algorithms to non-invasively assess sleep in large numbers of animals. We thus use video tracking initially (e.g. to characterise a new transgenic sleep model) and if there looks to be a phenotype (e.g. sleeps less than wild type controls), we then perform EEG recordings. This minimises the number of invasive procedures that animals are subjected to.

Project 68	**Cortical structure and information processing**	
Key Words (max. 5 words)	brain, neurons, synapses, neuronal networks, sensory physiology	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To uncover the organization and function of specific neuronal networks in the brain with emphasis on the interaction between external, sensory stimuli and internally generated models.	
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)?	The workings of the brain remain largely a mystery to us. One reason for this is the lack of generalized working hypotheses about what computations brains have evolved to do. We will test new theories postulating that brains constantly attempt to match bottom-up sensory input with top-down, internally generated predictions and the most common neuronal computation across many brain areas is the comparison of prediction and prediction error signals. Furthermore, by generating fundamental new knowledge about the structure and function of specific brain areas we will not only advance our knowledge of brain mechanisms in health, but also help understand what may go wrong in pathological states.	

What species and approximate numbers of animals do you expect to use over what period of time?	In order to gain understanding and test causality, complex systems need to be dismantled and probed invasively. These experiments can thus only be done in reduced model systems. Our system of choice is mice and we will use approximately ~8000 mice over 5 years. Due to the statistical nature of genetics, around half of the animals bred under this protocol will not undergo other regulated procedures but will be reintroduced to the breeding stock or terminated humanely.
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?	No procedure done on our experimental animals is expected to produce lasting harm. After all we are interested in how the brain works in healthy animals, thus the well being of our subjects is of paramount importance. After experimental procedures animals will be killed in a regulated and humane way.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our project aims at generating new knowledge about the brain. Fundamental knowledge can only be gained from intact brains and animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will make every effort to obtain the maximum amount of information out of every experimental animal. Pilot experiments and statistical methods will be used to determine the minimum numbers needed for scientific rigour and significance.
3. 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.	Rodents have been used extensively in neuroscience research because of relative similarity to humans. Previous research in rodents also provides a platform to build on. State-of-the-art recording and analytical methods will be used to interrogate neuronal function. We will constantly monitor international and local developments in refining surgical and experimental procedures.