Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 19

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

Project Titles and keywords

1. **Brain plasticity with experience and recovery**
    - Plasticity, learning, stroke, exercise, experience

2. **Neuronal network activity underlying behaviour**
    - Brain, neuron, plasticity, cognition, neuromodulator

3. **Neural mechanisms in health and disease**
    - Neurophysiological function, neurophysiological dysfunction, ageing

4. **Circuit mechanisms governing network oscillations**
    - Septum, cortex, oscillations, theta, synapses

5. **Affect and cognition in rodents**
    - Affect, cognition, consumption, microstructure

6. **Role of neuroinflammation in depression and cognition**
    - Depression, inflammation, inflammasome

7. **Genetically modified rodent models of neurodegeneration**
    - Alzheimer's; Parkinson's, Motor neuron disease; transgenic rodents; novel therapeutics

8. **Delta-protocadherins in cortical development**
    - Cortex, development, delta-2 protocadherins, Pcdh19, EFMR

9. **Plasticity and function of the visual system**
    - Primary visual cortex; amblyopia; neurodevelopmental disorders; glaucoma

10. **Brain systems for rodent memory**
    - Brain, systems, rodent, memory

11. **Genetic and functional studies in neurodegenerative disease**
    - Neurodegeneration, Alzheimer disease, Down syndrome, motor neuron diseases, mouse

12. **Drug modification of opioid tolerance**
    - Opioid: tolerance: respiratory depression

13. **Novel imaging applied to the study of memory**
    - Learning, memory, imaging, Synaptic plasticity, Optics

14. **Bioenergetics of the nervous system**
    - Bioenergetics, mitochondria, inflammation, demyelination, neurodegeneration

15. **Support procedures for neuroscience studies**
    - Transgenic, behaviour, substances, tissue

16. **Neuropharmacology of vulnerability to compulsivity**
    - Addiction, compulsivity, vulnerability, neuropharmacology, rat

17. **Rodent models of neurodegenerative disease**
    - Huntington's Disease, cognition, locomotion, mice

18. **Spinal cord injury and repair**
    - Spinal cord; plasticity; regeneration; rehabilitation

19. **Mechanisms of myelination and synapse formation**
    - Epilepsy, neuropathy, genetic programme

20. **Blood vessels in cortical interneuron development**
    - Neocortex, interneurons, development, migration, blood vessels

21. **Neurophysiology of reward**
    - Brain1 neurons, reward, learning, decision making

22. **Investigating the neural basis of spatial and episodic memory**
    - Memory, Episodic, Alzheimer's, Hippocampus, Behaviour

23. **Epigenetic regulation of neuronal development**
    - Brain, development, neurodegeneration, transcription

24. **Neuronal Networks and Pathways for Communication**
    - Communication, neuroimaging, neurophysiology, primate, neuronal mechanisms

25. **Molecular pathogenesis of neurodegeneration**
    - Alzheimer's O-GlcNAcylation neuropathology

26. **Neuronal and sensory functions of Tmc genes**
    - Sensory transduction, taste, touch, hearing

27. **Mechanisms of perinatal brain injury**
    - Preterm, term, hypoxia-ischaemia, inflammation, translational

28. **Cognitive-enhancing properties of nicotine and related psychoactive substances**
    - Nicotine, attention, withdrawal, dependence

29. **Mechanism of brain function and malfunction**
    - Cognition, cell assemblies, EEG, dementia, Transgenes

30. **Neuronal activity underlying sensory behaviour**
    - Brain, electrophysiology, imaging, information processing, somatosensory

31. **GABAAR, neurosteroids and stress in brain function**
    - GABA, neurotransmission, stress, depression, addiction

32. **Investigation of the in vivo action of G protein coupled receptors**
    - Neurodegeneration, physiology, drugs, cancer, diabetes

33. **Biological and Psychological Bases of Addiction**
    - Drug abuse, transgenic mice, behaviour

34. **Characterisation of novel therapeutics**
    - Drug Discovery

35. **Studying central nervous system repair**
    - Multiple Sclerosis, Cerebral Palsy, Central nervous system, Repair, Oligodendrocyte

36. **Studying myelinated axons in vivo using zebrafish**
    - Zebrafish, In vivo imaging, Nervous System Development

37. **Zebrafish models of movement disorders**
    - Parkinson's disease, mitochondria, dopaminergic neurons, parkin, PINK1

38. **Post-operative Cognitive Decline: Pathogenesis & Protection**
    - Surgery; Neuroinflammation; Cognition; Dementia; Alzheimer's

39. **Genetic analysis of axon guidance and maintenance**
    - Breeding, transgenic, neurodegeneration

40. **Analysis of Fish Development**
    - Eye, brain, stem cells, zebrafish

41. **Blood flow and tissue oxygenation in rodents**
    - Haemodynamic response, Imaging, pre-clinical models

42. **Biological and Psychological Bases for Addictions**
    - Drug abuse, transgenic mice, behaviour

43. **Molecular & cellular correlates of stress-induced behaviour**
    - GABA, mental illness, noradrenaline, serotonin, emotion

44. **Neuronal circuitry of the spinal dorsal horn**
    - Pain, Itch, Spinal cord, Interneuron, Projection neuron

45. **Cellular functions of myosin motor proteins**

- Cell function, neurodegeneration, transport

46. Perioperative medicine related uses of anaesthetics
- Rodent; Anaesthetics, Noble gas; Brain injury; Cancer

47. Cerebrovascular changes in the aged and diseased brain
- Ageing, Alzheimer's disease, blood vessel

48. Synaptic plasticity in normal learning and addiction
- Memory, hippocampus, accumbens, opioid, heroin

49. Misfolded protein and neurodegenerative disease
- Prion, amyloid, seeding, neurodegeneration, TSE

50. Neuronal and glial AMPA and $GABA_A$ receptors in health and disease
- Synaptic transmission; neurons: neurological disease

51. Studies to Find Improved treatments for Movement disorders
- Parkinson's disease Dystonia Neurodegeneration Neuroprotection Symptomatic treatment

52. Neurovascular coupling in health ageing and disease
- Neurovascular coupling, Dementia, Ageing, Epilepsy

53. Animal models of neurodevelopmental disorders
- Rat, behaviour, pregnancy, gut, brain

54. Antibodies to neuropeptidergic signalling molecules
- Antibody Neuropeptide Evolution Echinoderm

55. Basic mechanisms of chronic neurodegeneration
- TSE, neurodegeneration, mouse models

56. Connectivity and plasticity of developing and mature central nervous system circuits
- Brain development, neurons, synapses, axons

57. Structural and functional plasticity in cortex
- Brain, plasticity, degeneration, synapses, learning

58. Studying a human neurological disease-causing gene
- Epilepssy, motor neuron disease, autism

59. Spinal sensory processing
- Somatosensory, dorsal root ganglion, spinal, analgesia

60. The role of neuropeptides in behaviour
- Vasopressin, oxytocin, social behaviours

61. Memory in the rat

- Memory, learning

62. Neural basis on spatial learning and memory

- Neural, spatial, learning, memory

63. The neural basis of spatial cognition and memory

- Rat, mouse, single neurons, behaviour, spatial memory, navigation

64. Mechanisms contributing to analgesic use & misuse

- Opioid, GABA, neurotransmission, stress, nociception

65. Functions of the murine Trappc9 gene

- Brain development, stem cells, microcephaly

66. Mechanism-based targets for new analgesics

- Analgesia, molecular targets, somatosensory, sensitisation

67. Encoding Behaviour From Synapses To Circuits

- Behaviour, Neurons, Communication, Brain, Experience

68. Understanding successful brain repair in zebrafish

- Traumatic brain injury, tissue repair

69. Ion channel function and epileptogenesis

- Ion channels, epilepsy, brain

70. Neuroprotective treatments for traumatic injury

- Neuroprotection, spinal cord injury, regeneration, nerve injury, repair

71. Repairing the damaged peripheral nerve

- Sciatic nerve, axon regeneration, neuroprotection, scarring, nerve conduits

72. Cell-specific chromatin profiling in mouse cortex

- Cerebral cortex development; Targeted DamID

73. The Role of Central Sympathetic Control Neurones

- Cardiovascular control, paraventricular nucleus

74. The molecular and cellular mechanisms that underpin CNS plasticity

- Stem cells, myelin, regeneration

75. Recovery of peripheral nerve function

- Nerve, bladder, physiology

<table>
<tr><td>Project 1</td><td>Brain plasticity with experience and recovery</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Plasticity, learning, stroke, exercise, experience</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>We aim to understand how experience can change brain structure and function. The types of experience we will study include learning (e.g., learning a new movement skill), physical exercise, and rehabilitation after stroke. We will study brain changes using both brain scans (MRI) and post-mortem microscopic measures. Brain scans are commonly used to study brain change in humans, but it is not possible to know which microscopic changes are causing any observed change in brain scan measures. By acquiring both brain scan data and microscopic information in the same animals, our project will allow us to test which microscopic changes underlie observed changes in brain scans.</td></tr>
<tr><td>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)?</td><td>Brain scans are commonly used to study people with brain disorders. However, brain scans typically give us indirect measures of microscopic features of the brain tissue. For example, they might tell us about water content, rather than the number of cells in a brain area. Our work will help to interpret brain scan information in terms of the underlying biology. The underlying biology is relevant to understanding clinically important questions such as how people recover after stroke. We therefore hope that the work</td></tr>
</table>

	will help to identify measures from brain scans that could be useful clinically, for example in guiding rehabilitation after stroke.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 3375 rats and up to 3375 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?	The experiments will involve testing the ability of rats and mice to perform behavioural tasks in which they have to learn and remember new skills or new information, such as how to reach for a pellet of food from a food-well. We will also test the effects of different experiences of behaviour and brain function. For example, we may give some animals access to running wheels for physical exercise. This could involve single housing for periods of time. We also assess anxiety, for example, by testing whether the animal prefers to explore a new place or remain in a safe location. We will examine the effects of brain lesions, drug treatments and genetic mutations on these behaviours, and record signals of brain activity and brain structure using MRI scans under anaesthesia. The expected adverse effects would include brief periods of mild distress as a consequence of motivation to complete some of the behavioural tests (e.g. after a mild footshock). There may also be pain and discomfort after brain surgeries and specific expected effects as a consequence of the brain lesion such as difficulty moving. The experiments are of moderate severity. Animals will be humanely killed 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	In order to test how experience shapes brain structure at a microscopic level, and to relate this to brain imaging measures, is it necessary to do experiments in which brain scans can be taken in animals that can then be killed to perform histological measurements. This is not ethical (or practical) in humans. Computer simulations of the brain actually rely on the information that we will provide and so cannot replace the work that we do.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimize the numbers of animals used by making both the behavioural tests and the experimental manipulations (e.g. lesions, genetic modifications) as accurate and sensitive as possible.
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 work on rats and mice because they are the lowest vertebrate group which reasonably resembles humans. Operations on the brain are done very carefully and in state-of-the-art surgical theatres, and the animals are given pain killers after the operations until they have fully recovered. Soon after the operations you would not be able to tell the difference between treated animals and controls as they behave in their home cages. It is only on the sophisticated tests of learning and memory that you can begin to tell them apart.

<table>
<tr><td>Project 2</td><td>Neuronal network activity underlying behaviour</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Brain, neuron, plasticity, cognition, neuromodulator</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall goal of this research is to understand the processes and mechanisms that underlie neuronal network behaviour. In particular, we aim to understand how environmental cues and experience influence neuronal networks and the computations they perform. Ultimately, the findings of this research will be used to shape new strategies to improve cognitive function in people with cognitive impairment.

Our first objective will be to determine where specific neuromodulators are released in the brain at high spatial and temporal resolution during behaviour since this information is not currently available. This information is important to inform experiments to define the impact of neuromodulator release and experience-dependent plasticity on the network processes that underlie cognition.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the</td><td>We aim to understand how the activity of neuronal networks enables cognitive function. We also aim to uncover where and when neuromodulator transmitters are released in the brain and how they change cellular and network processes. Since most psychoactive medications act through</td></tr>
</table>

project)?	neurmodulatory systems, ultimately, the findings of this research will be used to shape new strategies to improve cognitive function in people with cognitive impairment.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse – 13000 Rat – 2000 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 level of severity? What will happen to the animals at the end?	To generate appropriate experimental animal models, we will breed genetically-altered rodents, including those which have genetic alterations which mimic human disease. Some of these genetic alterations have effects on brain function but do not cause pain or extended suffering. We will use expression of genetic material in the brain of some animals to enable measurements of brain function. This involves injection of genetic material into the brain under anaesthesia and its subsequent expression. There are small possibilities of adverse effects from the surgery used for injections but in the majority of cases the effects of the gene expression are not expected to cause any prolonged suffering. To enable us to record and manipulate brain activity, we will implant animals under anaesthesia with small devices in the brain or on the skull. This will include electrodes in the brain to record electrical activity. These implants are inserted with surgical precision to minimise suffering or pain and the implants themselves are designed to allow the animals to behave normally after surgery. Behavioural tests of cognition are designed to make use of animals' natural curiosity and therefore minimise stress. Although the procedures may cause mild pain and stress, such manipulations do not cause any long-lasting pain or suffering. All animals 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	To investigate the mechanisms underlying mammalian cognition we need to use intact mammalian brains. Invertebrate nervous systems do not contain the same level of complexity or enable the same behaviours as mammalian systems. Whilst we will use computer modelling to

	assist our investigations they cannot replace the sophistication of experimental systems. It is not possible to make the kind of invasive recording of neuronal activity in humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our goal is to design experiments to ensure the maximum statistical power is achieved for the minimum number of animals. To achieve this we will seek assistance from statisticians and our collaborators who have extensive experience in the design of experiments involving animals. In addition, we aim to record from genetically defined populations of neurons that reduces the variability of recordings and therefore enhances statistical power and reduces 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.	To investigate the bases for mammalian cognition we need to study the intact mammalian brain. Rodents offer the ideal combination of cognitive ability, genetic manipulation and accessibility to recording. In addition, the anatomy of rodent brains is comparable to human and many of the cognitive functions attributed to specific regions of the human brain translate directly to specific rodent brain regions. Furthermore, rodent models of disease enable pre-clinical proof-of-concept testing for strategies to reduce disease symptoms and/or progression. In particular the tg4510 mouse model of Alzheimer's disease has been well characterised and exhibits many of the expected cognitive deficits. Whenever possible, selective genetic manipulation of subsets of neurons will be used to reduce the risk of harmful phenotypes. During surgery, animals will be anaesthetised to minimise suffering and maximise the effectiveness and efficiency of procedures. They will also be given analgesia as appropriate and temperature and fluid balances closely regulated. To minimise stress during training and testing on cognitive tasks animals will be habituated to the task and the environment in advance. Tasks are also designed to allow animals to benefit from their innate exploration behaviours.

<table>
<tr><td>Project 3</td><td>Neural mechanisms in health and disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neurophysiological function, neurophysiological dysfunction, ageing</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
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<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To understand how networks of nerve cells in the brain give rise to physiological functions and to identify abnormalities in these networks that cause dysfunction associated with cognitive/emotional, motor or metabolic disorders.

There are many diseases rooted in the brain such as cognitive, degenerative or metabolic. A lot of research and many scientific discoveries have been made in the past years with the aim to understand causes, prevention and cure.

However, what has become clear is that due to the high complexity of the brain not only in terms of structures but also because of the enormous heterogeneity of types of nerve cells, it is necessary to tackle these issues using the most precise model organisms and up-to-date and sophisticated technologies.

This is mainly because to restore neuronal functions requires that we first understand how nerve cells function at the cellular and molecular level, and how they connect and form complex networks that</td></tr>
</table>

	underlie specific behaviours and functions.
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 provide new insights in the molecular mechanisms that control the activity of nerve cells, and will contribute to the understanding of high order functions such as learning and memory, emotion, as well as motor behaviour and metabolism. It will also advance our understanding about the contribution of specific molecules to these functions. Ultimately, advancing knowledge into these mechanisms may well lead to new or improved treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, based on our experience with the use of genetically modified mouse models we expect to use approximately 11,000 animals over the 5 years. Most of these will be in the breeding programmes that will generate the genetically modified mice that we need.
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 animals will be used in a protocol with a mild severity limit such as Protocol 1, breeding and maintenance of genetically modified animals. Animals produced under this protocol are not expected to exhibit any harmful phenotype. In the case of unexpected and unwanted harmful phenotypes the animal will be humanely killed, or in the case of animals of particular scientific interest with an unexpected harmful phenotype, advice will be sought from the **NVS/NACWO**. Three protocols have a moderate severity limit; one involves aging and the other surgery. A smaller proportion of the animals will be used in these protocols and we envisage that for many of the animals the actual severity will be mild. In particular, mice on Protocol 2 will be behaviourally tested at different stages and so mice will be aged. These animals are not expected to show specific problems as they age. In some, after 12 months of age learning will deteriorate quicker than normal mice. However, ageing animals will be monitored closely including weighing, clinical examination and body condition scoring in consultation with the NVS, and any adverse effect due to a particular behavioural test will be dealt with accordingly. Mice on Protocol 3, instead, could present adverse effect due to surgery; these will be treated according to the effect presented. Mice on Protocol 4 will be analysed for metabolic dysfunctions. The severity limit of this protocol is mild, and there are no major adverse

	effects expected. Protocol 5 deals with breeding and maintenance of genetically modified mice with a moderate severity limit. Therefore, animals produced under this protocol are expected to exhibit harmful phenotypes. These animals will be inspected carefully and closely monitored to ensure they do not exceed a moderate severity limit. Animals exhibiting any unexpected unwanted harmful phenotypes or in case the severity is likely to exceed moderate will be killed, or in the case of animals of particular scientific interest with an unexpected harmful phenotype, advice will be sought from the **NVS**/**NACWO**. The work has been organized in stages, first of all a precise analysis of the genetically altered mice will be obtained from mice humanely killed which will suffer only transient pain and distress. Once preliminary information are obtained we will then proceed with *in vivo* functional validation of the identified gene(s)/cell type involving stereotaxic injections into the brain. Good animal care, husbandry, health checks based on veterinary advice will be applied to all animals that go through surgery. This will help identifying any adverse effect as soon as it appears. In this event appropriate steps will be taken to minimize it. Clear end points have been established.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The type of investigation outlined in this project cannot be carried in cell cultures as these lack information about the networks of neurons that are found in an intact brain. Furthermore, cell cultures do not replicate disease progression. Manipulation of the mouse genetics provides a unique opportunity to study the molecular basis of complex cell-cell interactions *in vivo*. The increasing availability of cell-type specific promoters gives particular power to the generation and analysis of precise mouse models that undoubtedly will help dissecting the complexity of the brain.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Generation of very precise and specific genetically altered mice results in more defined, less variable and hence more relevant data. This correspondingly reduces the number of animals necessary to obtain significant results as well as improves the quality of life of the 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 general measures you will take to minimise welfare costs (harms) to the animals.	Mice remain one of the best species to study brain mechanisms in health and disease. Moreover, being a mammal, the similarities in the brain between mouse and man will allow the transfer knowledge to human more easily. We will provide an environment that will meet the animals' specific needs, such as enriched cage environments with as little stress to the animals as possible. This, of course, will allow us to obtain more reliable results. Finally, understanding these molecular mechanisms will undoubtedly impact on the development of therapies to improve high order functions such as learning, memory, emotion, motor behaviour and brain metabolism.

<table>
<tr><td>Project 4</td><td>Circuit mechanisms governing network oscillations</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>septum, cortex, oscillations, theta, synapses</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
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<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To understand how oscillations in the cerebral cortex are implemented by a region of the basal forebrain known as the septum. Neurons in the septum that release the neurotransmitter GABA target other GABA-releasing cells in the cortex but the activity of most kinds of septal neurons during behaviour is unknown. Moreover, the cortical targets of individual septal cells are unknown. It is well-established that acetylcholine-releasing neurons in the septum degenerate in Alzheimer's disease, but changes in GABA-releasing septal neurons and the behavioural consequences in neurodegenerative diseases is largely unexplored.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or</td><td>A greater understanding of the neural circuitry of the septum, the definition of cell types and their projections to the cortex. This includes projections to the hippocampus, a region of the cortex</td></tr>
</table>

animals could benefit from the project)?	important for learning and memory. If we define how oscillations are implemented by the septum, and how the cholinergic and GABA-releasing septal neurons change activity during behaviour in Alzheimer's disease animal models, we may benefit Alzheimer's disease patients, by understanding how to intervene for treatments at earlier stages of the disease, such as mild cognitive impairment. Influencing the activity of the GABA-releasing septal neurons may be key to these benefits.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use mice and to a lesser extent rats over 5 years. Maximum 5,000 mice (excluding surplus generated from breeding genetically-altered mice, ~5,000). Maximum 1,900 rats (excluding surplus generated from breeding genetically-altered rats, ~800).
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?	Since most protocols involve cranial surgery, we expect moderate severity. During our 2-4 hour surgeries, we will typically make very small windows in the skull to gain access to the brain, implant tiny screws and probes, then seal everything with dental cement. The animals rapidly recover within 1-3 days (depending on species and length of surgery). The actual experiments to collect data will be mild (e.g. behavioural tasks whilst recording brain activity with implanted probes). Most animals will be fixed with paraformaldehyde after completing the experiments in order to study the recorded or labelled cells in sections of the brain under a microscope. We may use genetically-altered rodents that model some aspects of a memory-related disorder (e.g. Alzheimer's disease). These animals will be treated in the same way as wild type animals. We expect performance in memory-related behavioural tasks to decrease with age. In rare cases, we may use genetically-altered lines that may develop some motor impairments (an additional phenotype of some Alzheimer's disease rodent models); these impairments develop later in life and it is more likely that we would use these animals to obtain brain tissue for comparative

	studies rather than *in vivo* recording experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are studying how the brain is put together and how individual nerve cells contribute to the networks of nerve cells in the brain, and studying how these change in models of disease. It is thus not possible to use an alternative approach.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will ensure that we use the minimum number of animals: - by careful monitoring of the breeding programme to ensure that we do not breed excessive numbers of animals - by use of our multidisciplinary approach that allows the maximum amount of data from individual animals - by good experimental design, the application of the most appropriate statistical analyses and regular consultation with the University statistical services.
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 rats and mice to study the septum and cortex in health and disease since the principles of organisation and operation of these regions seem to be similar throughout all mammals, they are thus a good model of the human septo-cortical system. The best characterised models for dementias such as Alzheimer's disease are in the mouse. We minimise welfare costs to the animals by the highest standard in animal husbandry, the highest standards in surgical procedures and peri-operative care that is equivalent to the highest veterinary standards.

Project 5	**Affect and cognition in rodents**	
Key Words (max. 5 words)	Affect, cognition, consumption, microstructure	
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)	Affective processes are a key determinant of behaviour, and responding appropriately to rewards (and punishments) as well as being sensitive to their environmental contingencies, is a vital part of flexible and adaptive behaviour. Failures of affective and cognitive processing are associated with a number of human psychiatric disorders. This project has three main objectives: to improve the understanding of the psychological and biological mechanisms underpinning normal affective and cognitive processes; to examine how and why these processes might break down in rodent models related to psychiatric dysfunction; and to examine how common laboratory techniques might impact on affective and cognitive processes in the context of laboratory animal welfare.	
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)?	Psychiatric disorders are a major health burden in the UK (and worldwide) with treatment-resistant aspects of the negative symptoms such as anhedonia a particular problem. One particular bottleneck in developing novel therapeutic strategies is a lack of targets for drug discovery and screening. This project will develop the understanding of affective and cognitive function in rodents, and assess	

	dysfunctions analogous psychiatric disorders. Such outputs are clearly beneficial to the drug development process by having the promise of identifying behavioural and biological phenotypes that as targets in the pre-clinical screening of potential therapeutic techniques and the development of drug-development targets. Many thousands of rodents are used annually in laboratory work based involving techniques such as injections. The welfare impact of these techniques has received little direct scientific investigation, meaning the aversive impacts, and means to minimise them, are not known. This project will provide information about how aversive some common techniques are and about ways to minimise or avoid that harm.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats – 1600 Mice – 300 Project length – 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 procedures used here are not expected to produce severe adverse effects. The key behavioural observations involve the detailed examination of animals' responses to foods and flavours – something that is not informative if there are non-selective impacts on the general welfare and behavioural competence of the animals. That said, some of the procedures will be aversive to some degree – e.g. producing transient pain or nausea. If any animal shows undue or extended distress during the study, we will consult with veterinary services and where appropriate humanely euthanise the animal. In all other cases, animals will be humanely euthanized at the end of the experimental procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Affect and cognition are fundamentally properties of whole organisms interacting with their environment. To investigate both normal function, and its disruptions, requires animals as cellular and computational models cannot yet capture the complexity of brain circuits.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise numbers by using the most powerful analytic techniques; by re-using animals (when it will not increase the severity of the procedures or compromise the integrity of the data); and by avoiding duplication of experimental or breeding techniques through collaboration with other 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 were chosen as they allow the application of the most sensitive and well understood techniques for assessing affective and cognitive responses, and are also suitable for biological investigation. Harms to the animals will be minimised by selecting the least invasive means for achieving any experimental goal. The selection of techniques will be informed by the research on the project itself concerning the refinement of laboratory methods and in consultation with animal support and veterinary staff.

Project 6	**Role of neuroinflammation in depression and cognition**	
Key Words (max. 5 words)	Depression, inflammation, inflammasome	
Expected duration of the project (yrs)	5 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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Depression and related mood disorders are among the world's greatest public health problems. By 2020 depression will become the second most important cause of disability worldwide (after ischaemic heart disease) according to the World Health Organization. Existing drug treatments are effective in around 40% of patients but are accompanied by delayed onset of action and a significant side effect profile. There is an unmet medical need to develop alternative treatments. Neuroinflammation is increasingly recognized to play a role in the depression. Following a bacterial or viral infection, people experience changes in mood, appetite and interest in their environment that resemble depression. These symptoms are mediated by chemicals called cytokines that initiate an inflammation as part of the body's normal response to infection. Cytokine levels are raised in patients with major depression and reduced by antidepressant treatments. In animal studies, exposure to a bacterial toxin (lipopolysaccharide, LPSLP) causes inflammation in the brain, including the release of cytokines. This has led to the idea that cytokines can trigger depression. In this project we will examine the signalling pathways involved in the inflammation response that	

	lead to depression
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 advance the science of depression by studying the basic cellular mechanisms underlying depression, induced as a result of inflammation in the brain. Specifically we will identify the contribution of a protein complex called the inflammasome, expressed in microglia cells, in developing depression. Our long-term aim is to identify drug targets, which new candidate antidepressants can be directed against to benefit depressed patients that are resistant to existing treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate that we will use 1000 mice per year, 5000 mice over 5 years, including genetically modified mice of mild phenotype.
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?	Genetically modified mice of only mild phenotype will be used. The majority of experiments are likely to be moderate in severity. The administration of LPS will induce inflammation and the animals will become sick transiently, evidenced as loss of body weight (10-14%) and decreased locomotion. 24 hours after the last LPS dose, the sickness resolves but a change in depression-related behaviour is evident. The severity of this procedure is limited by using minimally effective doses of LPS and daily monitoring of condition and behaviour of mice. Repeated stress protocols used to examine the interactions between stress and inflammation are also considered of moderate severity. Severity of stressors will be limited by using the minimally effective level of stressor and by daily monitoring condition and behaviour of mice following stress. Moderate steps in the protocol also relate to surgical procedures for implantation of depot devices for e.g. antidepressant drug delivery or implanting guide cannulae for injection into the ventricles of the brain. Good surgical technique, close monitoring and use of appropriate analgesia will limit the severity of these procedures. At the end of the procedure all animals will be killed to facilitate study of their tissues which will allow us to achieve our scientific objectives.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	Mood and its regulation is a complex behaviour involving a network of brain regions which can only be studied in a whole behaving organism. Dissecting the molecular signalling pathways implicated in the

use non-animal alternatives	inflammation response in depression cannot be done in humans as obtaining the appropriate samples would not be ethical. In vitro studies using isolated tissues and cultured cells are conducted in parallel and inform the in vivo studies in this project.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In this project we will use a multidisciplinary approach to maximize the data obtained from each animal e.g. we will identify molecular pathways (using ex vivo tissues for gene analysis, cell cultures) and behavioural effects of inflammation in the same animal. A power calculation, based on prior experience with the behavioural measures, will be used to establish the necessary sample size for 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.	Mice are the lowest vertebrate animals in which well-characterized, pharmacologically validated models of depression-related behaviours have been established. A wealth of literature on the anatomical circuits, neurophysiology and pharmacology of depression-related behaviours exists in mice. Mice also express the protein complex we are particularly interested in studying. The majority of the procedures in Protocol 1 and 2 are mild. The behavioural tests are non-invasive and exploit normal rodent behaviours e.g. exploration, escape from mildly stressful test environments. Moderate steps in the protocol relate to (1) surgical procedures: severity of the surgical procedures is limited by good surgical technique and appropriate use of anaesthesia and analgesia (2) repeated stress protocols: the variable stressor paradigm, including electrical foot shock has been piloted in mice (BALB/c). In pilot studies with C57/Bl6 mice we will establish the minimal effective stimuli required to obtain a depression-related phenotype. Welfare will be monitored and there are appropriate controls and humane-end points to limit suffering.

Project 7	**Genetically modified rodent models of neurodegeneration**	
Key Words (max. 5 words)	Alzheimer's; Parkinson's, Motor neuron disease; transgenic rodents; novel therapeutics	
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)	The objective of the project is to use better rodent models of Alzheimer's, Parkinson's, motor neuron disease and related disorders to understand the molecular basis of disease and to use for testing novel therapies. The work described in this project will directly address this by characterising new transgenic strains of mice and rats which develop behavioural symptoms and biochemical changes in the brain highly reminiscent of neurodegenerative disease. We will use these rodent models of neurodegenerative disease to elucidate the molecular mechanisms underlying disease processes. Finally, we will use these rodent models of disease to test the efficacy of potential novel molecular therapeutics for 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	Neurodegenerative diseases, such as Alzheimer's, Parkinson's and motor neuron disease, represent a growing public health problem due to our aging population. As our population ages, the number of cases of neurodegeneration will increase sharply	

project)?	over the next 30 years, placing a high burden on our healthcare services. Neurodegenerative diseases are devastating illnesses which reach us all through affected friends and relatives. If we are to understand more how the diseases develop, and how they may be cured, we need to explore new avenues of scientific research, including improved cellular and animal models of disease. The work in this project will give us an improved understanding of what causes neurodegenerative diseases and how we might develop new treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will support the work of many scientists across several laboratories looking at modelling a range of diseases. Over the five years we expect to use up to 20,000 mice and 6,000 rats, of which only a small proportion (approximately 25%) will be allowed to age and develop disease-related symptoms. The rest will be breeding animals of a young age.
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 study strains of rats and mice carrying forms of human genes involved in neurodegenerative disease, such as Parkinson's, Alzheimer's and motor neuron disease. Most of the animals we will use will be young breeding animals used to maintain the strains. As neurodegenerative diseases generally affect old people only animals which grow into old age will develop symptoms which in rat and mouse terms is about 2-3 years old. A proportion of the animals (about 1/3) will therefore be allowed to grow old and develop symptoms reminiscent of a neurodegenerative disease of old-age, such as motor deficits and memory loss. Animals may also be treated with compounds to model the disease. Once we have identified disease-relevant changes in our mice and rats we will then test new therapeutics delivered orally, or injected into the body, or injected directly into the brain using surgery, to attempt to cure the disease. Animals will be very closely monitored following the onset of any symptoms and the disease will only be allowed to progress to a limited degree. 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	The animal work we do runs in parallel with other complementary approaches in our laboratory. First, we study diseases using neuronal cells cultured in dishes which is useful for generating preliminary data on disease processes. In particular, we work with patient-derived stem cells which can be used to generate different neurons of interest, such as dopaminergic, cortical and motor neurons. This new stem-cell technology represents an exciting platform both to investigate mechanisms of disease and screen for novel molecular therapies which impact on cellular disease processes and may have therapeutic value. The work in cultured cells tells you how neurons may die but not how the brain as a "wired circuit" will respond to disease. Second, we study human post-mortem brain tissue donated by patients who have died from neurodegenerative disease. Both approaches are very useful but have limitations. Human patient post-mortem brain material is very valuable to study, but it does only represent the end-stage of the neurodegeneration, and provides limited information on the mechanism of disease progression over time. The human brain is an inaccessible and highly complex organ which makes it almost impossible to obtain patient "brain samples" during life. We therefore need to use animals in our project but only after we have obtained as much information as possible from those experiments which do not require animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Previous experience and statistical power calculations allow us to define the number of experiment we need in each experiment to observe alterations in behaviour or to detect biochemical or pathological changes. Approximately 10-20 animals per strain are allowed to grow into old age for each experiment and to develop symptoms, which in rat and mouse terms is about 2-3 years old. We will test behaviour, co-ordination and memory of the ageing rodents, perform imaging studies, and finally, analyse the brain in detail when they die. By performing many tests on each animal, we will minimise the numbers

	of animals we need to use.
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 in our work as we need to study the effect of disease genes on the mammalian brain. Mice and rats conserve many of the brain structures found in humans and affected by disease which we need to study and represent the simplest mammal we can work with. Modern accurate genetic techniques act as a refinement and allow us to manipulate genes in rats and mice and express human disease genes in the right part of the brain at the right time to lead to disease processes and symptoms highly reminiscent of the human disease being studied. As neurodegenerative diseases generally affect old people we expect the vast majority of the young animals we keep will not develop any disease symptoms. In each experiment approximately 10-20 animals will be allowed to grow into old age, which in rat and mouse terms is about 2-3 years old. Some of these animals may develop symptoms of disease towards the end of their life. Animals will be very closely monitored following the onset of any symptoms and the disease will only be allowed to progress to a limited degree.

Project 8	**Delta-protocadherins in cortical development**	
Key Words (max. 5 words)	Cortex, development, delta-2 protocadherins, Pcdh19, EFMR	
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 objective of this project is to investigate the functions of a particular subfamily of proteins, the delta-2 protocadherins, during development of the cerebral cortex. Different steps are necessary for the correct formation of the cortex: neurons of different types need to be generated at a specific time and in correct numbers. Then these neurons have to migrate to their final positions in the brain and find the appropriate targets to connect to. We will analyse the involvement of the delta-2 protocadherins in the different processes that contribute to the correct formation of the cortex. There is not much known about the functions of these proteins, but because of where and when they can be found in the brain, they are expected to be involved in the development of the cortex. A special focus will be put on one of the members of this family, Pcdh19, which is mutated in the human disorder epilepsy and mental retardation limited to females (often abbreviated EFMR, also known as Juberg-Hellman Syndrome). EFMR is a rare disorder, but it is believed that up to 1 in 10 girls that develop seizures before the age of 5 might have Pcdh19 epilepsy. The link between mutations in the *PCDH19* gene and EFMR was made in 2008, but so far the	

	function of Pcdh19 in brain development in humans remains unknown.
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 is basic neurobiology, but it could eventually be relevant from a clinical point of view in the future. The expected results would translate into two potential benefits: - On the one hand it will generate new knowledge about the function of the delta-2 protocadherins during cortical development, contributing to our understanding of brain function. - On the other hand, it will shed light on the pathophysiology of EFMR, providing information to affected families to help them understand the disease. That information could also be useful for clinicians in the development of new treatments. In addition, this project could help identify new potential genes involved in epilepsy if they interact functionally with Pcdh19.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5-year duration of the project, approximately 2450 adult and 9500 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 level 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 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 will most probably 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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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.
2. Reduction Explain how you will assure the use of minimum numbers of animals	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.
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.	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 9	**Plasticity and function of the visual system**	
Key Words (max. 5 words)	Primary visual cortex; amblyopia; neurodevelopmental disorders; glaucoma	
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)	We are aiming to achieve a better understanding of how the visual system works and develops at a cellular level, both in the young and the adult, in order to find new ways to tackle a range of disorders of vision as well as of brain development more generally.	
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. A detailed knowledge, at the single-cell level, of how one might enhance or restore the ability of the adult brain to adapt to change will help us in refining novel means of intervention in the treatment of amblyopia (‘lazy eye’) in humans. This is a condition affecting up to 4% of the population for which no established treatment works beyond the age of 7. 2. A better understanding of how genetic defects that affect the ability of the brain to learn and adapt can lead to conditions such as autism spectrum disorders will point the way to the development of drugs tailored to compensate for the molecular deficits, or to gene therapy targets. 3. An investigation of how input from higher brain areas to the visual areas affects vision will provide insights into the way higher brain functions such as attention and memory are interlinked with sensory	

	processing. 4. A better understanding, in an animal model of glaucoma, of the sequence of events that leads to to the death of cells in the retina and the role of nerve growth factors in maintaining a healthy retina may point the way to a future treatment option for the second most common cause of blindness in the UK (accounting for 18% of all cases of blindness).
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, 2500 animals 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 level of severity? What will happen to the animals at the end?	Animals will be surgically prepared, under general anaesthesia, for brain imaging studies. Post-operative pain of moderate severity is the most likely adverse effect. In rare cases, infections may occur. All animals will be humanely killed in 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 function of the visual system can at present only be studied in intact animals which can integrate sensory experiences over time and produce behavioural responses in return. While we can study certain questions in brain slices it is impossible to maintain a whole brain and eyes alive in a dish, and even if this were possible, we would be lacking a behavioural readout. Computer-based modelling can help us to interpret our results; however, real data collected in vivo are needed to feed into any models to ensure they have a sound basis.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Chronic (longitudinal) studies will be used extensively, an approach that reduces the total number of animals both by obtaining more data from each animal and by increasing their statistical power. In addition, we will use cutting-edge imaging techniques which enable us to gather information on whole areas of cortex and many individual neurons at the same time, again ensuring that fewer animals are needed.
3. Refinement Explain the choice of species	The mouse is our species of choice for all experiments. Compared with animals models previously used in visual neuroscience such as cats

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.	and ferrets they have a shorter developmental time span and allow easier visual access to the brain for imaging purposes. But above all, they offer the advantage of ready genetic modifiability not available for any other mammalian species. This is critical for examining the function of specific genes/proteins in cortical plasticity e.g. using knock-out models. For all surgical procedures, animals will be under general anaesthesia. Analgesics will be given prior to surgery. Post-operative care will involve the use of analgesics and antibiotics where necessary. During post-operative recovery, animals will be closely watched until anaesthesia has worn off. Afterwards their health will be checked regularly.

Project 10	**Brain systems for rodent memory**	
Key Words (max. 5 words)	Brain, systems, rodent, memory	
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)	Particular structures in the brain are assumed to be vital for memory but are not critical for a wide range of other mental abilities. This brain specialisation is revealed in amnesia, when the ability to learn and remember day-to-day events ('episodic' memory) is lost while abilities such as IQ are intact. For a long time researchers have sought to identify those structures critical for memory and discover how they might interact to form systems that support learning and retrieval. Current research is largely focused on just one structure, the hippocampus, despite overwhelming evidence that other sites also play a vital role in learning and memory. Outstanding goals are to identify those other key brain sites for memory, while also determining if and why they interact with the hippocampus. My research seeks to define and better understand the network of brain sites that support learning and memory. In order to answer this question it is necessary to study the brain at levels of resolution not possible in human imaging or clinical studies. For this reason the research examines learning and memory in rats and mice. The goals are to understand the fine details of how multiple brain sites are interconnected and how these patterns of interconnections reflect different roles in memory. Behavioural tests of learning and memory that mimic features of human memory are, therefore, used.	

	Consequently, the experiments often measure the impact of specific lesions in key brain sites or critical pathways, as well as isolating and measuring memory-related activity in groups of brain structures.
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)?	Normal people vary in their ability to learn. Part of the reason arises from variations in the status of particular brain structures and their interconnections. This same issue is highlighted in clinical conditions that affect memory, either selectively (e.g. amnesia) or in combination with other abilities (e.g. dementias). In all of these clinical conditions there is injury to structures that support memory. The task is to identify and better understand brain structures that contribute to normal learning, while identifying sites that may be vulnerable in neurological disorders associated with memory loss. By uncovering networks across the brain that support memory the research not only provides a more comprehensive picture of how our brains solve this vital function, but helps our understanding of conditions that impair memory. This second benefit is particularly relevant for those conditions that produce more diffuse pathologies, e.g. traumatic brain injury. The results thereby help to identify clinical targets for therapy.
What species and approximate numbers of animals do you expect to use over what period of time?	The research will use approximately 1870 rats and 270 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?	In order to understand rodent brain systems for memory it is necessary to include behavioural measures that show when experiences have been acquired. For this reason it is necessary to study the behaviour of animals not suffering additional problems that may affect basic sensory or motor functions, or alter levels of motivation, such as feeding or drinking. Any interventions will be of mild or moderate severity and will typically be acute. Any chronic adverse effects would be counterproductive to the planned research as behavioural changes could not be interpreted. To determine the roles of specific brain sites it is necessary to either measure or modify their activity. For this reason, techniques will include those that target individual brain sites. These techniques include both permanent and temporary surgical lesions, which will demonstrate how vital a specific structure might be. Animals are allowed to recover fully from surgery so that

	they can perform behavioural tests of learning and memory, without the potential confound of nonspecific effects consequent on surgery. In order to verify brain manipulations it is necessary to examine brains *post mortem*. In these circumstances, animals will be deeply anaesthetised and then killed using, approved methods. To analyse memory it is necessary to apply a variety of behavioural tests that address different forms of learning. Food and/or water restriction may be used to ensure reliable responding over many trials, so strengthening any results. Mildly aversive conditions, e.g. swimming in a water tank, are sometimes used in rat spatial tests as such tasks employ a natural behaviour (swimming) to create conditions for very specific forms of learning. More aversive stimuli, e.g., mild foot shocks, are only used very rarely and only when other methods are exhausted. Such techniques have a place when persistent learning following a single (or rarely repeated) event is required. At the completion of a study, rodents will typically be killed by overdose of a general anaesthetic so that their brains can then be removed and examined.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The goal is to understand the interplay between multiple brain sites and how they support learning and memory in the mammalian brain. The complexity of these interactions, embedded within the many unknown structural details of the brain, means that it is necessary to derive information from intact organisms. It is also necessary to validate learning, e.g., through behaviour. Specific issues concern the anatomical resolution of the proposed analyses and the fact that some of the key target areas, e.g., individual thalamic nuclei, are not prone to selective pathologies in humans and cannot be distinguished using non-invasive imaging techniques for humans, e.g. MRI.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By refining behavioural tasks, e.g., the 'bow-tie' maze, which provides reliable measures of recognition memory with reduced variance in group scores. Both attributes reduce the numbers of animals needed in an experimental group. By using techniques that allow the simultaneous examination of multiple brain sites (rather than study different sites in different experiments). An example is the use of advanced statistical methods (e.g. structural equation modelling) to model patterns of brain activity in

	multiple sites of interest (from imaging methods such as MRI or immediate-early gene activation). A related refinement is to combine data sets, where possible. An approach that is feasible with structural equation modelling. By combining techniques in the same animal, e.g. the mapping of brain connections with tracers alongside measures of their individual activity (e.g. from immediate-early gene expression). By combining tracing techniques that can be distinguished within the same brain, e.g. using multiple fluorescent tracers with different wavelengths. By the use of non-invasive imaging techniques (e.g., MRI) that permit repeat analyses, i.e., longitudinal studies
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 goal is to understand the human brain. Consequently, it is necessary to examine a brain that contains the same structures as found in the human, with similar patterns of connectivity. A mammalian species is, therefore, required. Rodents (rats and mice) are most appropriate for network analyses as we already possess detailed information about the interconnections between brain nuclei of interest. This information is at a level of resolution typically only available for rats or mice (and does not exist for humans). It is also necessary to examine animals with appropriate cognitive/behavioural abilities that can be related to human abilities. The research goal is to determine how and why structures interact with each other. We will, therefore, pursue new technologies (e.g. with viral vectors) that make it possible to target and temporarily disrupt just those nerve cells in structure A that project to structure B. This level of refinement provides precise disconnections that will determine the anatomical steps in any cognitive network, while removing confounds in current methods. For reasons already explained, the research centres on the measurement of behavioural change associated with learning and memory. Any procedure associated with chronic discomfort or changes in sensory-motor performance would be unusable as the findings could not be interpreted. Any manipulation would, therefore, have to involve, at most, only acute welfare costs.

Project 11	**Genetic and functional studies in neurodegenerative disease**	
Key Words (max. 5 words)	Neurodegeneration, Alzheimer disease, Down syndrome, motor neuron diseases, mouse	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Yes	Basic research
	Yes	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
	Yes	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Neurodegeneration is an increasing healthcare and social burden, and personally devastating for those affected and their carers. We have almost no treatments for any form of neurodegeneration, and certainly no cures. Such disorders are thought of as late-onset, but this is not necessarily the case. We have some route into understanding neurodegenerative disease through the familial forms of disease and the genetic cases. If we can 'model' these genetic forms of neurodegenerative disease, we can start to understand the biology of disease by studying mice carrying the same mutations. The diseases we are focussed on include the Alzheimer disease may arise as a consequence of trisomy 21 (Down syndrome) and include different forms of motor neuron diseases, including amyotrophic lateral sclerosis. However, we do not work with these disorders exclusively, as the overlaps between different forms of neurodegeneration are increasingly recognised. Our objectives during the course of this project are to provide new and useful mouse models for furthering our understanding of what goes wrong in neurodegenerative diseases specifically those of interest to us currently which are motor neuron	

	diseases, including forms of amyotrophic lateral sclerosis, and Alzheimer disease through its link with Down syndrome – people with Down syndrome are at increased risk of Alzheimer disease. Our objectives are also within our own laboratory and those of our collaborators to understand what goes wrong in these disorders. A much longer term objective, given the difficulty of understanding neurodegenerative disease, is to help provide information towards targeted therapies for 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)?	Mice carrying mutations in specific genes are extremely helpful for understanding how changes at the DNA level can lead to loss of specific sets of neurons. Ultimately by understanding what goes wrong, we will be able to come up with treatments to ameliorate these diseases. Such mice ('models of disease') are extremely important because generally we have no other route into understanding neurodegeneration but to work with animals – for example, even though studies of individual cells are becoming increasingly informative, we now know that neurodegeneration results from many factors such as interactions between different cell types, including non-neuronal cells. Further, aspects of individual disease can be modified by interactions with other factors such as the immune system or lifestyle choices (for example, smoking or drinking alcohol, weight levels, etc.). It is essential that we have the broadest analysis of each mouse strain that we can, to learn about all the different cell types and systems that may be affected in a single neurodegenerative disease. Understanding the many different components of disease in the mouse, which has a similar biology to humans, will help us identify important possible targets for therapy, including directed at non-neuronal cell types.
What species and approximate numbers of animals do you expect to use over what period of time?	We work with mice and over 5 years we may use up to 61900, although the vast majority of these mice, up to 47,000, will simply be used in breeding protocols. The reason we breed so many mice is that this project is based on mice with different genetic changes and in order to produce the combinations that are most informative, we have to breed lots of different animal genotypes together.
In the context of what you propose to do to the animals, what are the expected adverse	Most of the animals we work with are used for breeding and then for studying tissues, so they are humanely killed and then we can look at which genes

effects and the likely/expected level of severity? What will happen to the animals at the end?	and proteins are switched on in their tissues. Some animals carry disease causing mutations and a small proportion of these are allowed to develop the neurodegenerative diseases we are interested in, again to see what happens compared to unaffected mice and therefore to find out about the biology of the disorder. As these mice model human neurodegenerative diseases they may start to develop the same symptoms as humans, thus mice with genetic lesions known to cause Alzheimer disease in humans, can develop short term and long term memory changes, such that they cannot remember as well as unaffected animals. Mice that model human motor neuron diseases may develop paralysis. However, no mice are allowed to reach an endstage that is equivalent to the human endstage of the neurodegenerative diseases we study. We aim to use mice with the same genetic lesions as humans that are not as badly affected or the mice may be killed at an earlier stage, and have low level loss of neurons, or no neuronal loss at all, but have changes within cells that are still informative for us.
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 would replace working with whole animals over their 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 hold regular meetings with animal facility staff to maintain 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	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

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	animals to maximise the information gained from each mouse. We also try hard to develop protocols that detect early changes, such as behavioural changes, prior to major deterioration; such detection can only improve with time and as we learn more about the process of neurodegeneration including from the very earliest stages.

Project 12	**Drug modification of opioid tolerance**	
Key Words (max. 5 words)	Opioid: tolerance: respiratory depression:	
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)	To determine the mechanisms by which tolerance develops to the analgesic and respiratory depressant effects of opioid drugs (both prescription opioids and Street drugs) on prolonged administration. To determine how exposure to other drugs commonly abused along with opioids (e.g. alcohol) can modify the development, maintenance and decay of tolerance to opioid drugs.	
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 elucidating the molecular and cellular signalling pathways involved in opioid tolerance this research will provide knowledge of targets for the manipulation of the level of opioid tolerance This could lead to the development of agents that inhibit opioid tolerance and these could be used clinically to enhance analgesia in patients receiving opioids for pain relief while reducing unwanted side effects such as nausea, vomiting and constipation. Conversely, a mechanism by which tolerance could be induced or enhanced could prevent relapse in recovering opioid addicts as subsequent self administration of morphine or heroin would fail to produce the normal pleasurable effects for which these drugs are abused.	

	Detailed information on how other drugs of abuse (e.g. ethanol, cocaine, benzodiazepines, pregabalin) that are regularly taken by heroin addicts alter the respiratory depressant effects of opioid drugs will lead to better public health advice to current and recovering heroin addicts about polydrug use.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 2816 Rats 468
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?	Animals will undergo brief surgical procedures under general anaesthesia with recovery and receive non-lethal injections of opioid drugs. The level of severity is moderate. All animals will be humanely killed at the end of each experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will study analgesia and respiration. These physiological responses cannot be measured in non-animal alternatives
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical power analysis has been done to determine the appropriate number of animals in each group, in each experiment to obtain statistically reliable data. Analgesia and respiratory depression will me measured in the same 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.	The characteristics of the acute analgesic and respiratory depressant effects of oploid drugs such as morphine have been extensively characterised in mice and rats. The brain nuclei, neuronal pathways and neurotransmitters that control these behaviours as well as the anatomical distribution of opioid receptors in the brain show considerable similarity across mammalian species including man. Therefore these species are appropriate for the study of the development of tolerance to the analgesic and respiratory depressant effects of opiold drugs and how it can be modified by other drugs. Surgical procedures will be performed under general anaesthesia. The doses of drugs administered will be

	chosen to avoid adverse effects. Measurement of analgesia and respiration will be performed in freely moving animals.

Project 13	**Novel imaging applied to the study of memory**	
Key Words (max. 5 words)	Learning, memory, imaging, Synaptic plasticity, Optics	
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)	The project has two objectives; the first will attempt to implement a completely novel form of imaging in vivo, the second will use the new technology to explore the relationship between changes at the synapse and how these may manifest as memory.	
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 may be derived either from successful development and implementation of the technology or from the questions we will be able to address with the novel optical device. While the relationship between synapses and neurological disease are increasing well recognised, even resulting in the suggestion that many neurological diseases are synaptopathies, a more complete understanding of the fundamental role played by synapses stands to significantly enhance the way we tackle brain diseases. Our development and experimental use of this novel research tool also affords us the chance to explore whether the device is likely to be suitable as a diagnostic instrument for use in humans. Endoscopic imaging is a critical tool in the armoury of modern medicinal diagnostics raising the possibility that a minimal invasive imaging device of this type might	

	offer new opportunities that have hitherto been impossible for want of micro-instrumentation.
What species and approximate numbers of animals do you expect to use over what period of time?	Over a 5 year project we would expect to require approximately 2000 rodents (rats and mice). The majority of these will be used to form breeding colonies and will not undergo any form of invasive procedure.
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 design of our project will ensure that almost all *in vivo* brain imaging will be conducted under general anaesthesia, under aseptic conditions and with appropriate post-operative care. In this way pain and discomfort will be kept to an absolute minimum, and stress and discomfort will similarly be minimized. Only once it is completely clear that our *in vivo* technology is working appropriately and our surgical procedures exemplary will our studies extend to addressing novel biology in animals that undergo behavioural training and/or substance administration for the modulation of nervous system activity. For this relatively small cohort of animals stringent monitoring will ensure that stress and discomfort is avoided if at all possible to kept to an absolute minimum. Animals will be killed 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	It is an inescapable fact that demonstrable learning requires behaviour. If we are understand the neural basis of the learning process and perhaps what goes wrong within the brain as learning starts to fail, we need to be able to directly relate changes within the brain to behaviour. For this reason it is necessary to use animal models where leaning can be seen to occur and we can monitor changes within the brain that are linked to this learning. Rodents represent a powerful choice for this undertaking: they learn and have brain architecture that would strongly suggest that they achieve learning in a manner similar to human beings.
2. Reduction Explain how you will assure the use of minimum numbers of animals	As a principal goal of our optical developments is to permit repeated measurements to taken from a single animal we radically reduce animal numbers by the nature of our experimental design. Data are collected from single animals before and after a plasticity event, we therefore always have a within animal control. In this way the need for examination of large

	populations of animals to ‘smooth’ biological noise is removed.
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 (rats and mice) are a species with neural architecture that shares close resemblance to human beings. Animal welfare is foremost in the minds of the investigators. Animals will be examined in state of the art facilities using only best practice methods. All surgeries will be conducted under anaesthesia and monitored closely by animal welfare professionals ensuring that recovery is swift and minimally painless.

<table>
<tr><td>Project 14</td><td>Bioenergetics of the nervous system</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Bioenergetics, mitochondria, inflammation, demyelination, neurodegeneration</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</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><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>How and why tissue loss occurs in neurodegenerative disorders including multiple sclerosis is not known. Effective treatments are unavailable for many of the neurodegenerative disorders including progressive forms of multiple sclerosis. Overall purpose is to understand bioenergetic changes in the context of inflammation, damage to myelin, restoration of myelin sheaths, neurodegeneration and neuroregeneration within the nervous system. By understanding mechanisms of tissue injury within the nervous system and developing systems that model the changes evident in neurological disorders the hope is to develop novel treatments for neurological conditions such as multiple sclerosis, primary mitochondrial disorders, Parkinson’s disease and Alzheimer’s disease.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the</td><td>Greater understanding of how and why neurons dysfunction and degenerate is one important outcome of this work. The work will also provide an improved pre-clinical platforms to test agents as potential therapy for a number of neurodegenerative disorders including multiple</td></tr>
</table>

project)?	sclerosis.
What species and approximate numbers of animals do you expect to use over what period of time?	We intend to use mice (up to 5500 animals) and rats (up to 1000 rats) over the 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?	Genetic modification of metabolic components include those of mitochondria (the powerhouses of cells) may cause dysfunction and degeneration of the nervous system. The effected animals may demonstrate weakness in limbs with impaired locomotion. However, none of the animals will be maintained beyond moderate severity (i.e. when subdued with little peer interaction and before weight loss of 20% body weight over any period).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Transgenic mice are necessary to induce specific bioenergetics defects within specific nervous system cell types, in vivo. Rats are necessary to obtain nervous system tissue for cell culture studies, which aim to identify potential therapy before the use of such agents in live animals can be justified. Non-animal alternative do not capture the type of inflammation and animals demonstrate and importantly these non-animal systems do not poses a completely intact and interactive network of metabolic support from different cell types and circulation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The proposed animal numbers are the result of discussion with statisticians and have been derived from power calculations based on published findings and preliminary data. The host centre has extensive experience in study design and written protocols will be submitted for all experiments to minimize experimental variation. In situations where previous experience is lacking, we will do pilot studies to determine the variance of the primary outcome measure before deciding on the number needed to show statistical significance with a 80% power.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	Mouse models are necessary to genetically manipulate the energy producing organelles, mitochondria, in a manner that it best recapitulate what has been found in patients with neurodegenerative disorders. Furthermore, this

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	approach will inevitable decrease the severity of a clinical phenotype in animals as the method can target specific cells rather than all tissue types. The inducible genetic technique will minimise developmental abnormalities as the time of onset of the manipulation can be pre-determined to decrease the severity of the phenotype. Rat tissue is necessary to model components of the energy producing cell types that are effected in specific diseases, hence reducing the need to do experimental procedures in live animals. Induction of experimental loss of myelin and inflammation will impair neurological function. Animals will be closely monitored and, to minimise suffering, none of the animals beyond moderate (limb weakness insufficient to cause paralysis and locomotion failure and weight loss of no more than 20%) level of severity will be maintained.

Project 15	**Support procedures for neuroscience studies**	
Key Words (max. 5 words)	Transgenic, behaviour, substances, tissue	
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)	The overall aim of the work under this licence is to understand why the damaged Central Nervous System cannot repair itself, and to develop new treatments to repair it when damaged. This licence provides the followings: 1) a demand-matched supply of tissues and blood products (regulates in vitro studies and in viva studies of animals in normal laboratory housing conditions): 2) a demand-matched supply of genetically altered rodents (describes genetic manipulations and experiments for morphological and functional studies and for modelling diseases): 3) pilot data for new substances (describes how to design experiments to study substances to gain background data for further experiments).	
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 supported by this licence is relevant to Nervous System injury and repair and neurodegenerotive diseases. The work is designed to produce new treatments for patients with spinal cord injury and neurodegenerative diseases. Work under this licence provides: a demand-matched	

	supply of animals to provide tissues: optimal production of transgenic animals with breeding strategies designed on the demands of the group: guidelines for the administration of new substances of potential therapeutic use.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse: 15 320 animals Rat: 9 200 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?	Overall, "'45% of the animals maintained under this licence show no detectable adverse signs. Some transgenic lines may develop motor, sensory or cognitive defects, which may render the animals less effective in their normal life tasks ("'30% of the transgenics, i.e. 18% of the total). Of these, some animals may develop behavioural deficits such as motor disabilities or learning and memory defects, in which case they will receive special care. It is possible that some of the animals used for testing substances already tested in vitro develop motor, sensory or cognitive deficits ("'10% of the animals tested for substances, i.e. "0.02% of the total). On this protocol, up to 40% of animals may undergo a surgical procedure which is likely to have a short term adverse impact on their welfare. All these animals are intensively monitored and terminated when unable to move and feed, or presenting gradual weight loss reaching 20%, or unresponsive to minor NVS treatment. Animals which will be either transferred under another licence or killed by a schedule 1 method, exsanguinations, perfusion or decapitation. In case of wild-type animals, after genotyping they can be kept for prospective reuse.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The Nervous System is extraordinarily complex, and each part interacts with other parts. New treatments cannot be developed or their effects predicted from cell lines. Tissue cultures derived from the Nervous System will be used for many mechanistic studies and for developing new treatments. Ultimately, in order to find out if treatments are safe and if they will restore function to the damaged system, studies of a complete Nervous System in viva have to be performed. Mammals have to be used since their Central Nervous System is unable to repair, unlike

	lower species.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Increasing amounts of research are carried out in tissue culture models of increasing sophistication. Thus the amount of in viva experiment is reduced. Breeding is controlled to match experimental requirements. Animals are bought as required and, when possible, different tissues from the same animal are employed simultaneously by coordinating the experiments of the various members of the group in order to minimize the number of used animals. Pilot studies and power calculations refine 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.	Rodents are used as their Nervous System is close enough to the human biology. Their genetic manipulations allow the testing of molecular hypotheses and new treatments. Transgenic animals of increasing sophistication, and with genetic defects that can be turned on and off when required and in the organ required mean that fewer animals show behavioural deficits. If any procedure causes harm likely to exceed the moderate severity limit, animals will be immediately killed.

<table>
<tr><td>Project 16</td><td colspan="3">Neuropharmacology of vulnerability to compulsivity</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Addiction, compulsivity, vulnerability, neuropharmacology, rat</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Our research aims to identify the environmental, behavioural, neurophysiological, cellular and molecular substrates of individual vulnerability to develop impulsive /compulsive disorders such as drug addiction or Obsessive Compulsive Disorders to eventually develop new treatments for the people suffering from these disorders. This research project aims at providing strong proof of concept of the contribution of a specific mechanism or the effectiveness of a specific drug for translating into human studies.</td></tr>
<tr><td>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)?</td><td colspan="3">Impulsive / compulsive disorders cost the UK an estimated £32 billion per year. Thus, these neuropsychiatric disorders, that include obsessive / compulsive disorder, drug addiction, or dopaminergic dysregulation syndrome place a considerable burden on not only the affected individual, but also social and economic burdens on society. Our research will provide new insights into the behavioural, neural and cellular substrates whereby some individuals are vulnerable to these disorders and open new avenus for the development of new treatments for them.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">We use behavioural models in rats. We use the minimum numbers of animals possible to achieve biologically and statistically meaningful data. We anticipate that we will use fewer than 5350 over 5 years.</td></tr>
<tr><td>In the context of what you propose to do to the animals,</td><td colspan="3">For the majority of our animals (85%), we anticipate no more than transient discomfort and no lasting harm.</td></tr>
</table>

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	However, as some of the disorders we study cause distress in humans (e.g. obsessive / compulsive disorder, Parkinson Disease, or drug addiction) then some of our animals, namely those that are the most vulnerable to develop neuropsychiatric disorders (~15%), do experience more stressful conditions (including repeated exposure to stressors such as mild footshocks, loss of weight) (of no more than moderate severity) which reflect the spontaneous propensity to develop an aberrant behavior in the face of specific environmental conditions (such as drug exposure, frustration, stress). In some of our experiments, we have to manipulate the brain (e.g. by surgically damaging specific regions) in order to understand why and where neuropsychiatric disorders happen or to insert vascular cannulae to allow animals to self-administer non ingestive drugs. Both manipulations cause moderate severity. When we need to do these procedures, the animals are very carefully monitored for any signs of pain or distress. If the animals show signs of suffering and we are not able to ameliorate these very rapidly in consultation with the named veterinary surgeon, then we euthanize the animal. Fortunately, such instances are very rare. At the end of experiments, the animals are killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This research is only possible with the use of animals because human studies (e.g. brain imaging studies) are useful, but can only provide correlative data that do not address causation. Furthermore, it is not ethically possible to study the genetic and/or environmental factors that underlie predisposition to, and the development of, neuropsychiatric disorders in humans. Similarly, it would not be possible to develop new treatments for brain disorders without testing them in animal models first. *In vitro* models (e.g. brain slice preparations) or computer simulations cannot be used because the modelling of behaviour in these systems is not sufficiently advanced.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are fully committed to using the minimum number of animals required to obtain data that are statistically and biologically meaningful. We carefully design our experiments to maximise the behavioural data collected from each animal, and to minimise distress.

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 rats because they are the least sentient species which allow researchers to investigate marked inter-individual differences in sophisticated behaviours that capture core features of the neuropsychiatric disorders we are interested in. Additionally, the brain circuitry implicated in many neuropsychiatric disorders is highly conserved between rodents and humans. We take the welfare of the animals very seriously. Most of our animals run in long-lasting behavioural experiments in which they perform tasks for food or drug reward, and experience procedures, e.g. injections that produce only transient discomfort and no lasting harm. Animals are monitored frequently (often undergoing daily testing) and any adverse effects are discussed with the named veterinary surgeon. If these cannot be quickly ameliorated then animals are euthanized to prevent suffering.

<table>
<tr><td>Project 17</td><td>Rodent models of neurodegenerative disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Huntington’s Disease, cognition, locomotion, mice</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>We aim to use genetically engineered mice carrying a mutation for Huntington’s Disease to characterise the timescale of the different types of symptoms (psychiatric, cognitive, locomotor) and attempt to develop novel therapies to treat the different symptomatic stages and also the underlying cause of the disease.</td></tr>
<tr><td>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)?</td><td>This project will potentially benefit patients with neurodegenerative disease, primarily Huntington’s Disease although the mechanisms we are investigating here may also apply to other serious neurodegenerative disorders such as Alzheimer’s Disease, Parkinson’s Disease, spinocerebellar ataxias and amyotrophic lateral sclerosis.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Species used will be mice, approximately 10,000 over 5 years.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected</td><td>Huntington’s disease in humans is associated with progressive loss of motor function, which is ultimately fatal. Our subtle locomotor coordination testing will detect abnormalities in the mice, and</td></tr>
</table>

level of severity? What will happen to the animals at the end?	any benefits from treatment, before any difficulties in movement become apparent. The surgical procedures to allow electrical recording from, and the delivery of therapeutic agents directly to the brain are completely standard and safe.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Neurodegenerative diseases affect the brain and therefore some kind of animal or animal tissue is essential in order to study them. Many neurodegenerative diseases occur during ageing and therefore need to be studied in tissue of an appropriate age. Also to study human disease one does not just need to study molecular level processes (which can be done in immortal human cell lines or patient samples) but to see the symptoms at an overt systems level and assess whether or not treatments targeting specific biological pathways actually relieve symptoms.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will carefully plan and control our breeding colony in order to only breed the numbers and genotypes of animals required at the current time for experiments. Animals bred and then used for purely behavioural experiments may be returned to the colony for breeding purposes later if their tissues were not required after behavioural studies and they have not received neuroactive substances. We will use power analysis to predict how many animals will be needed to complete a behavioural, electrophysiological or biochemical study in order to produce statistically significant results and use the minimum number possible. We will use strategies that will allow collection of maximal data from individual animals, for example repeated behavioural testing at different age groups using spontaneous behaviours to measure memory; using multiple brain slices from each animal for electrophysiology, using crossover (within-subjects) designs for drug studies to halve the number of animals needed.
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	All animals will be housed in groups with environmental enrichment such as tubes and houses. Food and water will be continuously available in unlimited amounts. The only exception to this will be if we need to carry out behavioural experiments using food or water restriction in order to motivate the animals: however we will always try to use spontaneous non-rewarded and non-

minimise welfare costs (harms) to the animals.	aversive behavioural tasks in the first instance. Animals will be tested at similar times each day to allow them to incorporate this in to their circadian cycle and handled extensively before any experiments begin to minimise stress and maximise familiarity with the handler. Breeding animals will always have a shelter in the cage, males and females are not separated, pups will not be weaned until at least 21 days of age, extra nesting material ("nestlets") will be provided for further enrichment and cages with births will not be disturbed for the first 7 days to minimise stress and disruption. Behavioural tests will involve as little stress to the animals as possible. When potentially therapeutic agents are being delivered, we will favour the routes by which humans are normally treated (i.e. by mouth or by injection). Occasionally, in proof-of-principle studies, however, we may have to administer these compounds directly into the brain (for example, to avoid the complication of metabolism and activity elsewhere in the body).

Project 18	**Spinal cord injury and repair**	
Key Words (max. 5 words)	Spinal cord; plasticity; regeneration; rehabilitation	
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)	Spinal cord injury is a crippling condition in which the body below the level of the injury is paralyzed and without sensation. The injury damages many of the nerve fibres that connect the brain with the spinal cord and the body. In order to repair the damage, the damaged nerve fibres must be made to regrow across the injury to make connections below it. There are usually some undamaged nerve fibres, and these can potentially regain some function through stimulating intact fibres to sprout, bypassing the lesion. The project is designed to develop treatments that will repair damage to the spinal cord through promoting nerve fibre regrowth (regeneration) and/or fibre sprouting from existing or uninjured axons (plasticity).	
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 project is expected to provide novel information about growth-promoting molecules to aid neuronal plasticity and regeneration after neurological damage to the spinal cord. It will advance our knowledge regarding molecular and physiological processes that occur after injury to the spinal cord. These processes	

project)?	can then be targeted or manipulated to aid neuronal growth and functional recovery after injury to the spinal cord. This project will be a critical step in developing new treatments for patients with spinal cord injury, including the recovery of sensory, motor and bladder function after spinal cord injury, as well as examining combinations of treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	This project aims to only use rats and mice for anatomical and functional assessment. As the majority of our lab work involves tissue culture models, we aim to use animals only when necessary such as testing potential therapeutic drugs or neuronal tracers in a complex living system. This will occur where successful data has been produced from non-animal based data and justifies the use in animals. Thus, we anticipate using approximately 4000 mice and 6500 rats 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 aims to elucidate potential growth-promoting candidates that are developmentally regulated in wild type and transgenic animals. Neuronal connectivity can also be assessed via anatomical tracing and electrophysiological assessment and also with the use of transenic animals that have candidate genes overexpressed or eliminated. To accomplish this different fluorescent tracers or viruses can be injected into the spinal cord or areas that innervate the spinal cord to label different populations of neurons. Transgenic animals with the addition of viruses can also be used to either stimulate or inhibit neuronal signalling. Secondly, we aim to induce a partial injury to the spinal cord by inducing physical (cut or crush injuries) or chemical or electrolytic lesions and consequently mimic direct physical damage or focal lesions to neurons or supporting non-neuronal cells in the spinal cord. Clinical signs from spinal cord injury include sensory and motor impairments, which are subtle deficits that affect the ability to sense and move the affected limbs. General walking, grooming and feeding behaviour are not affected as lesions are only partial. If spinal cord injury is performed in lower spinal levels, urinary retention may occur (extra care is taken to manually express the bladder) however

	this impairment usually resolves after 10 days post-injury. The majority of animals will remain in the moderate severity category and only a small number (<10%) will reach the moderate severity limit. The progression and development of damage will be investigated and studied to assess differential changes before and after neuronal damage. Finally, different treatment strategies will be administered either directly into the spinal cord or into areas that innervate the spinal cord such as the brain, peripheral nerves or muscle to assess their effects both on anatomical reorganisation of neuronal connections and functional recovery. Treatment strategies include enzymes, viruses, antibodies, graft implants, etc. The majority of drug treatments are not expected to show adverse effects. Although in the case of graft implants, there may rejection from the host and immunosuppression may have to be used. Again, changes in neuronal connectivity and function can be assessed through anatomical tracers, behavioural function and electrophysiologicàl assessments. At the end of the study, animals are humanely killed. Tissue is collected for anatomical studies or used for molecular/protein assessment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The basic concepts and treatments for spinal cord injury repair are performed using tissue culture models, and the majority of papers from our laboratory involve little or no animal work. Concepts developed in tissue culture have to be tested and refined in a real spinal cord where the complex environment of the adult nervous system is present, and where functional recovery can be measured. No treatment for spinal cord injury could be tested in human patients without extensive prior vahdation in animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	No animal experiments are performed until a well developed treatment concept has been developed using tissue cultures. For animal experiments, we aim to achieve minimal variation between animals thus enabling the use of

	smaller experimental groups. Animal group size is determined from previous experience, pilot studies and statistical power calculations to ensure the number of animals used is sufficient to achiever statistical significance. Additionally, the partial lesions conducted have minimal effect on gross behaviour so the general well being of the animal is not affected.
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.	Our experiments are performed in rats and mice as their biology of spinal cord damage, repair and neuronal growth is similar to humans. Rats are also capable of complex behaviour and skilled paw use, making it possible to achieve good behavioural outcomes. Mice are used for some experiments because they can be genetically manipulated, allowing molecular hypotheses to be tested. Their behaviour is almost as good as that of rats. We minimise suffering by developing and/or using behavioural outcome tests of high resolution that pick up deficits in fine movement control. Therefore, it is not necessary to make large and disabling spinal cord injuries. We use well established lesion models that have been extensively used in our lab for a number of years, thus we have achieved a high rate of reproducibility with our spinal cord lesions (consistent size and outcome).

<table>
<tr><td>Project 19</td><td>Mechanisms of myelination and synapse formation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Epilepsy, neuropathy, genetic programme</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td>~~No~~</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Signals in the nervous system are passed on in the form of electrical signals along the length of the neurons axon. To ensure efficient and reliable transmission of the signal many axons are insulated by a myelin sheath, which is produced and maintained by specialized cells. Once the signal reaches the end of the axon the electrical signal is passed on to another neuron or muscle cell in the form of a small neurotransmitter molecule. This happens at a specialized structure, which is called the synapse. There a millions and millions of synapses in our brain and they can be inhibiting or activating the next cell. The insulating myelin sheath around axons and the inhibitory and excitatory circuits are laid down, modified and maintained during our adult live. It is therefore not surprising that disturbances in these structures either through genetic or environmental causes result in common neurological disorders such as epilepsy and white matter diseases. These diseases affect a large number of people and represent a considerable clinical burden. This programme aims to uncover common basic biological mechanisms that underlie myelination</td></tr>
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	and synapse formation and that are affected in inherited forms of these 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)?	Scientific advancements come from an understanding of the genetic basis of molecular and cellular interactions that shape key structural and functional aspects of the nervous system. It further provides an understanding of the disease processes in which these molecular and cellular interactions are disrupted, either by genetic or environmental causes or through surgical procedures. Additionally, it potentially explains why certain clinical phenotypes tend to occur together in a neurological syndrome thus opening new avenues for diagnosis and development of therapeutics that aim to cure or slow the progress of the disease.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will use mice and rats. The total number of mice will be around 7000 and 200 rats over a 5 year period. The vast majority of these animals will not be subjected to experimental procedures and will not develop disease symptoms. They will be used to generate and maintain breeding stock of genetically altered 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?	The genetic alterations in some mice are expected to result in moderate adverse effects that include tremors, problems with gait and locomotion (a drunk man's walk) and delay in postnatal development such as slower growth and weight gain. These behavioural effects will be quantified in simple tests and the underlying causes will be studied post mortem through histological examination, ex vivo nerve activity measurements and culture of cells. To study the role of genes in response to trauma, nerves will be surgically damaged and the repair process is studied over a number of weeks. The damage to the nerve causes a transient paralysis of the lower limb. Animals will be euthanized and their nerves will be dissected for further study. Some animals may be transferred to other projects.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	The development, function and response to trauma of the nervous system of vertebrates including mice and man cannot be fully studied in a petri-dish or with computer models. Mice have a sufficiently evolved nervous system to readily compare to

use non-animal alternatives	humans. Cell-culture models are intimately integrated in the current programme but still require the breeding of genetically altered mice and the isolation of Schwann cells from nerves of young rats.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers will be minimized through careful planning of breedings, taken into account proper controls and statistical considerations to ensure that the minimum numbers of mice are used to obtain relevant data. Lab staff will meet regularly to evaluate colony size for each mouse line. Non-GA mice will be obtained from a commercial source and mouse lines not under active investigation will be terminated. Procedures will be carried out by fully trained staff.
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 a well-developed nervous system that resembles that of humans in all key aspects studied. The ability to modify the genome of the mouse at will and the availability of sophisticated neurophysiological and behavioural paradigms make the mouse the species of choice to model human disease states relevant to the objectives of this programme. We use specific genetic techniques to minimise welfare costs for the individual animal and have defined clear endpoints to ensure that no animal will experience adverse effects beyond moderate. Animals subjected to experimental procedures will be closely monitored, post-operative pain will be relieved with analgesics and humane endpoints applied

Project 20	**Blood vessels in cortical interneuron development**	
Key Words (max. 5 words)	Neocortex, interneurons, development, migration, blood vessels	
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)	In man, the cerebral cortex is by far the most important part of the brain containing the majority of its neurons. In mammals, cortical neurons are divided into two broad classes, the excitatory pyramidal cells, connected to brain structures outside the cortex, and the nonpyramidal cells, the inhibitory interneurons, that form connections only within the cortex. These two cell types are generated in different zones of the embryonic brain and follow distinct forms of migration to reach their final positions in the cortex. All pyramidal neurons are generated in the ventricular zones that line the lateral ventricles of the dorsal telencephalon and migrate radially (i.e. perpendicular to the surface of the brain) using the support of glial cell processes (gliophilic migration). Interneurons arise in the basal telencephalon, outside the developing cortex, and follow long and tortuous routes to migrate into the structure. Once in the cortex, they chose one of two main tangential (i.e. parallel to the pial surface) migratory streams to eventually reach their destinations. It is thought that these cells use axons of pyramidal neurons to navigate from the basal telencephalon to the cortex (neurophilic migration). However, evidence has recently emerged to support vasophilic mode of	

	migration, where interneurons use the developing network of blood vessels as a substrate for their movement. Here, we propose to test the hypothesis that various factors, secreted by blood vessels, regulate the development and migration of interneurons. Specifically, we propose to combine mouse genetic tools, with cell and molecular biological techniques to investigate the roles of blood vessel secreted molecules on the generation, survival and migration of cortical interneurons, focusing on vascular endothelial growth factor (VEGF). In addition to studying the role of VEGF in cortical interneuron migration, we wish to assess whether this and other blood vessel secreted factors dictate the choice of migratory path by cortical interneurons. In summary, the proposed research programme will explore novel mechanisms involving blood vessels in cortical interneuron migration. The work is likely to contribute to our understanding of normal cortical development and provide new clues about the mechanisms that underlie cortical pathologies and neuronal migration disorders 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)?	Work contained in this project is part of our long-term effort to elucidate the molecular mechanisms which underlie the migration of cortical interneurons. This work will likely enhance our understanding of the aetiologies of developmental disorders of this area of the brain such as mental retardation, autism and certain forms of epilepsy.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice as most published studies in our field have been conducted in this species. Genetically modified mice will also be used as they will allow us to confirm results from molecular analyses on wild-type animals. We will use approximately 4,400 mice during the course 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 happen to the animals at the end?	The likely adverse effects of the procedures will be none other than transient discomfort during induction of anaesthesia or slight and transient pain from ear notching. The expected level of severity will be ‘mild’. At the termination of the proposed experiments, animals will be killed by Schedule 1 procedure (to take fresh tissue for molecular analysis or in vitro experiments) or perfusion when microscopy will be used to assess changes in the developing cortex.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of mice is essential for the work contained in this application on the development of a large and complex part of the mammalian brain, the cerebral cortex. In the proposed in vitro experiments, we will be examining the molecular interactions that affect the generation, survival and migration of cortical interneurons in the mammalian forebrain, and we can only obtain these neurons from pregnant animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experiments proposed here will involve examination of cultures of dissociated cells or fixed brain sections of wild-type and mutant mice at different stages of embryonic development. It is difficult to estimate the number of animals we are going to use because much of this depends on the size of the litters of the pregnant mice. However, as always, the number of animals to be used at each stage and in each set of proposed experiments will be just sufficient to achieve statistical significance (4-6 embryos per group). Wastage in the breeding colonies will be kept to a minimum by monitoring their size and adjust according to requirements.
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 experiments will be carried out in mice in order to compare the data with previous and ongoing studies from this and other laboratories. Much of the background information used in our proposed work is derived exclusively from developmental studies in mice and, thus, the use of this species will facilitate the interpretation of data in a meaningful way. Further, the availability of GA animals lends support to our choice of species. The expected severity limit will be ‘mild’, and attention will be given to minimising the irritation and pain felt by the animals where possible.

Project 21	**Neurophysiology of reward**	
Key Words (max. 5 words)	Brain1 neurons, reward, learning, decision making	
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)	In order to understand how voluntary behaviour is made, we need to know how the brain is engaged in economic (reward) choices that are the most fundamental processes for survival. This knowledge is also fundamental for understanding drug addiction, which is a disorder in reward processing and economic choices. Addiction to reward extends to obesity, which is the prevalent disorder in the early 21st century, similar in importance to infectious diseases a hundred years ago;	
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 brain signals for fundamental reward and decision variables such as utility, probability and risk would help to understand the basic particles of economic valuation and explain why economic decisions may go right or wrong. Any, even minor advance, in understanding of what goes wrong in reward disorders such as drug addiction, obesity and risk misjudgement would promote the search for treatments which would save thousands of lives (and billions of pounds). Brain signals might explain how we price and exchange goods as a fundamental human trait, which should contribute to enhancing welfare. As we receive many rewards in social situations, we need to understand how the brain recognises the rewards others get and how it recognises who cooperates and who not. Less	

	misjudging of social reward processes such as cooperation-defection or disadvantageous inequity (getting less than others) may reduce social conflicts. Thus the knowledge gained from these experiments would help us to understand abnormal processes in reward addiction, obesity, gambling, attention deficit disorder and other disorders, and provide a further step to improve the human condition.
What species and approximate numbers of animals do you expect to use over what period of time?	Macaca mulatta (rhesus monkey, old world monkey), 25 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?	All animals will have at least one cranial implant, which is tolerated without complications in most animals. Potential adverse effects may arise in a minority of animals from local infections around the implant and from minor transient seizures following insertion of electrodes into the brain. Despite very careful control, there is a limited chance of bites in group housed animals. Animals have controlled access to food and/or fluid and grow well and gain ample weight. At the end of the experiment, the animals are killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We investigate biological signals in relation to voluntary behaviour. Computer simulations, tissue studies and human imaging studies cannot replace behavioural experiments and do not permit one to relate the activity of individual neurons to specific behaviours, which is the key for understanding brain function. Other mammals such as rodents do not have the highly developed brain structures of interest (e.g. frontal cortex and connected structures), do not permit sufficiently precise distinctions between reward, decision making and movements to allow identification of reward and decision neurons, and do not permit the required focused behaviour extending over a few hours. Despite some progress in investigating neurons in humans, systematically controlled experiments require the use of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Each study requires a minimum of 2 animals. Sometimes 3 animals are necessary to obtain reproducibility is assured across reliable results (most numbers of neurons).

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.	Computer simulations provide guidance for us for more efficient animal experiments and reduce the period of behavioural training. Our experimental designs are informed by experimental procedures based on formal animal learning theory and economic decision theory. Human imaging studies provide information about the selection of brain structures and thus avoid unnecessary explorations. Data analysis techniques optimise the number of data to be gathered. Macaque monkeys show the necessary behavioural repertoire for studying the relation of brain activity to specific, well characterised behavioural processes involving reward and economic decisions (marmoset monkeys and rodents move around a lot and therefore make distinctions to movement difficult). Macaque behaviour is also sufficiently well controlled to permit recordings from individual neurons with moveable microelectrodes. In addition, they cope well with the conditions in experimental laboratories. Animals that are distressed or in pain will not perform in a behavioural task, even if they are hungry or thirsty. It is therefore important to keep the animals at ease and in good health. Poorly performing or distressed animals are either temporarily or permanently excluded from the experiment. Furthermore, macaque monkeys have brains that are closest to humans, and their behavioural capacities allow researchers to test similar brain functions as in humans in a controlled fashion. Data obtained in other species, such as marmosets and rodents, are less applicable to humans due to more different brains from humans. Our past progress concerns the housing of animals, voluntary entering into specially adapted primate chairs and laboratory, refinement of food and fluid control, reduction of impact of implantations by using different materials, well adapted cleaning and disinfection of implants, use of noninvasive monitoring procedures (infrared camera and face mask for eye monitoring, infrared lick detectors), and sharing best practice with other researchers.

Project 22	**Investigating the neural basis of spatial and episodic memory**	
Key Words (max. 5 words)	Memory, Episodic, Alzheimer's, Hippocampus, Behaviour	
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)	Learning and memory are two important functions of the brain and an enormous amount of research has been carried out to find the neural mechanisms and circuits responsible for them. Memory can be sub-divided into many different psychological processes. However, the process that best sums up what people most commonly refer to as memory is episodic memory, the ability to remember specific, personal events that have happened to us in the past. Our episodic memories, to a large extent, make up who we are and they are used frequently to guide our current behaviour. The study of the neural basis of episodic memory has centred on the medial temporal lobe of the brain and its associated structures, focusing on an area called the hippocampus. Studies have shown that people with damage to the hippocampus have severe anterograde amnesia, an inability to form new memories. This project will examine how the hippocampus and associated areas process episodic and spatial memory by examining the neural mechanisms involved in these psychological processes. From a systems perspective there is at least some agreement that the hippocampus has role to play in learning and memory but the specifics of how it interacts with other brain	

	structures to process episodic memory are still unknown. At a more fundamental level the mechanisms within brain areas like the hippocampus that support episodic memory are poorly defined. This work will address these problems at the level of the single neuron, networks of neurons and systems of brain areas. It will go on to examine the genetic factors associated with these types of memory and apply this to the study of disorders of memory including dementia and 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)?	This work will not only benefit the scientific community by furthering our understanding of learning and memory but will also provide potential therapeutic targets for disorders of memory like Alzheimer's disease. It will also test potential therapies for disorders of memory using appropriate behavioural models.
What species and approximate numbers of animals do you expect to use over what period of time?	The project uses rats and mice. We will use approximately 50-200 rats each year depending on grant funding and 0-750 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?	The majority of the experiments involve behavioural tests of animals which involve them learning to remember stimuli in their environment. A typical example would be a pot of sand in which they have to dig to find reward. The main adverse effect of this is stress caused by new environments. This will be minimised by habituating the animals to the environments in groups of their cage mates where possible. Some of the experiments involve either recording brain activity or manipulating the brain by infusing substances to affect brain activity. These experiments involve the animals having surgery and to minimise pain and suffering all appropriate analgesia and anaesthesia will be used. As with any surgery there will be a small amount of weight loss associated with the procedure but animals will quickly recover healthy weight gain. Some experiments cause small amounts of damage to specific portions of the brain. These may result in cognitive impairments such as deficits in memory. There may be occasional incidents when surgery does not go as planned but animals will be killed when such incidents become evident.

	Most experiments will only have a severity level of mild but some of them will be moderate. At the end of the study animals are given an overdose of anaesthetic.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The proposed experiments aim to examine the neural mechanisms underlying learning and memory. This involves studying an organism that can demonstrate that it has learnt information and that it can remember that information for long periods of time. The available options here are humans and non-human animals. Human subjects are used whenever possible to better define memory processes, however in order to examine how the brain processes memory we need to carry out experiments that monitor brain function or manipulate the system in some way (through lesions or drugs). Monitoring brain function can be done in humans using methods like fMRI. However, these methods do not allow us to examine very specific parts of the brain as activity patterns have to be averaged across relatively large areas that include millions of neurons. In order to gain a real understanding of how the brain performs psychological functions we must examine the activity of individual neurons. This is not usually possible in human subjects. Other alternatives include cell cultures, which are clearly unsuitable for examining psychological processes, and computational models. Models can be very useful for generating hypotheses and where possible these are used. However, current models are necessarily simplistic and so cannot yet simulate many memory processes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to carry out good research we must use sample sizes that give us sufficient statistical power to test our hypotheses and make meaningful conclusions. To reduce the numbers of animals we use whilst maintaining statistical power we use methods that maximise the data we can obtain from individual animals. As an example, single unit recording studies use neurons rather than animals as the unit of analysis. In this case increased statistical power is gained not by increasing the number of animals but by increasing the number of neurons from which we can record. We will still need to use multiple animals as there are individual differences between animals that might account for the firing patterns of neurons. However, the numbers would be reduced as this would be something to examine as a

	covariate with the primary dependent variable (cell response) rather than being the unit responsible for giving us statistical power. By using methods such as this we can reduce the number of animals used by refining the method to allow us to record from more neurons in each animal. We use power analysis to ensure that we are using appropriate sample sizes based on previous datasets. Where possible we use behavioural experimental designs that allow us to increase statistical power and so reduce animal numbers. For example, we will be employing a procedure that allows us to run multiple memory trials in one day for each animal. This will reduce variance, increase power and allow us to reduce sample size.
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 species used in the current application are rats and mice. These are used for a number of reasons. The rodent brain is highly analogous to the human brain with the hippocampus in particular being very well preserved across species. This ensures that findings from these studies can be used to make conclusions about memory processes in humans. They are the most well studied species in this area ensuring that the data produced can be evaluated in the light of previous data and they are relatively small making them cost effective to keep in the laboratory. We make sure to house the animals in conditions with environmental enrichment in groups whenever possible. We use all appropriate anaesthesia and analgesia whenever procedures have to be carried out.

Project 23	**Epigenetic regulation of neuronal development**	
Key Words (max. 5 words)	Brain, development, neurodegeneration, transcription	
Expected duration of the project (yrs)	5	
	Y	Basic research
	N	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	Y	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 understand the mechanisms that regulate brain development with the scope of discovering novel therapeutic approaches for neurodegenerative 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)?	Studies done in many laboratories over the past 50 years or so have shown that a family of signal proteins called neurotrophins have a major role in supporting the development of the brain. They also play an important part in promoting learning and memory in the adult brain and in promoting nerve regeneration following injury to adult peripheral nerves. Interestingly, a deficiency of neurotrophins may contribute to some neurodegenerative diseases. Many patients with Alzheimer's disease, for example, have significantly reduced activity of neurotrophins in their brain, and a recent clinical trial has shown that delivery of nerve growth factor (NGF) to the brain of patients with Alzheimer's disease results in dramatic neuronal regeneration and improved cognitive performance. Moreover changes in the levels of Brain Derived Neurotrophic Factor (BDNF), a neurotrophin express in adult brain, leads to a host of neurological and psychiatric disorders, including Huntington and Alzheimer's diseases, schizophrenia and bipolar disorder. Thus, an increased understanding of how neurotrophins influence neuronal gene expression, as	

	well as the identification of new proteins that promote neuronal development in mammals, should ultimately lead to the development of novel therapeutic strategies to prevent and treat neurodegenerative diseases, which will become an increasing personal and societal problem as human populations age.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats (200/year) and mice (600/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?	Transgenic mouse models described in the project may be associated with mild phenotypes in the tissue targeted by the genetic modifications. Mice will be monitored daily and will be culled if they show signs of illness. Potential adverse effects of *in utero* and postnatal electroporation and infection will be minimised by using state of the art facilities for surgical procedures available at the designated establishment and fully trained staff. As outlined above, animals will be closely monitored after the procedure for any signs of illness or discomfort. Intraperitoneal injections may lead to infections in a small percentage of cases. If animals show any signs of infection, they will be euthanized immediately. In sum, we are convinced that the expected adverse effects are scientifically justifiable because the planned experiments will provide a better comprehension of the key players controlling brain development and neurodegeneration. Therefore, the broad implications of these studies in our view justify the use of animals.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although we use neuronal-derived cell lines and primary neurons in the laboratory, *in vitro* model systems do not recapitulate the environment in which neurons develop *in vivo* (e.g. the interaction with several types of nonneuronal cells). Moreover, many different cell types cannot be always reliably purified and in some cases, culture conditions may alter the characteristics of primary neurons. In sum, it is difficult to model neuronal development and neurodegeneration in vitro, and this is mainly due to the complexity of the developing and adult brain. The intact tissues with their full complement of specialised

	cells are the only system in which mechanisms can be fully tested and therapies accurately evaluated.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before embarking upon any *in vivo* experiments, hypothesis will be tested using *in vitro* models in primary cell cultures. When *in vivo* experiments are appropriate, small pilot studies will be carried out to estimate the variability of the experimental data so appropriate statistical power analysis can be used to minimize the numbers of animals required for a validated result. For all experiments, we will use the smallest possible number of animals to obtain statistically significant data. 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, I will ensure that high standards of animal care, welfare are applied and will utilise the most appropriate breeding methods. Colony sizes will be monitored and adjusted to meet the requirements of the research programme. I will ensure that breeding colonies will be always kept to their minimum size so as not to over produce. I will also verify that a full search is carried out to ascertain that there is no other worldwide availability before setting up or creating new transgenic lines.
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 only mammalian species for which transgenic techniques are widely available and is the species that is most widely used in the international research community for studies of the *in vivo* functions of mammalian genes. Therefore in order for our data to have worldwide significance, the mouse is the most appropriate species to use. Importantly, in rodents nervous system development is for many aspects very similar to humans. Work conducted with this animal model will provide insights into the pathophysiology of the human nervous system. More generally, anatomy, physiology, genetics of the mouse are similar to humans, and mice represent a very powerful system where to model human biology and disease. Currently, the most effective way of investigating the function of a given gene at the level of the whole animal is through the generation of genetically altered mice. Although it is possible to inactivate genes and proteins of key physiological and developmental processes by administration of toxins and drugs to whole animals, in many cases this approach suffers from lack of specificity and gives rise to more wide-ranging adverse effects that cannot

	be easily controlled. It also does not allow the detailed dissection of the complete network of proteins involved in key developmental or physiological processes, unlike the use of transgenic animals. Personnel in the lab have had extensive training in all the regulated procedures in this licence and will use our experience to ensure suffering is minimal. We normally attend courses organized by the Biological Services at our institution to maintain best practice and to keep our knowledge of animal welfare up to date with the Home Office guidelines.

Project 24	**Neuronal Networks and Pathways for Communication**	
Key Words (max. 5 words)	Communication, neuroimaging, neurophysiology, primate, neuronal mechanisms	
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)	This project has the overall aim of improving our understanding of the brain mechanisms for human communication by relying on an animal model that can provide the level of specificity about neuronal circuits, which can only be obtained in nonhuman primates whose neurobiology and cognitive abilities are most like those of humans. To achieve this we rely on behavioural findings that clarify which cognitive abilities support human language and are also shared with nonhuman primates. This forms the basis for studying the neuronal circuit interactions between different brain areas supporting the cognitive abilities for communication. We will also use non-invasive brain imaging comparably in humans and the nonhuman primates to better allow us to translate our understanding of neuronal mechanisms to humans, adding additional value to the research. Moreover, the studies in nonhuman primates will directly inform our work with typical and language impaired humans. This can lead to benefits for humans, such as improving the prognosis of recovery and the treatment options for stroke patients who	

	have lost the ability to communicate.
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)?	Stroke is a leading cause of disability in the UK and internationally. The U.K. Stroke Association notes that there are over 152,000 new strokes in this country annually and given how extensive the language regions are in the brain, many stroke patients will have severe communication problems. Language problems are devastating because they result in social isolation. Treatment options are limited, in part because we do not well understand how the brain supports communication so that we can make more accurate prognoses of language recovery following stroke and improve treatment options. Animal models can help to advance our understanding of neuronal circuits and pathways, and we have been leading in revealing which cognitive abilities that support human communication are shared with nonhuman primates. The insights obtained from primates, as neurobiological models, will directly inform parallel work in stroke patients with communication disabilities. This could translate to better prognosis of language recovery following stroke and in the longer run help to improve the treatment options for communication disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	This project can be achieved using no more than 24 Rhesus Macaques 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 animals will be slowly acclimated to accept some restraint as they perform a behavioural task. They are motivated to perform the task by having controlled access to fluid (or food), which is provided to them for correct task performance. Fluid or food control is carefully regulated individually for each animal. The level of control is carefully chosen so that it just motivates the individual animal to complete the task without causing adverse effects on their health or wellbeing. The fluid/food control levels are regularly re-evaluated and adjusted. The expected adverse event with immobilisation is distress, which is minimized by slowly acclimating the animals to the procedures. Non-invasive brain imaging is conducted with MRI or EEG under temporary head immobilisation. These brain imaging approaches are used to directly link the

	nonhuman primate findings to those in humans, to better translate the understanding of brain mechanisms to humans. In the animals, recordings will then be made from the central nervous system during task performance. Access to brain neurons requires a surgically implanted recording chamber. Thus the animals will undergo an implant surgery once at the beginning of the procedures to implant any necessary chambers for recordings. This is important as it will allow us to show how neuronal circuits interact between different brain regions, which is not possible in humans. The use of neuroactive substances, injected via the chamber, will allow us to activate or inactive certain neurons, which is unlikely to be noticed by the animal. A potential adverse event is infection associated with the implant, which is minimised by regularly using mild disinfecting agents on the implant and healing creams. The protocol is classified as Severe. At the end of the experiments the animals will be humanely killed during a non-recovery procedure and then slices of brain tissue studied, adding additional value to the research.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project investigates the interplay of brain circuits while important information is being communicated to the individual and being acted upon. Thus such work must be carried out in intact, behaving organisms. The laboratory also conducts human brain imaging studies. However, brain imaging studies show us where the brain uses more oxygen but not how the neurons in these brain regions work, thus human studies alone cannot directly answer the scientific questions. Animals whose neurobiology and cognitive abilities are most like those of humans are required for this project. Cognitive abilities related to communication cannot be studied *in vitro* in slice preparations nor can they be computationally simulated with computers. However, the information that will be obtained here might lead to less reliance on animal experiments in the future, as we better understand the neural bases for the effects seen in human brain imaging studies (replacement).
2. Reduction	Power calculations indicate that we can complete experiments with 2-3 animals per study. Thus a

Explain how you will assure the use of minimum numbers of animals	relatively small number of animals are required to complete this project.
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.	Rhesus macaques have the cognitive abilities most closely related to human language and their neurobiology can be studied using approaches not feasible in humans. The procedures have been refined over many years and continue to be refined to allow the animals to stay healthy and in as high of a state of welfare as possible for the duration of the experiments. We will continue to refine our procedures on this project license, such as developing and assessing better refined options for motivating the animals to correctly complete the tasks, find better ways to immobilise the head that do not depend on surgical implants. We are also refining how we measure the impact of the procedures on the animals so that they are as minimal in severity to their welfare as possible. Moreover, we regularly attend primate welfare meetings to make sure that we contribute towards advances in animal welfare and to stay on top of international developments.

<table>
<tr><td>Project 25</td><td>Molecular pathogenesis of neurodegeneration</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Alzheimer’s O-GlcNAcylation neuropathology</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>3</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To understand the changes which occur in the brains of people with Alzheimer’s disease and other dementias and how brain glucose (the brains energy source) can impact on these changes.</td></tr>
<tr><td>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)?</td><td>The potential benefit of this work would be to determine if glucose dependent alterations are a viable target for therapeutic intervention to prevent the changes observed in Alzheimer’s disease brains.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mouse, approximately 650 mice over 3 years</td></tr>
<tr><td>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?</td><td>Mild cognitive impairment is the most likely adverse effect of the transgenic mice. The likely/expected severity of procedures is mild to moderate. At the end of the designed experiments animals will be killed humanely and tissue fully utilised</td></tr>
</table>

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The work proposed in this application has the potential to open new avenues to the treatment of Dementia in humans. The potential benefits are therefore immense, and for anyone who has experienced the decline in health of a family member with Dementia the benefit of a treatment to prevent further decline would be impossible to put a cost on. In order to achieve the goals of the proposal it is vital to study as physiological a system as possible, which unfortunately means the use of rodents. Using mouse brain is the closest approximation we have to studying changes that occur in the human brain. In all instances, cell culture and slice culture will be used as much as possible and animals will only be used if this prior work supports further investigation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The project has been designed based on our preliminary development of the inhibitor model, in order to minimise the number of mice needed to gain sufficient tissue. From our experience we estimate that 10 mice will be sufficient to perform each experiment in vivo including multiple experiments in parallel to ensure statistically significant information. If though the course of the experiments we can gain significantly relevant information with fewer animals, study design will be changed accordingly.
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.	At the cellular and systems level, most of the proteins found in mouse brain share similar functions to the proteins found in the human brain. This means that the major intracellular signalling pathways are highly conserved from mouse to man, and the higher functions of the brain, specific to mammals, dependent on regulation of such proteins are also conserved. This, combined with the relatively short lifespan of the mouse, along with the tools available for genetic manipulation, make it the most appropriate model of the complex neurological systems to be studied. Anaesthesia and analgesia will be used to ameliorate suffering. Osmotic pumps will be used to minimise distress and suffering that would be caused by daily injections of disease modifying agents.

Project 26	**Neuronal and sensory functions of Tmc genes**	
Key Words (max. 5 words)	sensory transduction, taste, touch, hearing	
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)	The transmembrane channel-like (TMC) family of ion channel-like proteins have been implicated in hearing in humans, with Tmc1 representing an important cause of inherited deafness. We have found that an invertebrate TMC gene encodes a sensor for salt taste. This project aims to discover the functions of other members of the TMC family in mammals, in particular in sensory processes such as the detection of taste and touch.	
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 molecular sensors involved in a number of human senses, including hearing, salty and sour taste and many forms of touch, remain unknown. We believe TMC proteins may be components of some of these receptors, and understanding them may therefore improve understanding and treatment of conditions such as deafness and chronic pain.	
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 4150 mice over the course 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	Knockout mutations of Tmc1, Tmc2 and Tmc3 in mice are known to have few adverse consequences to the animals, other than deafness in the case of Tmc1. Some procedures associated with breeding	

level of severity? What will happen to the animals at the end?	involve surgical implantation of embryos, while the behavioural tests we propose involve at most brief discomfort (brief prodding with a stiff hair). At the end of all experiments all animals will be killed humanely. Where possible, we will use tissues for biochemical and physiological experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	While the broader TMC family is found in all animals, the particular TMC genes found in humans (including Tmc1, Tmc2 and Tmc3) are all vertebrate-specific. Thus, studying the effects of knocking out these genes can only be performed in mutant mice. We will continue to address general questions about the TMC gene family in nematode worms; in addition, we will conduct fine-scale genetic analysis on mammalian TMC genes using transgenic nematodes expressing mouse Tmc genes in worm neurons.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will keep animal numbers to a minimum, and only breed numbers of animals necessary for behavioural testing and generation of tissues for experimentation in the laboratory.
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.	Among vertebrates amenable for laboratory experimentation, mice are the best model for the human senses of hearing, touch, and taste which we plan to study. We plan to use only mild behavioural tests, or alternatively perform experiments on tissues obtained from humanely-killed animals.

<table>
<tr><td>Project 27</td><td>Mechanisms of perinatal brain injury</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Preterm, term, hypoxia-ischaemia, inflammation, translational</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Brain injury occurring during the perinatal period is a common cause of life-long neurological disability including cerebral palsy, epilepsy, cognitive deficits and neuropsychiatric disease. Severe brain injury will result in the death of the child.

The causes of such injury are complex and multifactorial, but hypoxia-ischemia (HI; restricted blood flow and oxygen provided to the brain) and infection/inflammation are considered important causes or precipitating insults of preventable/treatable forms of perinatal brain injury. Genetic background, sex, and degree of brain maturation of particular regions all contribute to the various mechanisms involved in brain injury. Brain injury evolves over time, and different mechanisms are critical during the initial, subsequent and longer term injury phases. Indeed, recent experimental data suggests that interventions can be effective if administered hours, days, or even weeks after the primary insult.

The aim of the Perinatal Brain Injury Group is the rapid and efficient discovery and translation of new</td></tr>
</table>

	neuroprotection and neural rescue therapies to ameliorate the devastating consequences of such injuries. Such advances can only come through a better understanding of the mechanisms of perinatal brain damage. The proposed project aims to define the molecular basis for both preterm and term brain injury using a number of prevalidated and optimised rodent models of injury.
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 successful outcome of this project would result in the development of prophylactic and neuroprotective strategies to reduce mortality, neurological morbidity and increase quality of life for the children affected. As there is currently only one therapy for term HI (which only improves outcome for 1 in every 7 infants treated) and no specific therapies for alleviating the effects of preterm brain injury, this represents a critical unmet need.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use rodent (rats and mice) models of preterm and term brain injury. For breeding purposes we expect to use no more than 2500 mice and for each of the injury paradigms, no more than 2500 mice or 1500 rats over the five-year project. These numbers represent the maximum required but in cases in which the experiments are rapidly successful, we would require fewer 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?	We will induce brain injury in a number of ways, all of which are relevant to the perinatal condition. We will mimic term HI by restricting the blood flow to the brain and/or exposing the pup to a low oxygen environment. Neuroinflammation observed in preterm injury will be mimicked by injection of pregnant dams and/or pups with inflammatory agents or neurotransmitter-like compounds (brain signalling molecules). The expected adverse effects would be transient soreness around injection, wound or tissue sample sites (e.g. tail tip or ear notch), rarely motor deficits such as impaired balance or the ability of the animal to right itself, raised temperature/fever, growth delay, transient lethargy and hypothermia after surgery. Efforts are continuing to optimise anaesthesia, administration of substances and to avoid adverse effects.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our project aims to understand the mechanisms involved in perinatal hypoxic-ischemic and infectious/inflammatory brain injury and to test neuroprotective strategies. As such, we need models that allow us to mimic human perinatal brain injury. Animals have to be used, as to validate a mode of action, experiments are required that cannot be conducted in humans for ethical and scientific reasons. In addition, biodistribution in whole organisms, with intact physiological barriers and excretion mechanisms, is key to inferring potential clinical use. We have considered the feasibility of achieving our purpose by not involving animals, for example by using cell lines or *in vitro* recombinant methods, but no such alternatives are able to reproduce the brain injury we aim to investigate in this proposal. Where possible, however, we will replace whole animal studies with primary cell preparations or experiments in appropriate cell lines (e.g. neuronal SH-SY5Y, microglial BV2, oligodendrocyte CG4 lines).
2. Reduction Explain how you will assure the use of minimum numbers of animals	Under our previous project proposal, we optimised the conditions of a number of our protocols, therefore ensuring the reproducibility of the injury and reducing the number of animals needed for each experiment. Our intention is always to use as few animals as possible for ethical reasons. At the same time sufficient numbers have to be used to attain reasonable power. We will always plan experiments with the appropriate power calculations to ensure the minimum number of animals can be used to produce a statistically relevant result.
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 other animal models we could use, some in which the brain structure is more similar to that of a preterm or term human (foetal sheep, primates, piglets). However, we have decided to replace the use of such animals with rats and mice. These species are less expensive, easier to handle, breed easily and have a short gestation. Importantly, they share several important features with the human brain with regard to brain complexity and injury response in white and grey matter. Moreover, we have the possibility to use genetically modified animals, and a significant amount of genetic and

	physiological information are available for these species. Finally, we are studying perinatal brain injury that should match the degree of brain development in preterm and term newborns. Our experiments are always carried out with the welfare of the animals in mind. We will constantly monitor them for adverse signs, return pups to the dams as soon as feasible, ensure appropriate anaesthesia and provide optimal environmental conditions conducive to the animal's wellbeing.

Project 28	**Cognitive-enhancing properties of nicotine and related psychoactive substances**	
Key Words (max. 5 words)	Nicotine, attention, withdrawal, dependence	
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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Nicotinic receptors are found in key brain regions associated with cognition and administration of the natural alkaloid nicotine has been shown to improve memory and attention, cause changes in subjective mood states, and produce adaptations in the brain which leads to dependence. The mechanisms which are responsible for these various effects of nicotine are not clear. The project will analyse mechanisms through which nicotine produces changes on memory and concentration. These different phenomena will be modelled by various behavioural procedures in rats. Specifically, the project will identify: 1. Which subtypes of nicotinic receptor enhance performance on cognitive tasks using conditioned and spontaneous behavioural endpoints? 2. Where in the brain are these changes produced by nicotinic compounds and which neurotransmitter systems are mediating these effects? 3. Identify the sites of nicotine action using small animal imaging and assess neuroadaptive changes in	

	the brain following chronic exposure to nicotine and related nicotinic agonists.
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 from this project will come from further understanding on how nicotine acts in the brain to improve cognitive performance. These effects are of greater significance since patients with psychiatric disorders who present with cognitive impairments will gain benefit from this research as the nicotinic receptor is considered a therapeutic target for improving cognitive performance. Overall, this work will assist with the development of new cognitive enhancers and also assist individuals with cognitive impairment.
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?	Rats will be trained daily in sessions lasting up to maximum of 120 min in which they will learn to nose-poke, lever press or dig for food pellets in response to seeing a light or smelling an odour at a particular time and place – this task is a test of attention/concentration. Once the effects of nicotine and related nicotinic agonists have been evaluated, they will then be humanely killed. A small number of animals having been treated chronically with nicotine or another psychoactive compound known to affect cognitive function will be anaesthetized and then placed in a magnetic scanner for periods of up to 3 hours. These animals will receive injections of drugs such as nicotine or a related nicotinic agonist. Animals will be kept under the anaesthetic the whole time and at the end of the scan will be humanely killed. In terms of expected adverse effects, most animals will suffer no more than mild stress. The animals adapt to the behavioural task readily and they maintain their body weight under restricted access to food or water and stay in good health. Doses of drugs to be tested will be chosen so as not to have adverse effects. In some experiments, rats will be trained repeatedly following daily injections for weeks. Repeated exposure to the pharmacological compounds produces little adverse effect. In animals that have undergone surgery to have implantation of guide cannulae (fine tubes) for local drug injection or an osmotic minipump to deliver drugs

	chronically under the skin for up to 4 weeks, very few animals develop an infection where cannulae or minipumps are implanted, which is a rare event and veterinary advice will be sought on these occasions. On a daily basis, animals will be checked by technical and/or the scientific staff and body weights will be recorded twice weekly. As part of the experiment, the animals will also be handled and thus any adverse effects of drugs will be detected. The protocols are rated as moderate severity because of the minimal daily handling that could be a mild stressor and the pain associated with this and the minor surgical interventions described will be minimised and managed with appropriate use of anaesthetics and analgesics.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to assess the effects of drugs on memory and attention, only whole animals can be used. The rat is the most appropriate species for this work. A large body of literature already exists on the neurobiology of cognition and the effects of nicotine in rodents. There are currently no other alternative approaches available to measure this. It simply cannot be done in a test tube. While computational network modelling is being introduced within the field of cognitive neuroscience, it has not advanced to the level in which effects of psychoactive compounds on behaviour can be predicted. To date, there is no non-animal alternative to developing and evaluating cognitive enhancers.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments will be designed carefully to ensure minimal number of rodents are utilised. For example, comparisons will be made 'before' and 'after' the drug test for groups of rats. Also, pilot tests will be able to inform the number of subjects required to inform on a significant observation with confidence.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having	In order to assess the effects of drugs on memory and attention, only whole animals can be used. The rat is the most appropriate species for this work because the brain systems and the underlying neurotransmitter systems closely resemble that of the human. A large

regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	body of literature already exists on how the brain works to hold certain types of memory and how best it is possible to improve memory effects using drugs like nicotine. There are currently no other alternative approaches available to measure these types of memory. In the past, non-human primates were used for this purpose and over the last 20 years significant advancements have been made in understanding the optimal conditions under which rodents can be trained and tested with psychoactive substances. Rodent models of various cognitive domains have been developed and from human analog versions that inform on various types of memory and attention. These models have been extensively used to understand the neurobiological basis of cognitive enhancement and this project seeks to explore the role of nicotinic receptors within this with a view to understanding which nicotinic receptor subtypes are mediating this and where in the brain the effect is taking place. The animals adapt to the behavioural task readily and they maintain their body weight under restricted access to food or water and stay in good health. Doses of drugs to be tested will be chosen so as not to have adverse effects. In some experiments, rats will be trained repeatedly following daily injections for weeks. Repeated exposure to the pharmacological compounds produces little adverse effect. In animals that have undergone surgery to have implantation of guide cannulae (fine tubes) for local drug injection or an osmotic minipump to deliver drugs chronically under the skin for up to 4 weeks, very few animals develop an infection where cannulae or minipumps are implanted, which is a rare event and veterinary advice will be sought on these occasions. On a daily basis, animals will be checked by technical and/or the scientific staff and body weights will be recorded twice weekly. As part of the experiment, the animals will also be handled and thus any adverse effects of drugs will be detected.

Project 29	**Mechanism of brain function and malfunction**	
Key Words (max. 5 words)	Cognition, cell assemblies, EEG, dementia, Transgenes	
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 has three interlinked objectives: 1. to enrich our basic scientific understanding how the brain brings about behaviours with a particular emphasis on processes engaged during the formation and recall of memories; 2. place this information into the context of ageing and neuronal disorders in order to understand malfunctions underpinning degeneration and diseases such as Alzheimer's and Schizophrenia; 3. to develop new and more physiological experimental models of disease together with novel translational biomarkers for the testing of refined therapeutics.	
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 richer understanding of the neuronal processes of cognition, memory or amnesia is of vital importance. The difficulty to date arises from the fact that we have a lack of knowledge concerning the specific role of individual brain regions, and the information processing within each region. Add to that the fragmentary understanding of how these regions interact and this in part explains our difficulty in finding appropriate treatments for diseases. In the coming years, we seek to determine some of these	

	processes in normal subjects and by extrapolating into aged or diseases cohorts try to develop a detailed understanding of 'what goes wrong'. Ultimately, patients that suffer neurological / neurodevelopmental diseases will benefit from this work as understanding the principle functions of the brain leads to a clearer vision for treatment and eventually the emergence of better therapeutic.
What species and approximate numbers of animals do you expect to use over what period of time?	Our work is mainly concerned with rodents. Both rats and mice are suitable model organisms and the development and use of genetically altered subjects gives a preference to mouse. Overall numbers vary dependent on funding, drug availability, etc. but on average 2000-4000 animals are utilised each 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?	Loss of cognitive function does not mean there is physical illness. Consequently, we are particularly keen that disease models show no or little overt signs of anomalies and only highly sophisticated behavioural, physiological, imaging or cellular analysis tools can reveal significant differences from the norm. To determine specific brain functions, we administer drugs systemically or locally to inactivate brain regions, nerve cells or neuro-transmitters. In some cases surgical drug administration will lesion areas, cell types, and electrode implantations enable the record the global brain activity using EEG and/or behavioural tests (tests for spatial learning or for semantic knowledge, anxiety and cognitive flexibility). We have specific tests reflecting specific disease models, and one of the important goals is the development and testing of novel drugs for symptom relief. These drugs will be tested for palatability and side-effects such as fatigue and cachexia. Diabetes occurs in the elderly and is utilised as an inducer of cognitive decline. Upon completion of testing, we harvest multiple organs from all our subjects in order to conduct ex vivo analyses. We anticipate the use of more than 10000 animals with 60% of procedures being mild, about 40% being moderate and only 800 of severe severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Understanding behaviour and how the brain functions to induce specific reactions cannot be done without animals. Especially for the investigation of episodic and semantic memory functions, lower vertebrates (fish) or invertebrates (flies, worms) are unsuitable.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We have several strategies for reducing animal numbers. For example, several of our experiments (for example brain imaging) enable the repeated testing of subjects over several months. When tested young, animals considered normal, and as they age they develop neurological anomalies. Through this repeated testing, each animal functions as its own control and we can in some cases omit a control group. In addition, we have developed refined analysis tools for behavioural strategy detection in order to better classify anomalies. This allows us to work with smaller cohort sizes. Finally, we use multiple techniques for recording simultaneously with the aim to gather more information from one subject and correlate behaviour with physiology or cellular markers.
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.	Many of our experiments are conducted using genetically altered mice. The reasons are twofold: 1. They are the best laboratory tool for our experiments, have been widely used so that detailed pre-knowledge exists, and the most sophisticated tools have been developed; 2. We are constantly seeking to develop more physiologically relevant models of diseases (genetic, pharmacological or other) either in our own laboratory or though collaborations. As for the general health of our subjects, several peri-operative measures are in place (repeated and appropriate dosing with analgesic, group housing post-operatively, etc.) and both NVS and NACWOs are at hand to advise and provide support.

Project 30	**Neuronal activity underlying sensory behaviour**	
Key Words (max. 5 words)	Brain, electrophysiology, imaging, information processing, somatosensory	
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)	Distinguishing temporal structure in the wor1d – in particular things that happen in a specific sequence over time — is essential for survival: objects and scenes are distinguished by the pattern (order and interval) within which their elements occur over time. Recognising such patterns is crucial to understanding speech, musical pieces and even Morse code. Surprisingly little is known about how the brain converts basic patterned sensory information into the percept of a sequence. This is a major gap in our functional understanding of the brain. The goal of this project is to understand how representations of sequences emerge from the activity of neurons in the cerebral cortex — the part of the brain that combines information from the senses, assigns it a meaning and decides on a response. In achieving this goal, the project will provide insight into how neuronal circuits shape sensory representations and give rise to decisions and actions.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or	Achieving insight into how neuronal circuits give rise to behaviour is a basic scientific aim. Advances in basic brain science are needed to provide the framework for future improvements in how we	

animals could benefit from the project)?	understand and treat neurological disease. Our understanding of how sensory perception drives decisions and actions remains very limited, particularly in the cerebral cortex, the key region for percept formation, decision making and cognition. The project is designed to provide an approachable experimental framework for this overall question. Sensory processing and its translation into decisions is affected in many pathologies of the cerebral cortex, e.g. developmental disorders such as autism or schizophrenia. Our approach can provide a useful experimental model for testing the functional impact on sensory behaviour of disorders of cortical neuronal circuitry.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice, approximately 1500-2000 animals 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 end?	The experiments involve preparing and training mice to, perform sensory discrimination tasks while monitoring or manipulating neuronal activity in their brain. Adverse effects will vary between mild and moderate. Genetic or pharmacological modifications will be targeted to a small number of cells in the brain and no adverse effects are expected. Surgical procedures will be needed to allow- access to the brain via optical or electrical methods. The relevant surgical methods are well established, generally minimally invasive, and will be carried out under general anaesthesia with analgaesia being provided during and after surgery to avoid pain as much as possible. After recovery, animals will be trained to perform the sensory- guided tasks while positioned within a tube and held with a head restraint, and water or a sweetened fluid will be given to them as a reward in each trial. To ensure motivation, water access will be controlled or restricted and water given in combination with further positive rewards. Hydration levels will be controlled and animals will be given the chance to top-up their daily water ingestion; any evidence of dehydration will result in animals being taken off training. The procedures will end if there is evidence of distress from water control or from restraining the animal's motion during the task. Animals will be humanely killed at the end.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no realistic alternative to in viva data collection for understanding how the brain generates sensory function. Computer simulations can help guide predictions based on known data, but are not yet sufficiently constrained by experiment and cannot take into account unknown factors: to simulate the brain , we will need much more validated experimental data to generate meaningful output. Thus we cannot yet replace observation with simulations. In vitro experiments can provide basic information about how neurons and synapses work, but cannot reproduce the behaviour of an intact neuronal circuit. We do use in vitro work to test the effects Of cellular and synaptic mechanisms under controlled conditions, and this helps reduce further in vivo experiments. For in vivo work, we use electrophysiology and optical imaging because other techniques cannot provide the resolution needed to analyse the activity of individual neurons.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies involving the training and repeated monitoring of animal behaviour on a controlled task imply working repeatedly with the same animal, leading to a drastic reduction in the overall number of animals used. Results from each animal will combine behavioural and neurophysiological data and be much more powerful than if either type of data were collected separately. We have expertise in computational techniques for data extraction and will develop new tools to maximise the yield from each animal. Data sets will be made publicly available, so each experiment will potentially be analysed in a wealth of possible ways. The methods for recording neuronal activity will allow simultaneous measurements from tens of neurons in parallel, allowing us to use a significantly smaller number of animals. We will be able to measure both the sensory capacities of animals and the underlying brain activity at the same time, reducing variability and increasing reproducibility.
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	Rats and mice can perform behaviours previously thought to be only within reach of primates. Neuronal circuitry underlying early cortical processing is remarkably conserved across different mammals, which share principles of cortical organisation. Therefore rodents provide a good experimental model for the fundamental organisation of human sensory

minimise welfare costs (harms) to the animals.	processing. At the same time, rodents have perceived lower sentience than primates and provide a standardised model for laboratory biology. Much knowledge is available on basic rodent neuronal properties, and new work can build on this, avoiding the need for foundational experiments. Tools for genetically based targeting of neurons in mice permit more powerful and precise experiments. Consideration for welfare of the animals is integral to the project's design. Animals will be given analgaesics peri-operatively and monitored after surgery for signs of pain or distress. Animals routinely learn to perform the behavioural task without evidence of distress or ill health. Animals will be housed together; each animal will undergo behavioural training always in the same location, in a dark and quiet room, and will be acclimatised before starting training. Training will be done using positive rewards for motivation as much as possible. Fluid rewards will be defined using the least strict water intake restriction schedule that produces results.

Project 31	**GABAAR, neurosteroids and stress in brain function**	
Key Words (max. 5 words)	GABA, neurotransmission, stress, depression, addiction	
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)	We wish to investigate how the signalling molecule GABA regulates the communication between different types of brain cells in health and disease and how its action is influenced by steroid molecules that are naturally found in the brain ("neurosteroids"). We also wish to understand how negative experiences early in life can cause long-term changes in the communication between brain cells and how these in turn may predispose to the development of mood disorders e.g. depression and drug addiction.	
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 growing evidence in people that early-life experiences can predispose to serious disorders such as depression and drug addiction. By gaining a better understanding of how stress early in life may disrupt the dialogue amongst specific brain cells and the role played in this dysfunction by GABA and neurosteroids, this study may lead to the new treatments that could counteract the long-term effects of these experiences and perhaps protect people against these later risks.	
What species and approximate numbers of	The study will use mice and rats. We anticipate to work with a maximum of 10000 mice and 1400 rats	

animals do you expect to use over what period of time?	over 5 years. We use specifically genetically modified mice to understand the specific contributions of different genes and these animals will have to be bred in our facilities, hence their larger numbers. The stress models we use have been well characterised and do not cause significant outward signs of adverse welfare above a moderate severity (any such signs are usually much less). Such signs include an initial reduction in body weight, which then recovers to near-normal values at adult-hood. However, we expect to be able to measure more subtle behavioural and cognitive effects in our experiments. Some animals will undergo surgery under general anaesthesia but are expected to recover from the procedure with no lasting consequences. In all cases, at the end of the experiments all animals will be killed humanely, in order that we may retrieve brain tissues for further detailed laboratory study.
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 stress models we use have been well characterised and do not cause significant outward signs of adverse welfare above a moderate severity (any such signs are usually much less). Such signs include an initial reduction in body weight, which then recovers to near-normal values at adult-hood. However, we expect to be able to measure more subtle behavioural and cognitive effects in our experiments. Some animals will undergo surgery under general anaesthesia but are expected to recover from the procedure with no lasting consequences. In all cases, at the end of the experiments all animals will be killed humanely, in order that we may retrieve brain tissues for further detailed laboratory study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are currently no non-animal alternatives or simulated models that can faithfully reproduce the overall response of an individual to stressful challenges or the potential of reward. The use of mice and rats is appropriate as the processes of brain cells communication underlying an appropriate response to stressful challenges and the acquisition of rewards are well characterised in these species. They are, also very similar to the processes in the human brain (to the extent that these can be measured).

2. Reduction Explain how you will assure the use of minimum numbers of animals	Breeding programmes will be carefully managed to generate just the number of animals that we need. The sample sizes for our experiments are predictable, reducing the risk of using too many animals. Tissues harvested post mortem can be used in several different types of laboratory investigation, thus maximising the amount of data that can be obtained from a single 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 general measures you will take to minimise welfare costs (harms) to the animals	We use models that have been carefully refined to cause the least outward harm while generating molecular and more subtle behavioural changes consistent with the human states being modelled. All animals are closely monitored and, if any unexpected event occurs, the animal will be referred to our veterinary surgeon or immediately withdrawn from the study and killed humanely.

Project 32	**Investigation of the in vivo action of G protein coupled receptors**	
Key Words (max. 5 words)	Neurodegeneration, physiology, drugs, cancer, diabetes	
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 human body is made up of many trillions of cells. These cells make up organs such as the heart, liver, kidneys and brain. The cells in our body need to communicate with each other. They do so by releasing messenger molecules that attach to specific receptor proteins on the outside of cells. This project is interested in understanding these receptor proteins and working out how the messenger molecules activate of the receptor proteins and how this process changes the way the target cells (or organs) behave. The project will also aim to understand how we might make drugs that activate these receptor proteins and how these drugs might be designed so that they change the response of cells/organs in a way that help combat a number of human disease conditions such as memory loss in neurodegenerative disease, dysregulation of glucose levels in the blood in diabetes and in cancer.	
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 project will have the following benefits: • Scientifically – by studying the receptor proteins described in this proposal we will better understand one of the key mechanisms employed by our bodies to control numerous biological processes	

project)?	such as heart rate, learning and memory, hormone responses and even vision. • Medically – our study will provide a framework from which the next generation of drugs that target the receptor proteins described here will be designed. This will help because most drugs in drug discovery fail because either we have not got enough understanding of how the disease tissue works and how best to design drugs against the disease or because the drugs are toxic. In this study we will obtain the information that will allow for the better design of drugs to increase therapeutic efficacy and decrease toxicity • Economically – we work very closely with the pharmaceutical industry and the results of our study will benefit the generation of drugs against key diseases and as such support the activity of major drug companies.
What species and approximate numbers of animals do you expect to use over what period of time?	Time period is five year: Number of mice to be used = 21,000 Number of rats = 2400
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 mice will be used in moderate severity protocols which reflects the fact that we use a prion model of neurodegeneration and that the animals will be subjected to physiological protocols such as fear conditioning that have adverse stimuli. A small proportion of the mice may experience severe symptoms in the course of our experiments. Every effort will be made to ensure the length of time the animals have these symptoms is kept as short as possible. In the end all the animals in this licence will be killed by schedule 1 methods or through carefully prescribed non-schedule 1 procedures. In this licence the animals will be subjected to the following harm: • **Breeding genetically modified animals:** So of the genetic modifications described in this licence might result in a harmful phenotype such as distented bladder, or difficulty in saliva production. This will be mitigated by careful monitoring and making the animals more comfortable (e.g. by

	providing mashed up food to help with eating). • **Blood sampling:** This will be mitigated by good practice from well training of staff • **Anaesthesia** This will be mitigated by good practice from well training of staff • **Administration of drugs and substances:** In this licence we described a number of different routes of administration which include injections and placing very fine tubes into the brain. The route that will be chosen will be well tested in pilot studies to be the one that causes least distress to the animal and the most effective at delivering the drug. If necessary discomfort will be reduced by anaesthesia. Also good laboratory practice conducted by well trained staff will reduce suffering and harm. • **Animal behaviour testing** We describe a number of protocols where animals will be tested for memory loss and anxiety as well as the ability to feel pain. These tests involve the animals being subjected to mild electric shocks and placed in open environments or on run ways. We will reduce the harm to animals by exposing them to as low an adverse stimuli as possible. We also have extremely efficient equipment and very well trained staff that will reduce the harm to the animals. • **In vivo imaging** To monitor brain activity and tumour formation the animals will be placed in imaging equipment. The animals will be anaesthetised during these procedures to reduce harm • **Tumour development** The animals in this study will be induced to have cancer. Careful monitoring of the tumour growth and potential metastasis will reduce the suffering of the animals
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Mice provide the best model animals for the study of the receptors as they are readily manipulated genetically. Rats are the best animals to use in some of the metabolic studies described here because they have a metabolism that much more closely resembles that of humans than mice. There are no

	non-animal alternatives as the aim of this study is to take the information that we have gather in our non-animal work and apply it to animals to determine if the principles established in the non-animal models actually are of any significance in animals and potentially humans. This is due to the fact that in whole animals there is a complex interplay between organs and cell types that can not be replicated in non-animal studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experiments will be very carefully designed so that only the number of animals that result in statistically significant results will be used. We will also be piloting methods to reduce animal numbers by determining if we can monitor the action of our receptors in disease models in a more efficient manner. We will also maintain only those strains that we are experimenting on and keep all others as frozen embryos so that we reduce the number of animals that we breed and maintain.
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 main species used will be mice since it is possible to readily manipulate the genome of mice thereby changing the receptor proteins in this species in a way that reveals the function and modes of action of the receptors that relates to the function of these receptors in humans. Other model systems such as yeast, worms and fish do not approximate to that seen in humans in the same way as mouse. We will also use rats but not as many as mice. The rats will be used largely in metabolic studies since they these studies largely do not rely on making genetic changes but rather concentrate on the response of the animals to drugs. As this is the case then whereas mice are closely related to humans with regard to the way the receptors respond, rats are even closer. Hence, where we do not require genetic experiments we will use rats. We will employ disease models, such as prion disease, that are the closest model we have in rodents that mimick human neurodegenerative disease.

Project 33	**Biological and Psychological Bases of Addiction**	
Key Words (max. 5 words)	Drug abuse, transgenic mice, behaviour	
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)	Drug addiction and alcohol abuse are a major burden to health, society and the economy and are estimated to cost the UK £28 billion *per* year. The brain mechanisms underlying addiction are incompletely understood, especially how drug taking influences brain connectivity, and the consequences of altered connectivity. Without such knowledge, development of therapies is poorly founded. This project will investigate: 1) changes in brain and behaviour as a result of exposure to addictive drugs; 2) biological and psychological processes hypothesised to underlie addictive behaviour; and 3) potential treatments of addictions. We will investigate both the consequences of exposure to drugs of abuse (e.g. alcohol, cocaine and nicotine) on the brain at different developmental stages, and the neurobiological mechanisms underlying addictive behaviour. Since addictions reflect abnormal function of brain reward systems, we will also examine their normal 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)?	Drug addiction (both to legal and controlled substances) is a major cause of death and suffering (it is anticipated that liver disease arising from alcohol abuse will become the single most frequent cause of death within the next generation). It leads to a disproportional use of health facilities, and addiction	

	is associated with most criminal events. The project will provide a better understanding of the brain mechanisms underlying addictive behaviour, and how addictive drugs induce changes in brain function. In particular, we will provide a neurobiological account of how brain “incentive” pathways function and adapt to repeated drug use in ways that further strengthen addictive behaviour. Such knowledge will provide a new focus for research in addiction beyond the current emphasis on the role of dopamine. The work should ultimately lead to improved methods for identifying individuals “at risk” for addiction, provide targets for the development of therapies (either pharmacological or behavioural) for treatment of addicted individuals, and suggest ways in which addictions may be prevented.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 7500, 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?	Genetically altered animals are likely to show no or only mild signs (e.g. low birth numbers). The great majority of the behavioural tests are either observational in character (e.g. speed of movement) or allow animals to produce responses to obtain rewards, causing minimal discomfort, few adverse effects and mild severity. Where food restriction is required to maintain motivation, animals will be assessed for excessive weight loss daily and their diet adjusted. Where we use aversive motivation (electric shock to the foot) we are careful to establish and use the minimal foot shock levels required to obtain learning in control animals (fewer than 15% of mice (~900) will undergo foot shock training). Approximately 3,000 mice will undergo surgery under anaesthesia, in order to introduce drugs directly into the brain or bloodstream. Drug-specific effects will be short-lived (1-2 hours) and might include wobbliness and incoordination. Observable symptoms of drug withdrawal are likely to be rare, but could, if visible include convulsions, diarrhoea, teeth chattering. At the end of all experiments animals will be humanely killed and where applicable tissues collected and analysed.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Addiction is a maladaptive behaviour, reflecting drug induced-changes in the brain that lead to altered responses to reward-related stimuli. For this reason, the use of conscious animals that possess these abilities is essential. It is not possible to investigate such behavioural disorders in *in vitro* systems. Although mice do not display the full behavioural repertoire of humans, the brain systems thought to underlie the behaviours of interest are largely homologous. The project is part of a wider effort in which we make use, on the one hand, of patients and human volunteers, and on the other of *in vitro* cell culture. Within the narrower limits of the project as described, we are beginning to use brain tissue from experimental animals for electrophysiological work that will in the future guide our choice of drugs for testing therapeutic potential.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals to be tested will be the minimum number required to obtain statistically reliable results, based on previous experience in the laboratory, and the literature. Where appropriate, power calculations will be used to estimate the appropriate numbers of animals based on expected variability, and anticipated effect sizes. Where possible we use within-subject comparisons to increase the statistical power of the experiments, and to limit the numbers of animals used. By coordinating our behavioural work with those using brain tissues for molecular or electrophysiological analyses, we are often able to avoid the need for additional experiments and thus more animals by providing tissues for these *ex vivo* 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.	Our experiments use mice, as the lowest species that have sufficient resemblance in brain function and in specific behaviours (such as conditioning) to humans, to allow the knowledge acquired in experiments to be applicable to understanding human addiction. The use of mice allows a considerable knowledge of genetics, and manipulations of genetic makeup to be exploited, as well as the use of extensive databases on mouse behavioural genetics, reducing the need to repeat experiments carried out elsewhere. Careful attention is paid to the wellbeing of the animal, and where necessary animals that are

	severely affected are killed humanely. Surgery will be carried out under aseptic conditions, to reduce the risk of infection after which animals will be given pain relief.

Project 34	**Characterisation of novel therapeutics**	
Key Words (max. 5 words)	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 objective of the project is to permit the rapid evaluation of new approaches to therapies for various disease states. It will exploit compound activities at therapeutic targets that would be outside the scope of other project licences, and also allow us to pursue novel findings observed in ongoing 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)?	The aim of this project licence is to derive new therapeutic agents for disease states. The licence allows for rapid uptake and prosecution of new projects, to capitalise on existing expertise and knowledge, in therapeutic areas outside other projects. This should lead to quicker advancement to clinical trials and opportunities in novel therapeutic areas, which we would otherwise be unable to explore.	
What species and approximate numbers of animals do you expect to use over what period of time?	Over a five year period:- Mice 4800 Rats 5800	
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 likely level of severity is mild in the majority of animals, with some being moderate. Endpoints have been defined to minimise the risk of this level being exceeded. 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	Non-animal alternatives (e.g. genetically modified cell lines, iPSCs) are a major part of our screening cascade to identify efficacious compounds. These studies are accompanied by in silico screens and cell based screens to assess compound metabolism and likely *in vivo* distribution. In addition some work can be performed utilising whole tissues *in vitro* (e.g. brain slice or isolated nerve work). However the use of *in vivo* models is required for interrogation of complex systems (i.e. beyond the single cell level). Examples of this are:-effects on the cardiovascular system, overall compound metabolism and disposition, overall efficacy and indications of Therapeutic Index (the ratio of beneficial effect to any adverse effect).
2. Reduction Explain how you will assure the use of minimum numbers of animals	Rats and mice are the only species on this licence. A substantial body of background information exists on multiple aspects of their biology within the public domain, and within our company databases. The use of mice is particularly appropriate because there are numerous transgenic models of some of our targeted disease states available. These should have a greater veracity to the human state than chemically induced models, which should in turn enhance the scientific rationale. All the procedures performed in this project will be the least invasive and least harmful possible to achieve the objectives. Where more benign alternatives can be identified these will be investigated. Where these do not comprise the scientific outcome the alternative will be used. The use of terminally anaesthetised animals where possible is such an example.
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 and mice are the only species on this licence. A substantial body of background information exists on multiple aspects of their biology within the public domain, and within our company databases. The use of mice is particularly appropriate because there are numerous transgenic models of some of our targeted disease states available. These should have a greater veracity to the human state than chemically induced models, which should in turn enhance the scientific rationale. All the procedures performed in this project will be the least invasive and least harmful possible to achieve the objectives. Where more benign alternatives can be

	identified these will be investigated. Where these do not comprise the scientific outcome the alternative will be used. The use of terminally anaesthetised animals where possible is such an example.

Project 35	**Studying central nervous system repair**	
Key Words (max. 5 words)	Multiple Sclerosis, Cerebral Palsy, Central nervous system, Repair, Oligodendrocyte	
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)	In the central nervous system, nerves are surrounded by an insulation called myelin which is needed for their survival and communication of messages to other parts of the nervous system and muscles. Brain injury can lead to loss of myelin, leaving nerves uninsulated and open to damage; this can lead to major problems with sensation, intellect and movement as seen in the common disorders multiple sclerosis and cerebral palsy. This project is aimed investigating ways we can encourage the regeneration of myelin in the central nervous system, in order to identify new treatments for these disorders. More specifically, we aim to identify the important interactions between the cells that repair the myelin and other brain cells that control this repair, manipulate the molecules involved in these interactions or the cells themselves in order to increase repair, and discover new molecules involved in myelin repair that could be further developed into drugs to increase repair in diseases like multiple sclerosis and cerebral palsy.	
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)?	Current approved therapies for multiple sclerosis and cerebral palsy are only aimed at reducing the initial damage; these don't encourage the regeneration of myelin and are therefore only marginally effective at improving physical health. Thus there is a pressing need for treatments that cause regeneration of myelin. To do this, we must better understand what prevents and encourages myelin regeneration following brain injury. This could lead to the development of drugs to	

	stimulate myelin regeneration in people with cerebral palsy and multiple sclerosis to prevent nerve damage and improve their health. Our work over the last five years has made major progress in discovering important molecules that are involved in myelin repair and we hope to continue this work to progress towards their development as pro-repair drugs, and to discover additional molecules that could be similarly developed.
What species and approximate numbers of animals do you expect to use over what period of time?	Our research group anticipates using 7500 mice and 2500 rats over the course of 5 years, for all current members of the lab and future expansion of the lab. We use statistics to calculate exactly how many animals we need for each experiment to get significant results.
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?	There are 3 severity categories: mild (which means that there is no to little short term pain, distress or suffering), moderate (which means that the procedure may affect the animal's health but the animal recovers from the procedure and is able to feed and grow normally) and severe (which means the animal does not recover from the procedure and might experience severe discomfort). Our research only uses mild and moderate methods. This research uses mice and rats, using different model systems where we: 1. Grow cells and slices of the central nervous system in a dish to study myelin repair. This procedure is of mild severity and has no adverse effects, as we isolate cells/tissue from animals that are sacrificed humanely without any experimentation. 2. Maintain genetically altered animals for the purpose of modifying molecules and cells in models of brain injury. This procedure is generally mild as it involves breeding these animals, but can be moderate if changing the molecules/cells in these animals affects their health. Animals with poor health that are not considered to recover by the veterinarian are sacrificed humanely. 3. Carry out experiments on live animals to model brain injury. This procedure is of moderate severity as it involves surgery (under anaesthesia) to induce a small area of damage in the central nervous system. Adverse effects are rarely observed but may include temporary difficulties with movement. Animals are maintained after surgery for a short period (less than one month).

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This research can only be carried out in animals because of the complex and 3 dimensional structure of the central nervous system. We don't yet understand all of the interactions between the different cell types in the central nervous system nor do we know all the molecules they make that could impact myelin repair. For this reason, we cannot yet use computer models to do this research. We also need to be able to manipulate the injured brain to ask how specific cells or molecules contribute to myelin regeneration, which requires animals as it would be unethical to use human subjects. We also can't use human cells in a dish, because it is very difficult to get high numbers of the cells we need for our experiments, and because myelin repair involves the interaction of many brain cell types that is difficult to reproduce.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to use the minimum numbers of animals, we first do experiments using cells in a dish to optimize our experimental design, so that we can use less live animals for later experiments. We also use statistics to calculate exactly how many animals we need for each experiment to get significant results.
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 rodents because they are the smallest mammals that are used to model human disease, and many drugs/therapies now in use in humans were first tested in rodents, giving more confidence that if they work in these animals, they may work in humans. Also, there are many genetically altered rodents already in existence which are the best way to discover the role of particular molecules or cells in a biological process. More specifically, we work with mice because the protocols we use have been optimized for mice meaning we get the most data and experiments from one mouse, reducing the number of mice used. Also, there are numerous genetically altered mice already in existence that we can use to ask specific biological questions. We work with rats because we can get large numbers of cells from rat brains, maximizing the number of treatments we can test in one experiment, and reducing the number of animals used. We minimise harm to the animals by 1) performing only procedures regulated by the Home Office, 2) being trained appropriately for new procedures being carried out on animals, 3) by consulting the veterinarian prior

	to every surgery we would perform on animals, 4) by using anaesthesia and anti-inflammatory drugs to minimize pain and discomfort during procedures, 5) by closely monitoring the animals for signs of pain or discomfort and taking appropriate measures to eliminate those or end the experiment if necessary, and 6) by testing all drugs or treatments in a dish in the laboratory prior to giving to live animals.

<table>
<tr><td>Project 36</td><td>Studying myelinated axons in vivo using zebrafish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Zebrafish, In vivo imaging, Nervous System Development</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The aim of our project is to elucidate the formation, function and repair of myelinated axons. Our neurons transmit electrical impulses along thin cables called axons and the majority of our axons are covered by an insulating substance called myelin. Myelin is made by specialised cells in our central nervous systems called oligodendrocytes and damage to myelin contributes to numerous diseases of the nervous system, including multiple sclerosis, MS. We currently have a very poor understanding of how myelin is made by oligodendrocytes and how axons and oligodendrocytes interact to regulate when and where myelin is made. This lack of understanding impairs our ability to design rational therapies for the repair of damage to myelinated axons for the treatment of diseases such as MS.

We use zebrafish to study myelinated axon formation, because they are a relatively simple system with great relevance to humans, sharing over 70% of our genes. They also have innate properties that allow non-invasive lmicroscopy of the living animal.

Specifically, in this project we have 4 objectives.
1. We aim to identify the genes that are required for myelination. We also aim to identify chemical compounds that can promote myelination.
2. We aim to understand the complex cell behaviours and cellular interactions that occur during myelination.</td></tr>
</table>

	3. We aim to understand how brain activity can regulate myelination. 4. We aim to understand the cellular events that occur in response to damage to myelin and during subsequent repair.
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 identifying the genes that specifically regulate myelinated axon formation we will learn about the fundamental mechanisms of myelination and axonal development. We will also identify molecular pathways that could be manipulated for the treatment of diseases of myelin, such as multiple sclerosis. By identifying chemical compounds that can regulate and promote myelination we will gain further insight into mechanisms of myelination and may also identify compounds that could form the basis of treatments for disease. By gaining a better understanding of the cellular events that occur during normal myelination, in response to myelin damage and during myelin repair, we will gain insight into the events that are typically impossible to study in human disease or in mammalian disease models and thus gain new insight into important aspects of disease progression, pathology and repair. By deciphering how brain function can regulate myelination we will provide new insight into life-long function of our nervous system. It has recently been suggested that modulation of myelin throughout life by brain activity may affect learning, memory and higher order brain function.
What species and approximate numbers of animals do you expect to use over what period of time?	We use zebrafish in our studies and propose to use >100,000 over the course of this study. One very important point is that over 90% of the animals used throughout this project will be for breeding purposes only. These animals will live normal healthy lives with no adverse effects.
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 addition to animals that will be maintained for breeding and maintenance only, we also propose to analyse myelin formation through experimental protocols. In almost all of these cases this will be non-invasive microscopy carried out on young embryonic zebrafish. Because zebrafish embryos are very small and also naturally transparent, our community has used transgenic techniques to generate animals whereby cells of interest are made to fluoresce and it is now possible to watch myelin formation as it happens in the living animal. We can do this without any observable adverse effects on the

	animal, which is important as we want to observe how myelin forms in as close to the natural state as possible, and also how it is repaired following damage, in as close to a natural way as possible. To identify genes that regulate myelination we generate and maintain mutant zebrafish with mutations in specific genes. To study how such mutations affect myelination we use transgenic reporter animals that allow us to visualise various aspects of myelination in such animals using fluorescence microscopy. We have refined techniques used to generate mutations that in the past caused substantial adverse effects to treated animals, such that these now cause no substantial suffering to the animal. Although disruption to myelinated axons can have adverse effects we primarily study mutant and transgenic animals at early larval stages, and do not study animals with substantial adverse effects. Furthermore we do not maintain animals with adverse effects as adults. We also use a non-invasive transgenic system to ablate myelin producing cells, and we have found that animals are remarkably tolerant of this damage, and show minimal disruption e.g. to their normal swimming or feeding behaviour. This allows us to look at myelin pathology and myelin repair over time, without any surgical interventions in ways that almost impossible in other animal models, and of course, entirely intractable in humans. To actually visualise myelination in live zebrafish by fluorescence microscopy, we place zebrafish larvae under anaesthesia and embed them in a soft jelly-like substance and observe them under a microscope. At the early stages at which we carry out our experiments, the young zebrafish are entirely tolerant of this preparation, and can be removed from the jelly after microscopy, upon which they recover normal behaviour within seconds. This non-surgical method of imaging has in and of itself at most a mild effect on the animal, and the ability to carry out such imaging in this manner represents a great advantage of zebrafish as a model system.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex interactions between axons and glial cells that co-ordinate formation of myelin are hard to recapitulate in cell culture. However, zebrafish start to generate myelin at a very early stage of

	development, when they are assumed to be non-sentient. This allows us to carry out the vast majority of our studies at stages that are not protected by Home Office regulation. This replaces the need to carry out experiments on older animals or more sentient species and is a great advantage of the system. We need to maintain stocks of genetically modified adult zebrafish in order to generate the young animals that we study, but adults are not maintained if they exhibit significant adverse effects due to their genetic modification.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to minimise numbers of animals, we ensure the best husbandry practices, in order to raise as many adults from as few embryos as possible. We also carry out statistical analyses in the preparation of our experiments in order to use as few animals as possible.
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.	Myelin is a vertebrate specific feature. Therefore zebrafish are the simplest standard animal model that can be used for its study. The early onset of myelin formation in zebrafish also means we can carry out experiments at the least sentient stages of this species, when the animal is assumed to have minimal capacity to experience suffering. Almost all of our analyses involve non-invasive methods that cause little or no harm to the animal.

Project 37	**Zebrafish models of movement disorders**	
Key Words (max. 5 words)	Parkinson's disease, mitochondria, dopaminergic neurons, parkin, PINK1	
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)	Although neurodegeneration results in the loss of specific neurons, the exact mechanism or processes by which neurons die in Parkinson's disease (PD) or related disorders is typically unknown even where the exact genetic mutation that leads to the disease has been identified. Additionally, good models of disease that recapitulate many aspects of PD and related disorders and that are appropriate for identification of potential therapeutics are lacking. The goal of the project proposed is to develop genetic zebrafish models of neurodegeneration with specific focus on genes such as PINK1, parkin, glucocerebrosidasel or LRRK2 in initiating neurodegeneration. The genetic models developed will be utilized to determine commonalities of disease process between humans and fish. Additionally we will utilize zebrafish model to identify early changes in disease process and identify factors that modulate neuronal health using stress markers. The eventual goal of this study will be the use of the models developed to screen and identify potential neurotherapeutics to treat the disease.	
What are the potential benefits likely to derive from	We hope that the fish model would complement rodent models and thus provide a more rapid and high	

this project (how science could be advanced or humans or animals could benefit from the project)?	throughput system to study human neurological diseases. Due to their small size, transparency, rapid development and ageing, they can be used in screening drugs and treatments in addition to uncovering disease mechanisms. Specifically we plan to utilize genetic sensors to identify specific disease processes to allow visualization of disease process that would help in better understanding of disease process and in identifying novel drugs to impact the disease process.
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 65,000 zebrafish 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?	The most likely adverse events will be the development of symptoms of neurodegenerative disease, such as impaired swimming. These are likely to be moderate severity. Animals will be sacrificed humanely to provide tissue samples for our research.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To characterise molecular mechanisms of neurological diseases we must perform some experiments at the level of the whole organism. We use cells and tissue samples where it is possible to do so, but ultimately we need to understand how neurons die in their natural context. Neurons are highly specialised cells, which interact with a wide variety of other cell types both inside and outside the brain and spinal cord. For example a motor neuron in the lower spinal cord (small of the back) can send processes, over a metre long, out to muscles in the foot and in so doing makes unique and intimate interactions with at least four different cell types. Each interaction has its own complicated chemical and physical signals. Such complexity is impossible to replicate in culture systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We carefully design our experiments to minimise variability and this allows the use of the smallest number of animals required to produce statistically significant results. The experimental approaches we

	use have been discussed with statisticians and academic experts in 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.	We use zebrafish because they offer several technical advantages compared to alternative species such as mice for our experiments. Fish are vertebrates (i.e. they have a well characterized dopaminergic nervous system) so represent a simple yet appropriate model for studying human neurological diseases. We use close monitoring of adult zebrafish to monitor levels of distress. In the event that genetically altered zebrafish shows any distress, for example caused by abnormal swimming, this allows us to implement a humane endpoint.

Project 38	**Post-operative Cognitive Decline: Pathogenesis & Protection**	
Key Words (max. 5 words)	Surgery; Neuroinflammation; Cognition; Dementia; Alzheimer's	
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)	The objective of this project is to identify protective treatments that can quickly be transferred into clinical practice for older surgical patients who may be at risk of developing post-operative cognitive decline (POCD); a condition that can lead to dementia. At the point of pre-operative assessment, patients aged over 65yrs will be advised to undergo a short course of protective medication, typically for a few weeks preceding and following their surgery. This medication will form part of a comprehensive neuroprotective strategy (eg. including choice of anaesthetic), specifically tailored for the patient.	
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)?	At present there is no effective preventative or treatment strategy for POCD. The number of patients at risk of developing POCD is set to rise dramatically over the next few decades as over 65-yr olds are predicted to become the largest consumers of surgery, typically requiring procedures for age-related conditions such as knee and hip replacements. If a protective strategy for POCD can prevent just 1-2% of new dementia cases, it would spare over 100,000 lives annually, saving at least $2 billion in care costs worldwide.	

What species and approximate numbers of animals do you expect to use over what period of time?	Mice = 1000 (over 5 years) Rats = 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?	All protocols on this licence are mild or moderate procedures in terms of severity. The surgical procedures (either laparotomy, unilateral nephrectomy or tibial fracture repair) are fundamental to investigating how we can safely prevent post-operative inflammation from triggering cognitive deterioration. However, the aim is to replicate the hospital setting as closely as possible. Therefore surgery, anaesthesia & analgesia and post-operative monitoring will be carried out by doctors with the same level of care and expertise as given to a human. We expect adverse effects to be minimal based on our extensive experience and our continuing commitment to animal welfare (see 3R's section). At the end of these experiments the animals will be humanely killed by terminal anaesthesia (this is the same method used by vets for pet animals) and tissues collected for analysis. Any animal that shows signs of physical or psychological distress prior to this and does not respond to veterinary intervention, will be euthanised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The events which lead to POCD are complex, involving changes in immune system function, blood vessels and destabilization of specific brain networks. Human studies are useful, but can only provide associative information rather than address causation. Also, human drug trials have shown us that there is a very real risk that common drugs can make POCD worse and even increase mortality. We therefore need to assess new treatments first in a model that will provide an accurate insight into how human higher cognitive processes might be affected. We have considered using zebrafish as an alternative to small rodents, since they can be trained to learn simple tasks. At the moment we are unable to model more complex cognitive tasks or lifespan-related changes sufficiently well in zebrafish, however, genetic techniques may make this possible the near future. To replace living animals in some aspects of our work, we use computer simulations to model how a drug action may influence different cell types in the brain, and also use human cell culture models of the blood-brain barrier to select the most promising drugs before proceeding to animal testing.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We have been able to reduce the numbers animals used in this in this study by taking the following measures: (1) Good statistical design and planning: the sensitivity and power of experiments has been optimized to detect significant results with the smallest numbers of animals. This includes reducing statistical 'noise' by minimizing sources of variation. Eg. By using inbred strains with litter-mates as age-matched controls and using strict criteria to ensure all studies are performed to the same standards, including randomisation and blinding to avoid bias. (2) Use of non-animal alternatives such as computer simulation and human cell culture methods to predict which drug treatments are likely to be most effective has reduced the need to use animals by nearly half. (3) Animal breeding programs will be kept to the absolute minimum required, to avoid unnecessary animals being produced. Finally, (4) by increasing information sharing, such as making our tissue banks and raw data available to other researchers, this will improve the reliability of findings, reducing the need for further animal 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.	Mice and rats and are used as the least sentient mammals to model the cognitive functions and biochemical systems disturbed in human POCD. To model how vulnerability to POCD might develop in humans, we use older adult animals, some of whom receive a Western' diet containing slightly too much sugar or fats, or animals that have genes altered such human Alzheimer's disease mutations. Because we study these animals' cognitive ability for a long time, it is essential that they are well-looked after and have interest in their lives. For this reason, they live in small family groups and their cages are enriched (eg. with tubes, wooden toys, buried treats) to encourage natural behaviour. They also have regular sessions in larger play boxes containing running wheels, ladders, tunnels and interesting nest boxes. The animals are regularly handled so that they do not become unnecessarily anxious when they are placed in mazes to test learning. We aim to make the animals as comfortable as possible, eg. using low lighting and least distressing methods to collect information, eg. instead of injections, we always prefer to give drugs in a sugar droplet on the tongue. For surgery, we use minimally invasive techniques with the same type of anaesthesia, pain killers and post-operative care that a human would receive. Rodents recover much faster than humans and after a few days they can return to

	their home cages. We then monitor their cognitive ability again, starting with gentle tasks such as inquisitiveness and exploration; before they go on to do more complex tasks such as spatial navigation to find a hidden platform over the course of the next month or two. We have found that this approach produces findings that are highly similar to the way that cognitive problems can emerge after surgery in older humans, whilst minimising the distress to animals.

Project 39	**Genetic analysis of axon guidance and maintenance**	
Key Words (max. 5 words)	Breeding, transgenic, neurodegeneration	
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)	How is the brain built and how is it maintained? These questions are of fundamental importance in the quest for understanding not only the basic biology of brain function but also for understanding developmental and degenerative neurological disorders. Neurons, the cells that form the nervous system, make long-distance connections with each other via axons. These connection need to survive throughout the lifetime of an organism for the nervous system to function properly. Much is known about the biology of the cell bodies of neurons that contain the 'biological machinery' that is vital for cellular function, but little is know about the axons that connect these neuronal cell bodies. Previous experiments have shown that signals from the local biological environment is needed to establish these axonal connections, therefore it is important to study axons in the living animal. Using transgenic mouse technology will enable us to investigate the complex mechanisms that are involved in establishing these axonal connections, as well as the seemingly distinct mechanisms that are vital for their subsequent survival of these connections in a living 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	Diseases in which these axonal connections breakdown have devastating consequences such as major losses in motor and sensory function. Examining how these axonal connections form and subsequently survive, within the living animal may	

project)?	provide valuable insights for developing therapeutic tools for neurodegenerative disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	10,500 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?	The majority (90%) of the animals maintained under this licence are not expected to show any detectable adverse effects. A small number of the transgenic lines may develop neurological defects (e.g. partial visual impairment) however this is not expected may render these animals less effective in their normal life tasks, such as feeding and drinking. All animals will either be humanely culled, after which various tissues samples may be collected and analysed or genetically altered breeding stock may be transferred to another licence.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The nervous system is extraordinarily complex with numerous different cell types that form connections with each other via interaction with the local biological environment. We intend the study the complex molecular mechanic that underlie the formation and maintenance of these connections. It is therefore only possible to study these connections within their native environment, the brain of a living animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Breeding will be controlled to match experimental requirements and, when possible, different tissues from the same animal will be used for various experiments in order to minimize the number of used animals. Finally, pilot studies will be employed to refine 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.	The biology of the mouse nervous system is similar to that of humans. Studying the mouse nervous system therefore provides us with valuable information that will further our understanding of human biology and diseases. Transgenic mice technologies are becoming increasing sophisticated, where genes can be turned off within specific cells when required, whiles leaving the rest of the animal unaffected. This greatly reduces the risk of adverse effect to the animal. If any procedure causes harm beyond the moderate severity limit, these animals will be immediately humanely culled.

Project 40	**Analysis of Fish Development**	
Key Words (max. 5 words)	Eye, brain, stem cells, zebrafish	
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)	We use zebrafish to investigate 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.	
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 to derive from the projects outlined in this application are: a) several human congenital eye diseases (such as microphthalmia, anophthalmia and coloboma; MAC) arise from abnormal cell specification or 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 phenotypes. 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 develop our novel disease models and transgenic animals for drug screens. b) 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. c) Our study of neuronal circuit formation aims to link functions of neuronal subsets to animal behaviour. Our area of specific interest and expertise lies in brain asymmetry. Altered brain asymmetries are implicated in a variety of human disorders from schizophrenia, dyslexia, depression and other mood disorders, as well as neuro-degenerative diseases such as Alzheimer's disease. The zebrafish offers a valid alternative to using rodents for preliminary behavioural analyses and to test the efficacy of novel drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	We are using the zebrafish. Over the course of 5 years, the researchers in my group will use about 120,000 fish for breeding, 77,000 will be used in research 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?	The severity level for 99.5% of all used fish is undetectable or 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 (<1% or less than 200 larvae a year) it is possible, although unlikely, that there is a temporary moderate discomfort. Similarly, in a very small number of young fish (larva), we will need to use neuro-muscular blocking agents on tethered animals to prevent them from moving while we take images of their brain activity. 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	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 zebrafish are proving to be very useful for studying developmental processes in the whole animal. Our studies examine complex interactions between cells and their organisation into tissues in the developing embryo, the interactions between

	structures of the sensory system (e.g. eyes) and the brain, and behavioural responses to perceived stimuli in health and disease. These interactions and especially vertebrate behaviour, cannot be adequately modelled in an invertebrate animal system, such as *C. elegans* or *Drosophila*, *in vitro* or in other ways. Zebrafish is an ideal model because its neuroanatomy and circuitry is a simplified version of that found in mammals, and by using zebrafish, we are using the least sentient animal possible to achieve our research goals and to answer our scientific questions. Through our own research we have contributed to a replacement of mammalian species (such as mice and rats) by a lower vertebrate, such as the fish, to study animal behaviour including social behaviour.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For the vast majority of our work on brain and eye development we fixed tissue from embryos, at a stage before the nervous system is mature. We will endeavour to combine different molecular and histological techniques using the same fixed specimen to gain a maximum of information. For our work using older animals such as larvae, we are careful to plan our experiments so that very few animals are needed to obtain a qualitative result and we will use statistical analysis to achieve significance with the minimum number of animals. Where appropriate, we will collaborate with a statistician to plan experimental design and discuss results. Wherever possible, we will use sophisticated software allowing efficient data extraction as well as re- analysis. We will also collaborate and coordinate our efforts with other groups to avoid duplication.
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 choosing zebrafish, we use the simplest vertebrate model system in terms of evolutionary, anatomical and neurophysiological complexity that can be used to achieve our research goals. An advantage for using zebrafish is its optical clarity, which allows visualisation of cell behaviour in the intact unharmed animal simply using a microscope, and the availability of large numbers of eggs without the requirements of invasive intervention such as in rodents. 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 for any abnormal behaviour that would indicate. Any animal showing abnormal behaviour or abnormal distress will be culled by humane euthanasia methods according to guidelines.

Project 41	**Blood flow and tissue oxygenation in rodents**	
Key Words (max. 5 words)	Haemodynamic response, Imaging, pre-clinical models	
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)	In this project we aim to improve our understanding how the haemodynamic response changes in preclinical models of disease. The brain is critically dependent on its blood supply to maintain normal function. Interruption of the blood supply to the brain, as occurs during cerebral ischaemia ('stroke'), causes widespread neuronal death. This neuronal death makes stroke a leading cause of death and disability worldwide. At present, restoration of blood flow by thrombolysis with recombinant tissue plasminogen activator (rtPA) is the only licensed treatment. Since the health and welfare costs associated with stroke represent a significant burden, estimated in the region of £9 billion per annum in the UK alone, there is an urgent need for new treatments. But critically once the block is removed large areas of brain tissue die over the following hours and weeks in stroke sufferers which can lead to death or severe disability. With collaborators we want to investigate how neuronal inflammation and /or stroke change the dynamics during the acute phase and the following few hours. Colleagues have recently discovered in a genetically modified mouse model that has an eosinophil deficiency or increased that the changed number of Eosinophils leads to changes in blood flow and blood pressure in the periphery. In this project we want to investigate the effects of eosinophils on blood flow in the brain and in the periphery like the mesentery. This is important to understand as the changes to blood flow induced by different eosinophil	

	concentrations might be a clue why for example patients with inflammation have a worse outcome after stroke.
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)?	Outcomes from this research can inform the development of new treatment strategies in stroke patients. The research can also improve our understanding of the general aspects how the brain responds to increased brain activity by increasing blood flow to that area.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years we estimate to use about 300 mice and 100 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?	All animals will be maintained under general anaesthesia throughout the experiment. The animals will be euthanized at the end of the experiment. Therefore the severity of the experiment is non recovery. Animals will be monitored continuously for changes in physiological status. Appropriate measures to maintain surgical anaesthesia will be taken immediately if required.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The experiments proposed in this work involve the recording of neuronal activation and imaging of intrinsic signals in the rodent cortex. At the moment there is no alternative to in vivo studies to obtain information about the hemodynamic response of the brain and the underlying cortical activity and how this changes with disease. Currently there are no in vitro models or systems that replicate the complex interactions and architecture of the CNS and its communication with other potential influencing factors such as the immune and vascular systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have for many years been at the forefront of developing independent component analysis (ICA) methods to separate the signals from noise sources which allows us to reach statistical significance in our experiments with lower numbers of animals. Recently we were the first to demonstrated that these ICA approaches can not only increase the quality of the imaging data but are also capable of identifying the origin of different components in the recorded haemodynamic response In experiments that compare data between two

	groups or more a t-test or analysis of variance (ANOVA) will be used respectively. When measurements are taken over several time-points in the same cohort, repeated measures ANOVA will be used. Depending on what form the data take, statistical tests used will either be parametric (equal variance) or non-parametric (unequal variance). If there is any doubt on experimental design or statistical analysis, advice will be sought from a local statistician.
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 proposed experiments will be conducted on rats and mice. The mechanisms of the haemodynamic response are very similar in these species to humans. This allows us to draw conclusions from our research to the human model. The proposed studies could not be undertaken in lower species because they do not show such similarities to humans, and *in vitro* experiments do not allow the study of effects like the changes of blood flow. All experiments will take place under full anaesthesia without recovery which therefore is non recovery throughout.

Project 42	**Biological and Psychological Bases for Addictions**	
Key Words (max. 5 words)	Drug abuse, transgenic mice, behaviour	
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)	Drug addiction and alcohol abuse are a major burden to health, society and the economy and are estimated to cost the UK £28 billion per year. The brain mechanisms underlying addiction are incompletely understood, especially how drug taking influences brain connectivity, and the consequences of altered connectivity. Without such knowledge, development of therapies is poorly founded. This project will investigate: 1) behavioural and neural adaptations resulting from exposure to addictive drugs; 2) biological and psychological processes hypothesised to underlie addictive behaviour. We will investigate both the consequences of exposure to drugs of abuse (e.g. alcohol and cocaine) for neurobiological and behavioural competence, and the neurobiological mechanisms underlying addictive behaviour. Since addictions reflect aberrant function of systems serving reward, we will also examine the normal function of such systems.	
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)?	Drug addiction (both to legal and controlled substances) is a major cause of death and suffering (it is anticipated that liver disease arising from alcohol abuse will become the single most frequent cause of death within the next generation). It leads to a disproportional use of health facilities, and addiction is associated with most criminal events. The project will provide a better understanding of the brain mechanisms underlying addictive behaviour,	

	and how addictive drugs induce changes in brain function. In particular, we will provide a neurobiological account of how brain “incentive” pathways function and adapt to repeated drug use in ways that further strengthen addictive behaviour. Such knowledge will provide a new focus for research in addiction beyond the current emphasis on the role of dopamine. Such understanding will ultimately lead to improved methods for identifying individuals “at risk” for addiction, provide targets for the development of therapies (either pharmacological or behavioural) for treatment of addicted individuals, and suggest ways in which addictions may be prevented.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 550
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 great majority of the behavioural tests are either observational in character (e.g. measurement of fluid consumption) or allow animals to produce responses to obtain rewards, causing minimal discomfort, no adverse effects and mild severity. Mice may be administered two doses of psychoactive drugs, sufficient to cause temporary hyper-locomotion. Where food restriction is required to maintain motivation, animals will be monitored for excessive weight loss adverse every day and their diet adjusted. At the end of the study the mice will be killed and their brains used for ex vivo studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Addiction is a behavioural phenomenon that reflects disorders of motivational and executive control systems, as well aberrant learning. For these reasons, the use of conscious animals that possess these abilities is essential, It is not possible to investigate such behavioural disorders in in vitro systems. Although rodents do not display the full behavioural repertoire of humans, the brain systems thought to underlie the behaviours of interest are largely homologous. The project is part of a wider effort in which we make use, on the one hand, of patients and human volunteers, and on the other of in vitro cell culture. Within the narrower limits of the project as described, we are beginning to use ex viva materials for

	electrophysiological work that will in the future guide our choice of drugs for testing therapeutic potential.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals to be tested will be the minimum number required to obtain statistically reliable results, based on previous experience in the laboratory, and the literature. Where appropriate, power calculations will be used to estimate the appropriate numbers of animals based on expected variability, and anticipated effect sizes. Where possible we use within-subject comparisons to increase the statistical power of the experiments, and to limit the numbers of animals used. By coordinating our behavioural work with our immunohistochemical and electrophysiological procedures, we are often able to avoid additional experiments to provide tissue for ex vivo 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.	Our experiments use rodents, as the lowest species that have sufficient resemblance in brain function and in specific behaviours (such as conditioning) to humans, to allow the knowledge acquired in experiments to be applicable to understanding human addiction. The use of mice allows a considerable knowledge of genetics, and manipulations of genetic makeup to be exploited. Careful attention is paid to the wellbeing of the mice, and where necessary mice that are severely affected are killed humanely.

Project 43	**Molecular & cellular correlates of stress-induced behaviour**	
Key Words (max. 5 words)	GABA, mental illness, noradrenaline, serotonin, emotion	
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)	Our brains control our behaviour. However, our life experiences also impact on brain function which in turn can determine our behaviour. Thus, the function of our brains and thus our behaviour is a manifestation of native brain function and how that has been shaped by ourexperiences. What we do not know is how specific brain regions and cells within these regions cooperate to bring about coordinated brain function and the behaviour of the individual, We also do not know what chemicals are expressed by.these different cells and brain regions and how they influence brain function. Finally, we do not know how different life experiences, either nurturing or traumatic, influence the expression of these chemicals and thus the activity of the brain and the behaviour of the individual. This is important to know since many devastating mental illnesses such as anxiety and depression arise due to traumatic life events, such as severe stress. Intriguingly, some individuals who are exposed to severe stress appear to be adept at handling such challenges without any overt signs of disease. Thus, the overall aims of the project are to determine how life stress influences the expression of the proteins which control the cells within brain regions important for dealing with emotional challenges and what are the fundamental mechanisms which determine either resilience or vulnerability to	

	developing stress-induced mental illnesses.
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 will be a greater understanding of the chemicals within the brain which determine emotional behaviour, and how adversity, at different stages of ones' life, influences the expression and function of such brain chemicals. This knowledge will allow scientists to rationally design therapeutic strategies for brain disorders which arise from traumatic life experiences.
What species and approximate numbers of animals do you expect to use over what period of time?	I will use rodents, rats and mice. Based on my ongoing experience, I expect to use 600 animals over the 5 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 happen to the animals at the end?	The large majority of the animals will be killed humanely using general anaesthetics, thus rendering them insentient. Therefore, suffering will be minimal. However, we will use validated stress paradigms on a subset of animals some animals, the severity of which will be moderate. This is unavoidable since the only way to understand the native mammalian stress response s to expose animals to stress. Most animals will be humanely killed shortly after they are exposed to the stressors. All animals 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	The aim of the study is to understand native mammalian brain anatomy and function and how this determines behaviour. The study of native brain function can only be determined by studying the brain itself as there are no suitable models to replicate its anatomical and functional complexity.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Consideration at every stage of the project will be given to ways in which the number of animals used can be reduced. Results obtained from this project, for example, the description of the expression of different proteins could facilitate further research without the use of animals. As soon as I am aware of the presence of a certain protein in the brain regions I am interested in, non-animal models will be used to further characterise the function of said protein. This can be done by expressing recombinant forms of the proteins in various cell-lines and theirs functions being further

	characterised. Furthermore, the characterisation of the expression and function of specific proteins in animal brains will allow me to compare expression in post mortem tissue. This allows extrapolation of the likely functional roles in humans, thus minimising the need for any further animal 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.	I have chosen to use mice and rats since they are the most widely used research animal species. This will ensure that my data are comparable to those of other scientists and thus of benefit to the body of scientific knowledge. All the protocols are routinely used, highly refined and well validated throughout the world. This ensures that they can be used with a high degree of proficiency thus ensuring that the least amount of animals required, are used. Most of the protocols are mild and will induced only minor discomfort primarily due to the novelty of handling by the investigator or the relatively inert behavioural assays. However, part of the project involves investigating the effect of stress on the brain. This necessarily. involves the exposure of animals to stressors. All such stressors have been compressively validated and are used routinely. Furthermore, we will use the least amount of stress to assess its impact on brain function with the animals being killed humanely soon after the exposure to such stressors.

Project 44	**Neuronal circuitry of the spinal dorsal horn**	
Key Words (max. 5 words)	Pain, Itch, Spinal cord, Interneuron, Projection neuron	
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)	Although the spinal cord plays an important role in transmitting and modulating sensory information that is perceived as pain and itch, we still know relatively little about how this information is processed at the spinal level. The objectives of the project are to identify and characterise functional populations of nerve cells in the spinal cord that are involved in pain and itch, to establish how they are organised into nerve circuits that either increase or decrease these sensations, and to determine how they contribute to pathological pain after nerve injury.	
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 lack of effective treatments for both neuropathic pain and chronic itch, and these therefore represent major unmet clinical needs. Understanding the nerve circuits that convey pain and itch, and characterising the different cells that are involved, is likely to lead to the recognition of potential targets for new analgesic and anti-pruritic treatments. In addition, understanding changes that occur in the spinal cord following nerve injury is necessary if we are to develop improved treatments for neuropathic pain.	
What species and approximate numbers of animals do you expect to use over what period of time?	These experiments will be performed on mice, because of the availability of genetically-modified animals that allow specific types of nerve cell to be identified and have their functions altered. Approximately 5000 mice will be used during the 5 year course 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 happen to the animals at the end?	Most of these mice will be used to for breeding and maintenance of genetically modified lines, and since the vast majority of these animals will have no behavioural abnormality, this is classified as "mild". Many of the mice will undergo procedures that are carried out under general anaesthesia, from which they will not recover, and these are therefore classified as "non-recovery". A further group of mice will undergo procedures such as injection of harmless tracer substances into the brain or spinal cord, or spinal injections of agents that will activate or inactivate different nerve cell populations. These procedures are performed under general anaesthetic. These animals will receive post-operative pain-killers and should experience no more than transient discomfort resulting from the operation. Some mice will either have a nerve injury operation or injection of irritant chemical into the hindlimb, both of which may lead to a mild form of pain with increased sensitivity to touch or warm stimuli, or in some cases increased itch. The animals' ability to move around their cages is not reduced after these procedures, and they eat and drink normally. These last two groups of procedures are classified as moderate. At the end of the study, the animals will be killed, for example by perfusion fixation, while under terminal general anaesthesia, or else by a humane killing method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Since the aim of the project is to investigate the organisation and function of nerve circuits within the spinal cord, it can only be carried out on animals. It would not be possible to obtain appropriate samples from humans, and it is not possible to use cultured cells, since these do not have the complex organisation and interconnections of the intact spinal cord.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Numbers used for anatomical studies are usually around 4-5 per group, to minimise the risk of inter-animal variability. For physiological recording studies the experimental numbers will be determined by the need to obtain a sample that is sufficient to identify the various populations of nerve cell in the spinal cord or to determine whether the responses to a specific treatment are expressed to a significantly different extent between different groups of cells. Behavioural experiments are normally carried out on group sizes of 5-8 animals as this compensates for variability between animals. In all cases the number of animals

	used will be the minimum required to provide statistically significant data, and these will be determined by the use of power calculations where appropriate.
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 the lowest animals in the evolutionary tree on which these experiments could be conducted. Most similar studies have been performed on mice or rats, and mice are now increasingly being used because of the availability of genetically-altered lines, which allow identification and targeting of specific populations of nerve cells. All of the neuropathic, inflammatory and pruritus models that we will use are well-established and widely used in studies of pain research. They have all been refined during the course of previous studies by many laboratories to model clinical conditions. For those models that would cause prolonged discomfort, survival will be limited to a maximum of 3 days. None of the models should cause any significant alteration to the behaviour of the animal, and this will be monitored regularly.

Project 45	**Cellular functions of myosin motor proteins**	
Key Words (max. 5 words)	Cell function, neurodegeneration, transport	
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)	A significant proportion of our ageing population is affected by the late onset of neurodegenerative disorders such as Alzheimer's, motor neuron or Parkinson's disease. A better understanding of what causes these neurodegenerative diseases could lead to new therapeutic and preventive strategies. More and more research now suggests that motor neuron disease and some forms of dementia are linked to defects in a cellular pathway that is used to digest abnormal proteins or defective parts of the cell. This can lead to an accumulation of protein aggregates, which may clog up transport of cargo along nerves and eventually causes the nerve cells to die. Our research is focused the molecular motor proteins, which drives cargo transport along tracks to specific sites in the cell, rather like a train running along a railway network to its specific destinations. We thus aim to identify the precise roles of myosin motor proteins and its adaptor proteins in the pathway that is important for clearance of protein aggregates.	
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 advance our fundamental scientific knowledge on the importance of myosin motor proteins and associated adaptor molecules to maintain cellular homeostasis in neuronal and cardiac tissues. Characterising the critical cellular roles as well as the pathological functions of these motor proteins in neurons, astrocytes and cardiomyocytes wili allow us to assess whether they are suitable targets for the development of new therapies for neurodegenertive as	

	well as heart disease.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice for our work and intend to use not more than 3630 mice over 5 years, the duration of this work.
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?	For this project we will use naturally occurring mutants as well as genetically engineered mice to investigate the cellular functions of molecular motor proteins. The majority of mice will have similar genetic disorders as have been described for human patients; they are deaf and show balance problems. In addition the mice may show some mild clinical signs of neurodegeneration such as loss of grip strength or changes in gait. However, this phenotype is only very mild, due to the reduced life span of mice. In humans dementia as well as defects of peripheral nerves in the limbs, often only occur in later life. Therefore, we will carry out carefully planned experiments to detect any sign of central or peripheral neurodegeneration by performing for example treadmill experiments with these mice. The overall severity expected in these experiments is mild. Finally we will kill our mice humanely to isolate highly differentiated cells and tissue to perform experiments in vitro using tissue and organ culture methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The main focus of our current work is dementia and heart disease, which of cause can only be assessed in the whole animal. Mice are an excellent model of human disease and mutations in molecular motor proteins give very similar phenotypes in human and mice. For our cell biological work we will use tissues and specialised cells such as neurons, heart cells and also astrocytes from our mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To ensure that we only produce and use the smallest number of animals, we use good breeding techniques and apply statistical analysis to determine how many animals we require in our experiments to ensure a significant result. Whenever possible we perform pilot experiments in immortalised tissue culture cell lines and maximise the number of experiments and tissues that can be used from a single animal.
3. Refinement Explain the choice of species	We work with mice because they are a good model for the human nervous system and out of all the available

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.	mammalian animal models, mice have the lowest sentience. Our mice are housed in excellent animal facilities, cared for by trained animal technicians.

Project 46	**Perioperative medicine related uses of anaesthetics**	
Key Words (max. 5 words)	Rodent; Anaesthetics, Noble gas; Brain injury; Cancer	
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)	This project is to investigate the objectives stated below in which anaesthetics and other interventions can be implemented to preventing/treating disease-like conditions before, during or even following anaesthesia and surgery. The main objectives are: 1. To determine the nature of interventions to attenuate brain damage in the young and adult age. 2. To determine treatment in preventing/treating organ injuries before, during and after anaesthesia and surgery. 3. To test the impact of anaesthetics and/or tiny particle treatments on cancer progression.	
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 hopes to develop and test a range of interventions in different patients' illness. If these proposed inventions or strategies are effective, then they can be introduced to clinical practice for patients' benefits. As most people will be exposed to anaesthetic agents at least once in their lives, research into the true potential of these drugs to cause any lasting benefits or harm need to be	

	carefully considered. This project hopes to determine in a number of scenarios which anaesthetic agent may be the most appropriate to use to reduce adverse outcomes for patients.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice are the most common animals used for the types of work proposed in this license, and so the entirety of this project will use these two species. Throughout the 5 year duration of the project, over 5 protocols we expect to use approximately 12000 animals in total.
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 have a surgery reflective of realistic clinical scenarios under anaesthesia, such as nephrectomy. Others may be given clinically relevant doses of anaesthetics or analgesics under a variety of clinical scenarios such as labour analgesia, or pre-/post-kidney transplantation. All procedures are either mild or moderate severity. All animals will be killed after experiments in a humane manner. The majority of adverse effects associated with this license are mild in nature and are not expected to be seen, such as following simple anaesthetic gas exposure, as all staff are trained in the administration of these agents. The more severe adverse effects may be seen following surgical procedures but, by following strict aseptic technique as advised by the home office, incidence of this should be rare, and there are detailed instructions for the care of animals and any humane endpoints to be used to ensure minimal animal suffering. If in any rare case, animals suffer an unexpected level of severity, they will be culled immediately in a humane manner. Actual severity will be recorded for each individual animal to allow us to closely monitor the progression of each protocol and ensure close tracking of animal welfare, as well as experimenter competence.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have considered the feasibility of achieving our purpose by not involving animals, but no such alternatives are able to reproduce the whole body responses we aim to investigate in this proposal, as described above in detail. As the studies involved in this license all address clinical issues it is necessary to observe system-wide responses to any given intervention to validate in vitro findings as well as to ensure no unexpected side-effects occur in animals.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Under the previous licence, our group has had extensive experience in carrying out such research activity in animals. Active efforts have been made to achieve minimum use numbers of animals. Many parallel projects will be under way using *in vitro* techniques to best inform the *in vivo* work, such as which agents appear to give the best results, thus reducing the animal numbers used for drugs which may show little promise. Pregnant dams will be obtained time-mated from suppliers to minimise the risk of early spontaneous birth rendering the animals unusable 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.	We have chosen rats and mice to carry out this project due to the vast data already obtained in these models to inform our research, For example, our protocol regarding tumour progression uses nude mice, which are a well validated model allowing for more accurate comparisons, increasing the relevance of our research. The same can be said of our other rodent models, which closely resemble a number of current and previous research models. Based on previous work done by us, along with discussions with veterinary staff, the projects have been designed in a way as to minimise suffering of the animals. Animals will be housed and maintained in groups, where possible, and given environmental enrichment and access to ad libitum food according to best practice as advised by the animal technicians in the facility used. Each protocol has a strict list of possible adverse effects, humane endpoints and procedures to follow when there is any doubt over the welfare of an animal. Wherever possible the mildest pretreatments and surgical interventions will be used to obtain the most relevant data. Where a more harmful protocol must be followed, each animal will be closely monitored to ensure their health and welfare is at acceptable standards, and the welfare of the animal will be placed before scientific outcomes. In some protocols, the nature of our studies investigating anaesthetics and analgesics may even reduce pain and suffering the animals might experience.

Project 47	**Cerebrovascular changes in the aged and diseased brain**	
Key Words (max. 5 words)	Ageing, Alzheimer's disease, blood vessel	
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)	Alzheimer's disease (AD) affects more than 800,000 people in Britain and 35 million people worldwide. Current treatments for AD do not stop or reverse the disease. Risk factor for AD include old age, genetic factors and having metabolic disease (like diabetes and high blood pressure). However, it is still not understood how these factors increase the risk of developing AD. One of the hallmarks of AD is the build-up of 13-amyloid (A13), a toxic protein that kills brain cells. A13 is made by nerve cells and normally cleared from the brain efficiently in healthy individuals. However, as we age, the brain's capacity to remove A13 slows down. The neurovascular unit (NVU) plays an important role in the clearance of A13. The NVU is a collection of cells that form the wall between the blood vessels that supply the brain and the brain cells. Previous studies have found that damage to the NVU can slow down the removal of A13 from the brain. Therefore, it is possible that factors that increase the risk of developing AD do so by damaging the NyU, leading to increased amounts of A13 in the brain. The objectives of this project are: 1) to understand how the NVU works to clear A13 clear from the brain under normal conditions, 2) how age, genetic factors and diet the affect the function of the NVU and how that alters Af3 removal and 3) to evaluate the ability of NyU- and metabolic- based compounds to improve removal of A13 from the brain. To carry out these experiments, we will use mice and rats that have changes to their DNA sequence that	

	replicate those that are seen in human AD. We will also feed mice and rats diets that are high or low in fats and proteins. We will then inject these animals with A13 and look at how it is removed from the brain. We will also repeat these experiments in animals that have been treated with new drugs that improve the function of the NVU.
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)?	Old age is the strongest risk factor for the development of AD. As the proportion of people over 60 years old is growing faster than any other age group, it is predicted that over 115 million people will have AD by 2050. Current therapies do not stop or reverse the disease progression. Understanding how Aj3 is removed from the brain under normal and diseased conditions is essential to understanding how AD develops. The findings from this project will give a better understanding of how factors such as age, diet and genotype affect the efficiency of A clearance from the brain. This will provide new information for the scientific and medical community as a whole and will highlight a new direction for effective treatments for AD to be developed.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 5500 mice and 3500 rats will be used 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?	Some transgenic mouse models experience premature mortality. To minimize this effect, animals will be bred onto a background that reduces the mortality or used before the age at which they will have a risk of death, Although the risk is very low, some animals may experience infection, weight loss, dehydration, or more severe brain damage than intended after surgery. If animals lose more than 15% body weight or show signs of distress or severe brain damage, they will be killed by a humane method (e.g. overdose of anesthetic, exposure to carbon dioxide in rising concentration). Feeding a diet that contains high and low amounts of protein and fat will likely result in weight loss or weight gain and/or an increase or reduction in blood pressure. We will try to match the caloric values of the diets to ensure that the same amount of food is eating by every animal. Any animals losing considerable

	body weight, i.e. more than 10 % body weight over a 3 day period, will be killed by a humane method (e.g. overdose of anesthetic, exposure to carbon dioxide in rising concentration). Female animals that are fed a modified diet may have altered behaviours towards their offspring, including aggression or neglect. Any offspring that show signs to illness or distress or that considerable body weight, i.e. more than 10 % body weight over a 3 day period, will be killed by a humane method (e.g. overdose of anesthetic, exposure to carbon dioxide in rising concentration). At the end of the experiments, animals will be or transferred for continued use under this or another project.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex nature of the brain makes it difficult to study using non-living models or lower-order animals. The rodent brain functions in many similar ways to that of the human brain. Many aspects of AD can be accurately modelled using genetically altered rodents, which can also be used to test potential new treatments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals to be used in the project has been calculated based on the minimum number of mice that are necessary to obtain scientifically relevant and reliable 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.	Mice and rats are the model organism of choice to study AD, because the structure and function of the rodent brain is similar to that of the human. Further, the mouse and rat genome can be easily used to make genetic changes that replicate features of human AD. Rodents also breed easily, with a short generation time, facilitating multigenerational and ageing studies. Finally, protocols for rodent care, health and welfare management are well established and can be easily consulted. The research procedures in the project will not exceed the Moderate severity level. To minimise suffering, all animals will be assessed daily for signs of distress or ill health. Vigilant monitoring will be done in animals following surgical procedures. Any

	animals exhibiting weight loss of 10 % body weight over a 3 day period, or showing signs of distress and/or pain will be killed humanely. Handling will be minimised to routine husbandry and procedures required for the project.

Project 48	**Synaptic plasticity in normal learning and addiction**	
Key Words (max. 5 words)	memory, hippocampus, accumbens, opioid, heroin	
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)	One aim of this project is to further our understanding of the way in which memories are formed and stored as changes in the strength of synaptic connections among neurons. Although there is a large amount of evidence that such changes are necessary for memory formation, there is still little information about the formation of these synaptic changes, as they happen, in the course of learning. A second, related, objective is to study the way in which the mechanisms of normal learning—particularly the association of certain cues with reward—can be subverted in drug addiction. Changes in synaptic strength between the hippocampus, a brain structure with a key role in memory, with the nucleus accumbens, part of the brain's reward circuitry, may be critical both for the formation of natural memory-reward associations, but also for the association of drug-associated cues with opioid reward—a factor that is likely to play a large role in relapse. This project would aim to improve our understanding of this processes and how it can be controlled.	
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 central aim of this project would be to identify and study the way in which certain forms of memory—particularly memories linking spatial and reward memory—are stored in the brain. This is important both for scientific reasons, but also because future medical treatments targeting learning and memory will only be possible with a detailed an understanding of the cellular mechanisms underlying these	

	processes. Part of the proposed work would involve an attempt to identify potential targets for therapeutic intervention in human opioid addiction and relapse. Opioid addiction is a major public health issue, and even former addicts who succeed in remaining drug-free for long periods are highly susceptible to relapse, particularly when they encounter 'trigger' situations, contexts, and cues that are associated with previous drug use. By studying the synaptic mechanisms of this process in rodents, I hope to identify potential targets for intervention in order to reduce the likelihood of relapse in human opioid addicts.
What species and approximate numbers of animals do you expect to use over what period of time?	I expect to use 1120 rats and 640 mice 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 level of severity? What will happen to the animals at the end?	Behavioural testing is very unlikely to have any adverse effects, and most of the tasks will involve food reward as an incentive to perform. However, surgical techniques, such as the implantation of recording electrodes and drug infusion cannulae, have the potential to cause pain and distress. To limit this, all procedures will be carried out by trained personnel, anaesthesia will be correctly maintained during surgery, and post-operative pain relief will be given. The animals will then be closely monitored for signs of discomfort or ill health. Repeated opioid administration will, when stopped, result in a withdrawal syndrome of malaise and reduced food intake. No lasting harms are anticipated, and animals will be checked and weighed throughout the period of drug administration and withdrawal. At the end of the experiments, the animals will be humanely killed, and their brains will typically be studied in order to assess, for example, that recording electrodes and infusion cannulae are correctly located.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The aim of this project is to observe learning-related changes in the strength of synaptic contacts between neurons as they occur. This is only possible in the living brain, and cannot be studied, for example in cell-culture preparations. It is also not possible for ethical reasons in human participants owing to the need for electrodes to be inserted into deep

	structures of the brain. However, if the project is successful, it might be valuable to begin collaborative research involving human functional brain imaging. However, this approach cannot not, by itself, provide a window into the synaptic mechanisms involved in the learning process—the central aim of this project.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the minimum numbers of animals necessary to draw statistically meaningful conclusions from the results we obtain. In many cases, the experiments would involve the use of multi-electrode arrays. These allow the collection of far more information from each animal than would be possible using conventional electrodes that record data from only a single location in the brain. To reduce numbers, we will also try to collect data across different experimental conditions within the same group of animals, rather than using separate groups of animals for different experimental conditions.
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.	Most of the animals used in this project will be rats, predominantly the 'Lister-hooded' strain. These animals have good eyesight, and are capable of learning a wide range of behavioural tasks. Mice would be used in a smaller number of cases, owing to the greater availability genetically altered mice compared to rats. Whenever possible, animals will be group-housed to allow social contact. In many cases, the incentive for behavioural performance will be reward (e.g. food), rather than punishment. Painful stimuli, such as electric shock will not be used. Surgical procedures will be carried out by competent, trained personnel, using sterile techniques to avoid infection. Post-operative pain relief will be given, and the animals will be monitored closely for signs of discomfort or ill health.

Project 49	**Misfolded protein and neurodegenerative disease**	
Key Words (max. 5 words)	Prion, amyloid, seeding, neurodegeneration, TSE	
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)	Several neurodegenerative diseases of humans and animals are linked with the accumulation of misfolded protein aggregates in the brain. These aggregates are found in both infectious disease such as Transmissible Spongiform Encephalopathy (TSE or Prion disease), and non-infectious diseases such as Alzheimer's disease. The actual role these protein aggregates play in neurodegenerative disease is unknown, however in TSE disease the misfolded protein is thought to be the infectious agent. This project aims to examine how these aggregates form and spread in the brain, and how the brain deals with misfolded proteins as an individual ages. We will also continue to examine the nature of the infectious agent (the prion) responsible for TSE disease and the risk posed to humans from TSE diseases that are still present in 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)?	Neurodegenerative diseases associated with protein misfolding represent a major problem in the aging human population. This project will examine the early mechanisms involved in protein misfolding and clearance, and how this changes as an individual ages. Identifying how cells stay healthy may provide potential targets for the development of diagnostic tests and new treatments. Further characterisation of the nature of the infectious prion responsible for TSE disease will ultimately facilitate better diagnostic and therapeutic strategies in humans and animals. We also aim to continue to	

	characterise new TSE isolates in animals to determine whether such isolates pose a risk to humans. Identifying the specific properties of a TSE agent responsible for human infection will allow rapid assessment of newly identified TSEs that could pose a risk to humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse (wild type and genetically manipulated). Up to 6,000 mice over 5 years Less than 5% of these mice will undergo surgery to allow imaging of protein aggregates in the brain.
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?	Following exposure to infectious prions, animals are expected to develop clinical signs of TSE disease. These may include lethargy, hyperactivity, changes in limb movement, aggression, and weight loss or gain. Animals are monitored daily and scored weekly for development of these clinical signs. Animals are then humanely euthanized at a defined clinical endpoint (2 consecutive positive clinical scores), or at a pre-determined experimental endpoint.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At present there are no alternatives to the use of animals to study TSE infection and the nature of the infectious agent. Experimental systems that model protein misfolding in the laboratory rather than in the mouse are available, but cannot model replication and spread of a TSE agent through multiple cell types within an infected animal. Similarly, such systems do not control for cell aging and other cellular processes that aim to clear misfolded protein from the cell. We will however continue to develop and utilise model systems such as neuronal cell culture and brain slice culture to analyse early stages of prion infection and amyloid plaque formation. These systems will partly replace the need for live animals in this project and will inform which aspects of the project should be further analysed in whole animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Group sizes are calculated to keep the numbers of mice used to a minimum, whilst ensuring sufficient numbers are used to provide statistically significant data. Numbers are reviewed based on experience and statistical advice.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	Neurodegenerative disease is complex, involving interactions between cells and tissues of the body and brain. Due to advanced age of patients, long disease duration, and an inability to diagnose the

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	disease in its very early stages, early stage tissue is not available for study. Mice are the least sentient species available for our studies and have been used previously in studies of neurodegeneration. Disease can be monitored throughout lifespan either from birth or from the point of inoculation through to the development of clinical signs of disease, and early stages of disease can be studied. Animal suffering is minimised by the use of anaesthetics and analgesics during and after procedures as required. Animals are monitored daily and scored weekly for signs of neurodegenerative disease using a well-established scoring system. Animals are humanely euthanised at a pre-defined clinical endpoint (2 consecutive positive clinical scores).

Project 50	**Neuronal and glial AMPA and $GABA_A$ receptors in health and disease**	
Key Words (max. 5 words)	Synaptic transmission; neurons: neurological 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
		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 is made up of trillions of nerve cells that communicate with each other by releasing chemical transmitters. Nerve cells contain thousands of synaptic connections at which such communication occurs, and which represent the basic unit in this important signaling process. The simple amino acid glutamate is the major excitatory neurotransmitter in the brain, while GABA is the major inhibitory neurotransmitter. When released, these chemicals activate 'receptive' molecules present in neighbouring nerve cells. Synapses and their constituent receptors are the sites in the brain at which the processes of sensation, thought, emotion, and memory are all generated. Because of the vital role played by glutamate- and $GABA_A$ receptors ($GABA_A$Rs) in synaptic signalling, their dysfunction causes a number of serious neurological and psychiatric disorders. Understanding the regulation and fundamental properties of glutamate- and $GABA_A$ receptors ($GABA_A$Rs) and the various proteins associated with them is essential if we are to dissect their role in disease processes, and inform new efforts in drug discovery and treatment.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or	Our work will provide a better understanding of the processes and molecules involved in brain cell communication. It will also caste light on the various protein molecules that underlie normal and diseased	

animals could benefit from the project)?	synaptic transmission. The work we are doing focuses on and will provide a better understanding of motor neuron disease, brain damage following stroke, multiple sclerosis, brain tumor growth, Alzheimer's disease and Juvenile Batten disease.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use roughly 9,500 mice and 550 rats over a 5 year period. Many of these animals will be used purely for maintaining of breeding colonies.
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 work involves mild procedures in which animals are culled. A small amount of our studies involve procedures of moderate severity, or use of transgenic mice. These animals do not show abnormal stress and feed, drink and behave normally. A very tiny percentage of mice (>1%) may undergo weight loss, which can be quickly identified to minimise suffering. Expected adverse effects might include post-operative stress which is minimised with appropriate analgesia, where possible. At the end of each procedure animals are culled according to Schedule 1 or other itemised humane methods, and tissues will be isolated for later study. We also aim to minimize animal usage by coordinating experiments, and producing sufficient material from a single animal to allow two or more lab members to run experiments in parallel. Whenever possible we make use of cells in primary culture. This means that tissue from a small number of animals is maintained and used over extended periods of time.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Many of our experiments aim to determine properties of native glutamate and GABA receptors, in nerve cells and glia in various neurological disease mouse models as well as normal brain. Our experiments on synaptic transmission examine synapses *in vitro*. The use of acute slices of brain and spinal cord are therefore essential for these experiments. Currently there is no realistic alternative to the use of slices of brain and spinal cord containing synapses if we wish to understand the functional behaviour of native receptors and synapses, and unravel their role in a variety of debilitating neurological diseases which affect many millions of patients.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We make use of neurons and glia in primary culture to minimise animal use. Whenever possible, cultured cell lines permanently or transiently expressing receptors and related proteins are used. These allow us to examine the properties of defined recombinant receptors. Use of these various tissue culture approaches helps to minimize the number of animals used. We also use the minimum number of animals consistent with achieving our experimental aims. At each stage the animals are tested in multiple paradigms and experiments are co-ordinated to minimise numbers used, and to maximise the data collected. However, it is important to stress that interpretation of our data on cultured cells inevitably leans heavily on our studies on synapses in acute slices of brain tissue.
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 mainly use mice because there is considerable background information available on gene expression, structural/molecular biology and functional properties of glutamate and $GABA_A$ receptors in nerve cells and glia in mouse. Furthermore, they appear functionally identical to those in human, so that data generated by our experiments are directly relevant to the human brain. Finally, the use of mice allows us access to a host of genetically modified mice which provide excellent and very specific neurological disease models, thereby allowing us to address the causes of various debilitating human diseases (including brain damage following stroke, multiple sclerosis, brain tumor growth, Alzheimer's disease and Juvenile Batten disease and motor neuron disease). Transgenic mouse strains that express visible markers allow clear identification of specific cell types in the brain, greatly enhancing the usefulness of the information we can extract. Great care is taken when using appropriate anaesthetic/analgesic regimens to minimise any potential suffering. All procedures are carried out by highly trained staff.

Project 51	**Studies to Find Improved treatments for Movement disorders**	
Key Words (max. 5 words)	Parkinson's disease Dystonia Neurodegeneration Neuroprotection Symptomatic treatment	
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)	There are a number of movement disorders which are poorly treated and for which there is rio cure. The most common of these are Parkinson's disease (PD) and Dystonia, both of which involve changes in the activity of the areas of the brain that control movement called the basal ganglia. Parkinson's disease is the result of cells, which control movement, dying in the brain and results in the symptoms of slow movement, stiffness and shaking. Dystonia is the result of changes in the activity of nerves in the areas of the brain that control movement and results in slow or permanent, often painful contraction of muscles resulting in strange postures. This project aims to find new treatments to slow the progression, treat the symptoms and reduce the incidence of chronic side effects of the existing treatments in Parkinson's disease (PD) and to find better treatments for dystonia. PD affects approximately 1 in 500 of the general population and 1-3% of individuals over the age of 60. At any one time there are approximately 120,000 individuals afflicted by this disorder in the UK. PD is primarily due to the slow and progressive loss of nerves in specific areas of the brain that control movement, resulting in progressive loss of control of movement as well as other symptoms including anxiety, depression, sleep disturbance and constipation. Although less common, dystonias significantly affect quality of life of sufferers	

	from children to adults, and the contorsions that result are often painful and debilitating. The symptoms of both PD and dystonia can be treated, however, the treatments of both are associated with side effects, some of which are irreversible. Therefore, there is an unmet clinial need for treaments for these disorders that are not associated with undesirable side effects, and this is the first objective of these studies. In addition, if the progression of the PD could be slowed, then the quality of life of the sufferer would significantly improve and the burden of care to family and society will be reduced. Thus the second objective of these studies is to find new treatments that can slow the progresson of PD.
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 benefit of this work will be measured in the improvement of the treatment of Parkinson's disease and dystonia. We aim to find new treatments that can better treat the symptoms of the diseases with reduced side effects, and to find drugs that will slow or stop the progression of Parkinson's disease. It takes some time to see the clinical benefit of this work, however, work performed on previous licences have resulted in the clinical use of a number of drugs and drug combinations in the treatment of PD including ropinirole (Adartel, Requip), rotigotine patches (Neupro), and the 1-DOPA-entacapone combination therapy (Stalevo). In addition we have contributed to work that has resulted in the clinical use of the A2A antagonist, istradeffyline, in Japan.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use common marmosets. For the symptomatic studies we plan to compare the effects of drugs in parkinsonian marmosets reflecting the long-term nature of PD and dystonia. We, therefore, plan to use a maximum of 50 animals. For studies searching for neuroprotective agents we will only start these studies if we have positive data from rodent experiments, and so expect to perform no more than 3 studies over the 5 year period. For that reason we will use a maximum of 36 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?	For Neuroprotection studies we plan to induce a partial lesion of the nigrostriatal dopamine pathway using a toxin called MPTP. This will produce a maximum of 70%, but typically 50%, loss of the nerves which will have little or no effect on the ability of the animals to move and look after themselves. Control animals will receive placebo and their movement will be unaffected. These animals will also

	be treated for an extended period with the neuroprotective agent. Usually this will be systemic administration, by mouth or under the skin, but possibly also directly into the buccal cavity (i.e. under the tongue) or into the nose, but it may also include injection into the brain under general anaesthesia with recovery, or via an implanted cannula attached to mini-pumps located under the skin. These procedures are expected to be moderate in severity. For Symptomatic studies we plan to use animals with between 70 and 100% loss of nerves in one pathway in the brain using MPTP reflecting what is seen in Parkinson's disease. These animals will be transferred to this licence from a previous licence with the authority to MPTP treat the animals. Animals will be treated with drugs that aim to improve their symptoms. This mainly includes drugs that are administered by mouth, into the nose or under the skin, but may also include injection into the brain via an implanted cannula attached to small pumps placed under the skin. These procedures are expected to be mild to moderate in severity. However, the overall severity of the procedure remains severe.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will continue to perform studies using cells from human and rodents grown in flasks to investigate the effects of drugs and toxins. Similarly, we will continue our analysis of drug activity in test tubes, by investigating the effect directly on the tissue. However, brain function and behaviour is extremely complex and neurodegenerative diseases such as Parkinson's disease and dystonia present an equally complex neuropathology with associated changes in movement as well as other aspects of life including sleep, depression, bowel and bladder function. It is, therefore, vital to confirm the positive effects that may be apparent in experiments performed in test tube in a whole organism. Searches on www.frame.org.uk confirm that there are no alternatives to the use of animals for the investigation of these complex disorders of the brain. Although they are presently using Parkinson's disease as a model in a feasibility study to phase out primate work, so far no alternatives are available. These studies provide a vital link in the progression of treatments from the preclinical to clinical environment.
2. Reduction Explain how you will assure	The use of rodents in our assessment cascade for symptomatic/neuroprotective drugs has facilitated the

the use of minimum numbers of animals	reduction of the number of marmosets receiving MPTP treatment. In addition, refinements in our procedures in the last licence have allowed reductions in the number of animals used by approximately 50%. Importantly the animal act as their own control in all symptomatic studies, reducing the requirement for large numbers of repeat experiments and allowing repeated measures assessment and reduced numbers of animals to be used where appropriate. In addition we continually assess our experimental design and statistical methods, and have consulted both the senior college statistician and experts from the pharmaceutical industry on a number of studies in order to allow us to use optimal animal numbers. We have a wealth of data on which we use power analysis in order to calculate the number of animals required for studies, and do this as appropriate where different levels of effect are expected. We have, in the past, maintained the animals for up to 6 years after MPTP treatment with no detriment to their health. In addition, we plan to investigate the effect of aging on motor and non-motor parameters in this model. For this reason, we propose to remove the time limit after MPTP treatment. This will reduce the overall number of animals we use, and will add to our database of information from individual animals that can be drawn on to reduce the number of repeat experiment we have to perform, and it will give us the opportunity to study the effect of aging on motor and non-motor parameters which is highly relevant to this age-related disorder.
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 changes in the brain induced by the toxins we will use reflect that seen in PD and dystonia and therefore provide a basis for studying complex biochemical and behavioural changes. With respect to PD, the animal model that best recapitulates human Parkinson's disease is the MPTPtreated primate, however, it is unacceptable to use this model at early preclinical stages of therapy. Hence, rodent preclinical models are accepted used for the investigation of symptomatic and neuroprotective treatments at this stage, however, the primate MPTP-models more closely resembles the disease in man and therefore its use is restricted to later stage preclinical trials. Animal models of dystonia are poor, however the MPTP-treated primate shows dystonic posture both on and off drug, and these respond to existing clinical therapies. For this reason this model is appropriate to

	assess the effect of potential new classes of drugs for dystonia treatment. Following the intensive care period after MPTP treatment, the animals are able to look after themselves, and maintain a stable body weight, and we know that this is achieved for to 6 years. No additional care is required during this post recovery stage, however, we provide environmental enrichment in the form of play toys in the home cage, and challenging tasks such as memory and motor skills tasks for reward.

Project 52	**Neurovascular coupling in health ageing and disease**	
Key Words (max. 5 words)	Neurovascular coupling, Dementia, Ageing, Epilepsy	
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)	Modern neuroimaging techniques such as functional magnetic resonance imaging (fMRI) have revolutionised the field of cognitive neuroscience by being able to take discrete measurements of brain activity. However, they don't measure neural activity but the change in hemodynamics associated with the neural activation. This relationship is called neurovascular coupling. The mechanisms and mediators of neurovascular coupling are still poorly understood and the research in this project will directly address this question by providing fundamental scientific data. Recently it has also been suggested that the breakdown of neurovascular coupling could be important in many disease and trauma states such as Alzheimer's and stroke. Therefore a second major objective of this research will be to understand how neurovascular coupling is changing in ageing and disease. We will also use our multi-modal neurovascular imaging technology to help understand general disease mechanisms (such as the generalization of seizures in Epilepsy) and	

	implementation of novel treatment strategies.
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)?	Research on this project will lead directly to an improved understanding of human functional brain imaging. In particular, the technique of blood-oxygen-level-dependent functional magnetic resonance imaging was only conceived 20 years ago, and yet there are few areas of neurology or cognitive neuroscience upon which it has not had a significant impact. However, the full potential of this, and related techniques, can only be realised if the relationship between brain activity and the imaging signals is properly understood. Research such as that proposed here is fundamental to improving this understanding. Our research will also address the critical question of whether neurovascular coupling is breaking down in disease. If this is true for example in Alzheimer's disease we may develop a novel biomarker for the early detection of the disease. Early detection will allow more time for existing drugs to work and our results may isolate the cells that need to be treated to slow down or even cure the disease.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use rats and mice. The rat experiments will mainly consist of acute anaesthetised preparations whereas the mice will consist of a mix of acute and longitudinal chronic experiments. We anticipate an overall number of 2700 animals. This is based on the numbers used in my previous license and the current size of my team. The large number of transgenic animal's accounts for the fact that due to the genetics involved only 50% of the Alzheimer's J20 mice will have the correct genotype for the disease to be used in 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?	All of the acute experiments will be performed under terminal anaesthesia so these experiments are non-recovery. All animals will be sacrificed at the end of the procedure. For the chronic experiments the maximum severity we expect is moderate as this usually involves surgery to implant an imaging chamber. Animals very rarely show any adverse effects to the surgery such as infection

	and recover quickly. Some of the transgenic mice who have a disease (Alzheimer's, Motor Neurone disease) will be carefully monitored to ensure that they do not deteriorate beyond the moderate severity. All chronic animals will either be killed by schedule I or undergo an acute terminal anesthetised experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To understand the relationship between neural activity and hemodynamics in the brain, it is necessary to generate relevant biological data. The use of animals is therefore unavoidable. Rats and mice are the animal models of choice for research in this area due to the well- defined anatomical structure and function of the sensory cortex. That is, the sensory cortex has a spatially segregated structural topography with distinct peripheral regions represented in well-defined cortical areas. Our expertise in using these animal models for research has been developed over a 15-year period. Consequently, the expertise and skills are already in place to ensure that the number of animals used is minimised by a consistently high quality of practice from the outset. We will also use specific transgenic mouse strains that reflect human disease such as Alzheimer's and they will provide important information about disease progression. As a general strategy, acute anaesthetised preparations will always be used in the first instance to address all objectives, and where new or modified techniques are employed. New techniques may then be introduced into the awake preparation. This will ensure that the number of animals required for chronic awake studies is reduced and that minimal stress is placed upon the awake animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We strive to make every effort to use the least number of animals for our studies along 2 lines 1. In all studies the number of animals used will be minimised by using carefully controlled repeated measures designs to maximise the amount of data obtained from individual subjects. Powerful randomisation statistical techniques will be used to

	maximise the likelihood of achieving statistical significance with small numbers. Thus to date, most published experiments from our research group contain less than 10 animals per study. The Department is a great source of advice for statistical analysis who will be continually involved in the project to ensure the most appropriate statistical measurements are made that result in fewer subjects being needed. If further statistical advice is required help from Statistical Services Unit will be sought. 2. Since it is extremely important for our scientific aims to be able to record multiple signals simultaneously a natural consequence of this is to reduce animal numbers. We are at the forefront in developing such concurrent recording procedures.
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 the barrel cortex model in both rats and mice. We have over 15 years' experience in the rodent somatosensory system and it still represents the animal model of choice for the research questions we feel the need to address. Under protocol one (and final experiments in protocol 2) all animals will be under terminal anaesthesia which will be carefully monitored throughout the experiment. For protocol 2, the chronic preparation, additional care will be given during training, recovery surgery and in the testing stage to ensure minimal suffering to the animal. If any procedural complications do arise veterinary advice will be sought immediately. Advice on anaesthesia and analgesia has been sought in the past and will continue to be sought from the named veterinary surgeon.

Project 53	**Animal models of neurodevelopmental disorders**	
Key Words (max. 5 words)	Rat, behaviour, pregnancy, gut, brain	
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
	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 establish animal models of neurodevelopmental disorders (NDDs) Brain disorders cost €141 billion per annum in the UK, with a total 2010 cost of psychotic disorders of €16,717 (in million € purchasing power parity-PPP). Existing drugs are not effective enough and have unpleasant side effects reducing compliance. Better treatments are therefore required, particularly for memory and social communication in schizophrenia, while there is no drug treatment currently recommended for Autism Spectrum Disorders (ASD). Critical for the development of improved treatments is improved understanding of the cause and biological basis of such disorders which can only be achieved through carefully validated animal models. Existing animal models only allow testing of new treatments in adulthood once the illness has become established. Our new models will allow testing of new treatments at an earlier stage of the illness, even before the illness manifests itself, particularly for schizophrenia at the prodromal stage, with the overall aim of finding a biological target for identification of the illness before it progresses into psychosis. A further aim of the new model is to identify the link between gut and brain abnormalities in ASD. There is some evidence to support the theory that this may be the cause of some of the brain disturbances and a new target for drug therapy. This project aims to establish new animal models of brain disorders that have a	

	developmental basis, ie that are partly produced by a disturbance in pregnancy.
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)?	Humans will benefit from the project in a variety of ways. Psychotic disorders and ASD reduce the quality of life for patients and carers dramatically and have a large economic cost. Improved treatments have not been successfully developed due to a lack of understanding of the biological basis of these diseases due to limited animal models. For any disorder, prevention is better than cure and early treatment is more successful than late treatment. Our work aims to identify a biological target for early identification of the illness, early treatment and prevention of the illness becoming manifest as eg psychosis. This will limit the detrimental effects to the patient's social and academic life. The benefits will be economic and in quality of life for patients and carers. Animals (in our lab and others) will benefit from development of improved, food-rewarded ethologically relevant tests, see section on refinement above.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats approx. 2 700 over the 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?	The level of severity is expected to be moderate. Pregnant rats will be given an agent (reduced activated virus or bacteria or valproate, a drug used for epilepsy) at various times of pregnancy. This is likely to induce a mild and short-lived infection or pharmacological response in the mother, lasting less than 24 hours (increased body temperature and mild sickness). Behavioural techniques, applied to the offspring, are generally not stressful and can, in certain cases, be considered enrichment for the animals. We intend to stress the animals using an ethological and disease relevant stressor such as mixing up cage groups or short-term social isolation or introduction of a relevant parasite into the GI tract. At the end of the study, or as part of the experimental procedure (eg to perform assessment of brain changes induced by the interventions) rats will be killed humanely and quickly.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Studying the risk factors and mechanisms involved in neurodevelopmental disorders (NDDs) is extremely complex and involves understanding the interactions between several physiological systems (e.g. nervous, gut, immune). In addition to the changes in the brains of patients, these disorders are characterised by deficits in social behaviour, memory and mood, such key aspects of NDDs are not possible to model using cells or simulations. This work must entail the use of whole animals as behaviour is a central feature of the project. To date there is no suitable alternative to the use of whole animals for behavioural research. It is necessary to use whole live animals both to model NDDs and to measure behaviour, particularly complex behaviours such as memory and social interaction. We will perform extensive analysis of tissue samples from all animals as we are searching for biological and behavioural changes induced by our maternal infection. Some cell assays may provide valuable information about these biological changes and will be used where appropriate.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise the number of animals we use by testing the same animals at various stages of development, re-using the same animal in different behavioural tests, testing more than one drug in the same animals. All these repeat studies will only be conducted following an extensive examination of the animal by the veterinary Surgeon. We will use males and females from each litter as both men and women suffer from these disorders. We will consult a statistical expert to ensure we have minimum number of animals for maximum statistical power.
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 are a popular choice for much preclinical work because of the detailed existing knowledge of their brain structure and function and its similarity to that of humans. The rat has been chosen as a subject for the present work for several reasons. Primarily much is already known about memory and brain mechanisms controlling complex behaviours in rats. We have extensive experience of studying behaviour in rats, all our current tests are validated for rats and our tissue analysis systems are validated using rats. Rats are larger than mice and more suitable for longitudinal imaging studies, from weaning. We have refined our techniques in the continued review of our current work. Welfare is critical for successful

	experiments and we have extensive experience in behavioural analysis of rats which we will use continuously to ensure that all our rats are subjected to the minimum adverse events and that undue stress is minimised at all times, particularly when rats are handled and dosed. Specific on-going refinements include: reduced use of food restriction, increasing use of ethologically relevant tasks involving enrichment, improved dosing regimens, handling and dosing techniques, including reduced restraint.

Project 54	**Antibodies to neuropeptidergic signalling molecules**	
Key Words (max. 5 words)	Antibody Neuropeptide Evolution Echinoderm	
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)	Neuropeptides are chemicals in our brain that control how we feel and behave; they also regulate processes in the body such as digestion and blood pressure. Neuropeptides perform these functions not only in humans but also in other mammals, including rabbits. But when did these important molecules first appear in the history of life on earth and what functions do they have in other types of animals? Answering these questions was, until recently, very difficult because of a lack of data from many animal types. However, recent advances in genetics have enabled discovery of neuropeptides in an increasingly wide range of animals. For example, work leading up to the project proposed here has identified many novel neuropeptides in the common European starfish Asterias rubens. The aim of this project is to use this new information to discover the functions of neuropeptides in starfish; this will be the first study of its kind.	
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 knowledge we will obtain will provide new insights on neuropeptides as regulators of animal behaviour with respect to the history of life on earth. Also we will obtain fascinating insights on how neuropeptides are used to control behaviour in the context of a five-sided animal that doesn't have a head or a brain — the starfish. In addition to these advances in scientific knowledge, there are potential practical benefits of this research for	

	humans. The common European starfish feeds on economically important species such as mussels that are important foodstuffs for humans. Another starfish, the crown-of-thorns starfish, feeds on coral and this is causing damage to the Great Barrier Reef in Australia. By learning more about how the starfish nervous system works and how starfish behaviour is controlled by neurochemicals such as neuropeptides, it may be possible to develop novel ways of controlling starfish that feed on economically or environmentally important species.
What species and approximate numbers of animals do you expect to use over what period of time?	Rabbits will be used to obtain antibody proteins that bind to specific neuropeptides. These antibodies will then be used to determine where in the body of starfish the different neuropeptides are produced. For each neuropeptide that is studied, two rabbits will be immunised. Currently (2014) funding has been obtained to generate antibodies to twelve neuropeptides so for this work twenty-four animals will be used. In the course of the 5-year programme of this project licence, the expectation is that the maximum number of animals that would be used is approximately fifty.
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?	Antibody production in rabbits is a mild procedure, with minimal side effects and if these occur they are comparable to those experienced by humans when vaccinated. At the end of the procedure the animals are humanely killed to enable collection of blood for isolation of the antibodies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no suitable alternatives to animals for generation of antibodies for our research purposes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will immunise (inject) only 2 rabbits per neuropeptide. It is necessary to immunise more than just one rabbit per neuropeptide because based on our experience of antibody production over a period of 25 years, some immunised rabbits do not generate antibodies. In the past we have typically immunised 3 rabbits per antigen peptide. However, we are reducing this to 2 rabbits per antigen peptide because in our experience it is rare that more than one rabbit in an immunisation programme does not generate

	antibodies.
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.	Use of rabbits enables production of a quantity of antibodies that is sufficient for the research, by comparison with smaller (e.g. mice) or larger (e.g. sheep) alternatives. The method of antibody production (polyclonal) will provide antibodies of a quality and type that is suitable for the research, by comparison with alternatives (e.g. mouse monoclonal antibodies). The procedure (immunisation) is mild with minimal harm. Use of a topical anaesthetic during blood collection will minimise discomfort.

Project 55	**Basic mechanisms of chronic neurodegeneration**	
Key Words (max. 5 words)	TSE, neurodegeneration, mouse models	
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)	This research programme aims to understand the basic mechanisms underlying the development of neurodegenerative diseases, primarily focusing on transmissible spongiform encephalopathies (TSEs) or prion diseases. Neurodegenerative diseases currently have no cure and as a result create an enormous social and economic impact. Current therapies have a modest impact on symptoms and limited or no impact on disease progression. The prevalence of these diseases is predicted to rise dramatically over the next decade. The earliest events in neurodegeneration are poorly understood although there is evidence to suggest that there are common molecular and cellular pathways leading to the destruction of central nervous system cells called neurons. A common feature of a number of neurodegenerative diseases is the presence of misfolded proteins. Although different misfolded proteins are found in different diseases the pathway to neurodegeneration is thought to be similar between the different diseases. We will use unique rodent models which allow direct experimental control of the amount, position and accumulation of misfolded protein. We will use these models to determine the early cellular events involved in these diseases as well as the potential protective effects factors that may help to slow down disease progression. Our programme of work will therefore;	

	• Define the pathways leading to neurodegeneration • Define host pathogen interactions in TSEs • Define interactions between neurodegenerative pathways and the immune system which might exacerbate neurodegeneration • Identify normal function of proteins involved in neurodegenerative 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 aim of our research is to advance our understanding of poorly understood cell mechanisms that underlie the destruction of the brain and neuron function in neurodegenerative diseases. The potential benefits associated with understanding these basic mechanisms are significant both scientifically and medically. It is anticipated that the information gained will contribute to world wide efforts to understand these life destroying diseases as well as contributing to the ongoing search for options for medical intervention and treatment.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use a maximum of 10000 mice over 5 years. The size of experimental groups will be based on previous work discussed with a statistician. This will be used to estimate the minimum number of rodents required for establishing significant differences between groups.
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 detailed here are to be performed in rodents and do not exceed 'moderate' severity. The animal models and protocols to be used here have been developed by us and our colleagues over numerous years in order to study progression of neurodegenerative disease. All experiments are to be performed by appropriately trained staff and are essential for the success of this project. Well defined clinical scoring regimes are in place in order to give a defined humane endpoint and prevent needless suffering. Animals will be humanely sacrificed at the end of experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As neurons are not present outside the animal kingdom, studies on protection and degeneration of the nervous system require the use of animals and there is no feasible alternative that would entirely replace the living animal and would allow the objectives to be met. To study the interactions between cell types in the progression of TSE disease it is necessary to use animal models. Additionally, the volume of information available relating to the rodent model as well as our extensive experience with this

	model makes this the species of choice for our purposes. It is unfortunately not possible to accurately study the involvement of these complex systems in neurodegeneration without the use of animals and the models used in this project are the best available to address the main experimental aims. At present there is no more reliable method that allows TSE infectivity to be estimated than mouse models. However new studies examining cells studied outside of the normal environment (for example in artificial culture media) are being developed and we will continue to optimise these techniques and aim to introduce their use if they are found to be as reliable as our work on mouse models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All studies involving animals are subject to completion of a study protocol form which must be approved by both veterinary staff and the unit manager. Where a study involves a novel technique or large numbers of animals it must also be approved by selected members of the AWERB including researchers, statistician and TSE researchers independent from the study prior to commencing studies. The use of mice where specific genes have been altered may also show shorter disease incubation periods and thus may allow the use of fewer animals in studies. The use of imaging technologies will also allow us to examine the same animal at multiple time points, thus removing the need for culling multiple groups of mice at set time-points. We also have collaborations with other scientists whereby maximum use is made of animal tissues at the end of studies.
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.	Animal suffering will be limited in our studies by our strict monitoring of severity limits which are very well developed and are identified by experienced staff. We also use many procedures that do not produce significant trauma or suffering. Substances and treatments will be administered at non-toxic dosages and if unknown, this will be tested in a carefully graded dose-finding protocol.

Project 56	**Connectivity and plasticity of developing and mature central nervous system circuits**	
Key Words (max. 5 words)	Brain development, neurons, synapses, axons	
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 aim of this proposal is to understand how the brain is formed and how neuronal experience modifies how neurons connect to each other. We ultimately aim to understand how errors during this early period result in 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)?	This proposal will help understand the mechanisms behind the wiring of the brain. We aim to find important targets (sites, processes, organelles, genes) that are important for the proper formation of the brain and can be exploited when looking for drugs targets to ameliorate neurodevelopmental disorders.	
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 7000 mice 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?	The rodents will mainly be used to breed genetically altered animals (mild severity only at most). Additionally much smaller numbers will undergo surgical procedures which are not expected to have any serious adverse effects and every effort will be made to ensure minimal suffering (good anaesthesia and post-operative pain relief). Animals will be humanely killed at the end.	

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To do the experiments required to improve our understanding of the underlying mechanisms for these diseases, we cannot ethically perform these in humans. Although we will also use non-animal alternatives, such as cells taken from humans, these have significant limitations and so many experiments can only be conducted in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Firstly we will maximise the data from each animal by doing many experiments from multiple different cells and/or tissues after humane killing. We will use tissue from genetically altered animals of both sexes and all genotypes after humane killing, meaning we will generate far more information without any additional numbers of animals or suffering. Also, we will use the optimum experimental design and statistical tests to minimise 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.	We will do these experiments in rodents as these offer the best compromise between between relevance to humans and sentience. The regions of the brain that are known to be important for neurodevelopmental disorders are relatively similar in rodents, and it is possible to measure behaviours relevant to these disorders. Also, mice are ideal due to the number of transgenic (genetically modified) mice available including both disease-relevant mutations as well as reporter lines, and increasingly transgenic rats will be available as well. Working with rodents also builds on the wealth of knowledge and research already available and minimises unnecessary repetition. To minimise animal suffering, the vast majority of animals will only undergo a single procedure, and much of the work will be done in fixed tissue or ‘in vitro’ (ie. not in the live animal) using tissue. All animals undergoing surgery will have effective anaesthesia and be given additional pain relief to minimise suffering. The system we will use to deliver genes to animal tissue has been shown to result in optimum survival and minimal tissue damage. Also, many of our preliminary experiments will be done in cell culture or tissue taken from wild type rodents which will enable us to plan experiments and minimise animal usage and suffering.

Project 57	**Structural and functional plasticity in cortex**	
Key Words (max. 5 words)	brain, plasticity, degeneration, synapses, learning	
Expected duration of the project (yrs)	5 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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Plasticity in the brain underlies the ability to learn, adapt to novel environments and recover from injury. The disruption of plasticity is at the basis of many brain diseases. Plasticity has two components, functional - the strengthening or weakening of existing connections between brain cells, or structural – the creation of new connections or elimination of existing connections. Disrupted structural plasticity is at the basis of numerous diseases, including Alzheimer's disease, Parkinson's disease, Prion disease, mental retardation and epilepsy; however, how these structural changes occur in the healthy brain is still poorly understood, making it difficult to isolate the problem in the diseased brain. We will investigate how changes in brain activity change the connections between brain cells during learning or changes in environment in the healthy and diseased 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)?	Dendritic spines form the connections between brain cells (called synapses) and their changes are at the basis of our ability to learn and adapt to novel environments. Pathology of these dendritic spine changes is a hallmark of many brain disorders, including Alzheimer's disease, Prion disease, Parkinson's disease, mental retardation, epilepsy, and schizophrenia. Our current understanding of dendritic spine changes (structural plasticity) and how it relates to	

	activity changes in the brain is limited. The work proposed in this project aims to characterize the relationship between changes in brain activity and dendritic spines. By understanding which behavioural, developmental and activity changes induce or prevent dendritic spine plasticity, we can begin to understand potential mechanisms that are critical for the initiation and maintenance of changes to these spines. Then, using mouse models for these diseases, we can determine which of these mechanisms are faulty in the disease states mentioned above. Furthermore, by understanding whether activity levels that change across the entire brain, or just in individual cells are responsible for changes in these dendritic spines, we can determine whether treatments developed for these diseases should be targeted to affect the entire brain or only the affected cells. The experiments outlined here are an important first step in determining the mechanisms of dendritic spine plasticity, which will be critical in the future development of treatments of a number of neurological disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	We will do these experiments in mice because of the number of available mice that transgenically altered. Using transgenic mice has the distinct advantage that we can use mice with the phenotypes for diseases, such as Alzheimer's disease, Parkinson's disease and autism that have disrupted brain plasticity. We will use approximately 3000 mice that undergo any protocols 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?	The animals will undergo surgery of moderate severity. This means that the mice will be quiet and move less for a day or two after surgery. The animals will be given multiple types of pain relief after surgery. Animals might lose a bit of weight, but will typically regain that weight within two to three days. In the event of infection or at the end of the procedures, the animals will be humanely sacrificed in consultation with the veterinary staff.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Measuring the relationship between brain activity and changes to dendritic spines is quite new, and as a result, there are a limited number of studies that have been done thus far. Therefore, there is not enough information to examine these relationships with computational models alone. We work with multiple computational modelling groups, which helps us to

	extract the most information from our experiments and make predictions for the best experiments to do, thus limiting the number of experiments in animals that will need to be performed.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We and others have developed a way to measure the same brain cells (neurons) repeatedly over time that minimizes the suffering that an animal undergoes (as there is only one surgical procedure that could cause discomfort and the animal is given four weeks to recover from the surgery). By using repeated painless recordings in the same animal, we will obtain more data from each individual animal. Additionally, by measuring the same brain cells and dendritic spines over time, we have greater statistical power in our measurements, and thus will need to use fewer animals to determine our results. Finally, we will perform all experiments in designated surgical procedure rooms, which will increase the quality of our data. We will also consult statisticians as necessary to ensure that our experimental analyses are sound. We will re-use our data for multiple studies where it is scientifically feasible.
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.	Our protocol for repeated measurements from dendritic spines and neurons has been developed because it only requires one surgical procedure, after which the animal can undergo measurements for weeks and months without any pain. After the initial surgery, the animal will be given pain relief and allowed to recover from the surgery for at least four weeks before any further procedures are carried out. These precautions will minimize any suffering the animals might have otherwise experienced. The most common adverse effects from either the surgery or the sensory deprivation would be infection and are expected to occur in a low percentage of animals (>1%). The animals will be closely monitored throughout and treated humanely in consultation with veterinary staff in the event of pain or infection. Otherwise, the animals may be quiet and less active for a day or two following surgery, after which they return to normal behaviour.

Project 58	**Studying a human neurological disease-causing gene**	
Key Words (max. 5 words)	Epilepsy, motor neuron disease, autism	
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)	There are two linked objectives, based around analysis of the same genes. One is to discover whether we can manipulate the expression of the genes in nerve cells in such a way that we could protect against the development of motor neuron disease. The other is to make a mouse model of a new cause of epilepsy, severe intellectual disability and autism. This will be used to work out how the mutations seen in humans actually cause the disease, and ultimately to test therapeutic approaches.	
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 have a greater understanding of how the two genes work to control the development of nerve cells and their networks, and of ways in which we might be able to intervene in future to treat people with motor neuron degeneration or children with epilepsy.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, up to 4200 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	Animals with some of the mutations are likely to experience adverse effects, involving muscle wasting or occasional seizures, depending on the mutation. This will only occur at a severe level in a small number of animals in total, and most will be euthanised if the seizures reach a moderate level of	

end?	severity. All animals will be killed by Schedule 1 methods at the end.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The switch between these genes in nerve cells can't be replicated in cells in culture, and the techniques used to introduce mutations of the type seen in humans are much better established in mice than in other species.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The key to minimizing numbers is to keep tight control on the breeding schemes to ensure that enough animals are available for analysis to be significant, but without generating large numbers of animals simply in order to keep the stock going. We have a lot of experience of this, and have kept our animal numbers some way below that anticipated in the course of previous project licences. We will use small pilot studies in order to get good assessments of the numbers of animals needed, and not use more than necessary. We use the latest techniques for genome modification to make sure our methods are specific and do not generate random mutations. We will use inbred strains for generating mutants to avoid the need to carry out more matings simply to get a uniform genetic background.
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 is no real choice other than to use mice for this work, because the only model in which a deletion of the relevant gene has been found is the mouse, and because the genetic manipulation technology we need to use is much better developed in the mouse than in any other species. We will use mating schemes that mean that we only generate affected animals when they are needed for specific experiments. When this happens, mice are monitored on a daily basis to ensure that we minimise suffering. The genome editing technique we propose to use is being refined all the time, and we are careful to keep up to date with the relevant literature and websites. In order to measure seizure activity, we will use a wireless technique that enables mice to move around freely during recording.

Project 59	**Spinal sensory processing**	
Key Words (max. 5 words)	Somatosensory, dorsal root ganglion, spinal, analgesia	
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 seeks to find ways to further our understanding of painful conditions in order to improve pain relief. Such conditions occur because injury alters the way in which sensory information is processed in peripheral nerves, spinal cord and brain. Current treatment options are only partially effective or patients may not respond at all to treatment and many of the available medications have adverse side effects and/or abuse potential. Novel therapies are urgently required. This is well illustrated by the neuropathic pain that occurs as a result of damage to nerves by chemotherapy treatment in cancer patients. These conditions can be so debilitating and poorly managed by current therapies that they can lead to cessation of life prolonging treatment. The aim of this project is therefore to 1. Identify the ‘altered sensory processing’ that mediates painful conditions 2. Provide the mechanistic information required to improve translational capacity of ‘potential’ pain relieving medications 3. Provide novel information about the poorly understood mechanism of action of established pain relieving medications	

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)?	In the short term, the primary benefit will be advancement in our understanding of the mechanisms and circuitry underlying painful conditions. The consensus in this research field is that new knowledge regarding the mechanisms underlying these conditions is urgently required in order to improve treatment of these debilitating conditions. In the longer term, mechanistic insights or pharmacological targets identified in these studies may aid identification, validation or improve translational capacity of prospective therapies. Work from the lab has identified a potential therapy for chemotherapy induced neuropathic pain for which we are actively collecting the experimental data required to inform a planned clinical trial in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	To do this work we will use about 1150 rats and 450 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?	We will produce a mild-moderate painful condition in a single limb by producing a localised inflammation in a single paw or by eliciting peripheral nerve damage surgically / using chemotherapy drugs. We will study the nervous system in post mortem tissue from these animals, which will be humanely killed, to try and identify why the pain system malfunctions in these conditions with the aim of identifying new therapeutic targets. We may then administer promising agents to these animals with the aim of alleviating these painful conditions which we will assess by both monitoring their behaviour and similarly studying their post mortem tissue.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As the aim of this work is to identify the components of the pain system that malfunction in these conditions with the aim of improving the development of pain relieving medications. The normal functioning and wiring of this system is not as yet fully understood and so these pathways cannot be modelled in vitro or theoretically, so there is no feasible alternative that would entirely replace the use of a living animal. It is not ethical to conduct such experiments on humans, especially those requiring removal of parts of the nervous system for ex-vivo investigations. 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	Multiple postmortem tissue preparations can be prepared from an individual animal, minimising the number of animals used. In many cases the uninjured side can be used for comparison rather than separate control animals, again minimising the numbers of animals used. Importantly, the numbers of animals required for a given study is worked out using statistical analysis of pre-existing data or if this is not possible in preliminary data collected from a very small group of animals to ensure that subsequently only the minimum numbers that are required for the study are 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.	Rats and mice are chosen for this work given that much of the current understanding of painful conditions has been carried out in these species, knowledge that is an essential foundation for the studies proposed here. The models proposed are well established, conform to the ethical standards of the relevant professional bodies and have been used in studies published in major scientific journals (Science, Nature, PNAS). Notably the animals will be closely monitored using behavioural sensory testing approaches to define the shortest time window following injury that can be utilised to minimise suffering. Moreover a carefully selected array of behavioural assessment tools will be employed to ensure maximum likelihood that the findings of this project will translate well to humans.

Project 60	**The role of neuropeptides in behaviour**	
Key Words (max. 5 words)	Vasopressin, oxytocin, social behaviours	
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)	This project examines the importance of specific biological molecules known as neuropeptides, in particular those called oxytocin and vasopressin. Within the brain, these peptides are synthesised by different populations of neurons and these populations are responsible for different behaviours e.g. feeding, sexual and aggressive behaviours, social interaction, maternal care and bonding. This is important, as manipulation of these peptides may be valuable therapeutically. For vasopressin and oxytocin, there is evidence of links with neuropsychiatric disorders, including obsessive-compulsive disorder, eating disorders, post-traumatic stress disorder, anxiety, depression and autism. Thus, the main aim of our research lies in understanding how these peptides can trigger clear behavioural effects. In addition to the known populations in the brain, we have recently discovered new populations of vasopressin neurons in the olfactory system and the retina. This program of work is designed to understand the physiological importance of these cell populations. Our most general aim is to understand how these peptides affect neural brain networks, and how they produce coherent changes in behaviour. Our hope is that the results of this study will provide a platform of knowledge from which we and others can	

	further investigate the mechanisms underpinning neurological disorders which are modulated by vasopressin and oxytocin.
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)?	Social interaction and behaviours are of fundamental importance. Therefore, understanding how neuropeptides generate behaviours and what patterns of brain network activity underpin these behaviours is an important step towards understanding the mechanisms of neurological disorders. The aim of this research programme is to increase knowledge and understanding of how individual neuropeptides create coherent specific behaviours. We will build on previous rodent, primate and human data by providing a more in-depth understanding of the mechanisms of neuropeptide actions in rodents. It is our view that only by understanding how neuropeptides function in health will we be able to make significant advances in generating designer drugs and therapeutic interventions in disease. The first direct beneficiary of this work will be the neuroscience communities. We will present our data at national and international meetings with a view to forging lasting collaborations with others interested in peptide actions in health and disease. We will ensure that our work will be passed on to the Universities' Research and Innovation office to be publicised to their discretion. The mission of this centre is to enable discoveries made in the University to be commercialized through technology licensing, collaborative research and consultancy services.
What species and approximate numbers of animals do you expect to use over what period of time?	This programme of research will exclusively use rats and to achieve the outlined aims we will require approximately 8-12 animals per week, 50 weeks per year for 5 years = 2500 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?	Some rats will be used for breeding purposes only. The experiments proposed in this study require surgery, for delivery of drugs to specific brain regions and measurements of peptide release. For these experiments, pain will be controlled during surgery by general anaesthesia and pre and post-surgery by analgesics. The highest severity rating of this programme of work will be moderate but the majority of experiments will have a mild rating. Deaths resulting from anaesthesia or surgical complications are uncommon (<1%) and will be minimised by correct dosing of anaesthetics, by accurate weighing and maintenance of body temperature during and post-

	surgery. Risk of infection will be minimised by aseptic techniques. At the end of each protocol, animals will be killed by using approved humane methods and tissues from these animals may be used for *post hoc* histology. All rats will be monitored closely by experienced staff during the protocols and humanely killed at the end of each experiment, or in the unlikely situation that they present with clinical signs of illness.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The aim of this programme of research is to understand how individual and networks of neurons in the brain produce behaviour. To truly understand how the brain processes information it is imperative that experiments are done at the level of the whole animal. For this reason it is impossible to avoid the use of animals when addressing the aims of the outlined proposal. However, large amounts of work will be done on *in vitro* preparations. There is on-going work in the laboratory using computer models of single neurons. Neuronal models will be used to generate predictions and testable hypotheses based on existing biological data. The use of simple analytical approaches and complex computer models provides a useful method for exploring possible outcomes that can then be tested in rats. However, these approaches are unlikely to replace the need for suitable animal models in which to generate new physiological data.
2. Reduction Explain how you will assure the use of minimum numbers of animals	At the experimental design stage, all studies in the project will be subject to statistical power analysis to determine the minimum number of animals necessary to show the effect of treatment. Being able to calculate the power depends in part on the variability of the responses between animals. We have estimates of this variation from earlier studies.
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 will be used throughout this programme of research. The reasons for this are: 1) Rats have been extensively used as a model organism in this line of research, in our lab and by many others. In term of brain regions and peptides involved in regulating behaviours, the rat is well understood and is comparable to the relevant systems in humans. 2) Using rats lets us avoid repetition of earlier work, builds on current knowledge, and allows direct comparisons of our studies with others.

	3) Advances in molecular genetic techniques to identify or manipulate single neuron activity or neuronal network function have been developed for the rat. Thus, using rat models provides us with the ability to manipulate brain function in a highly cell-selective manner. For all of our studies we will ensure best working practice, consult the NC3Rs guidelines and monitor improvements in refinement when published online. For each study, as part of good laboratory practice, we will prepare a detailed experimental protocol that is discussed with members of the research team. This ensures we work to specific objectives with clearly defined hypotheses and that only work that is necessary to draw conclusions is performed.

Project 61	**Memory in the rat**	
Key Words (max. 5 words)	Memory, learning	
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)	The precise way in which memory is stored and maintained in the brain is not yet known. This is in part because patients with amnesia often have damage to many different regions of the brain. The project will use new tasks in rats which allow us to assess types of memory that are critically impaired in disorders such as Alzheimer's disease and ageing and to make careful and selective damage to particular systems in the brain to test hypotheses about how these systems store and represent different types of memory.	
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)?	In understanding how and where in the brain specific types of memory are stored, we can better target drug therapies to these regions, increasing our chances of being able to improve memory in our old age and in disorders where memory becomes impaired (such as Alzheimer's disease).	
What species and approximate numbers of animals do you expect to use over what period of time?	The project will use approximately 400 rats over five years.	
In the context of what you propose to do to the animals, what are the expected adverse	Many animals will be used to develop critical behavioural tasks that allow us to target key systems in the brain. These tasks will require some control of	

effects and the likely/expected level of severity? What will happen to the animals at the end?	the animals' diet to allow them to be motivated for food, but this control generally improves the quality and length of life for the rats, preventing them from becoming obese. These animals will have a mild severity limit. For other animals there will be lesions made within important systems in the brain to assess the role these structures play in memory. For these animals the surgery is carried out under anaesthetic, with drug relief for any pain experienced after surgery and careful monitoring for infection. Adverse effects are rare, and potential pain and discomfort and pre-empted by providing pain relief and fluids to the animal before and during surgery as well as afterwards
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Precisely placed lesions are the only way of demonstrating the requirement of a region of the brain to perform a particular function. However, the study of precisely placed lesions in humans is impossible, with surgery or accidental damage normally involving multiple regions. Therefore lesions in animals currently remain the only way to examine the necessity of a particular brain region to a particular behaviour.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have developed a range of behavioural tasks to reduce the number of animals used to assess memory. These involve maximising the data retrieved from every animal used and has led to an approximately 40% reduction in animal numbers required in these studies.
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 are chosen as a widely used behavioural model of human memory. A lot is understood about the brain and memory function of a rat, meaning any new information can be understood without having to repeat experiments in new species. The new behavioural techniques we use reduce handling of the animals, which reduces the stress on the animals within the tests. All surgery is carried out in sterile conditions and using techniques to minimise the time in surgery, reducing the risk of infection.

Project 62	**Neural basis on spatial learning and memory**	
Key Words (max. 5 words)	Neural, spatial, learning, memory	
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)	We will record the activity of neurons in the hippocampus, a brain area within the temporal lobes using brain slices and brain imaging techniques in live mice and rats. We will study how neuronal activity changes with the animal's experience, and how different neurons interact with each other and with neurons in other brain areas. This will help us to understand how hippocampal neurons transform the information they receive from other brain areas, and how the complex representations emerging in the hippocampus are derived from simpler inputs.	
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 work will deepen our understanding of how the brain represents space (places, directions and distances) and how an animals learn to localise themselves in space during development. These studies will teach us to which extent spatial constructs are derived from early experiences. The study of how hippocampal processing is affected by neurodegenerative diseases has the aim of identifying early pathological markers that could be used for both diagnostic and therapeutic goals.	
What species and approximate numbers of animals do you expect to use	Approximately 1600 rats and 2300 mice will be used over the five years of the project.	

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?	Rodents will have microscopic electrodes implanted in their brains to record neuronal activity. In some cases drugs will be injected into the animals' brains to modify brain activity. Animals undergoing any surgical procedures will be anaesthetised to a sufficient and appropriate level and analgesia will be provided to minimize pain and discomfort. Some animals will take part in behavioural experiments, which involve food reward and mild food restriction/aversive measures to motivate them to take part in the experiments. Behavioural experiments are designed such that discomfort and harm are minimized. Animals will be humanely sacrificed at the end of the experiments. The overall severity level is moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Rats and mice are used in these studies since is not possible to study the role of the hippocampus, by recording the activity of neurons while engaged in real world navigation without using behaving animals. Our studies include the use of computer models which guide our experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We minimise the number of animals used in these experiments through the continuous implementation of technical developments which allow us to record the activity of ever larger number of neurons in each animal, thus reducing overall number of animals needed. The use of computer modelling also helps reduce the number of animals, by guiding our experimental hypotheses.
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 and mice are used in these studies since there is a long history of studying their learning skills. We also understand well their brain anatomy and physiology. Rodents are extremely good at navigating through space and have an excellent memory for events that take place in each environment they have explored. Pain will be minimised through the use of anaesthesia and analgesia. Discomfort and distress will be kept to a minimum at all times, as participation in behavioural experiments requires the animals to be well motivated and in good health. We will use the minimum number of animals required in any procedure.

Project 63	**The neural basis of spatial cognition and memory**	
Key Words (max. 5 words)	Rat, mouse, single neurons, behaviour, spatial memory, navigation	
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 aim of the project is to understand how neurons in the brain's navigation network encode information about complex space — enabling, for example, the sense of direction" to be maintained when an animal moves from one region of the environment to another via a complex route.	
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)?	Although these experiments will be conducted in rodents, much evidence indicates a similar network in humans, which also has the function of supporting memory for life events. Understanding how this network functions thus could benefit both understanding of navigation behaviour (relevant for example in designing large complex buildings, or in understanding how drivers, pilots, mariners etc compute their position) and also understanding of lifelong memory (of the kind that degenerates in Alzheimer's disease).	
What species and approximate numbers of animals do you expect to use over what period of time?	Rats (approx. 360) Mice (approx.. 40)	
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 adverse effects mostly relate to the surgical implants, and things that might go wrong with those including anaesthetic death, post-operative pain, post-operative infection and occasionally, as a result of infection, post-op implant failure. These potential	

happen to the animals at the end?	effects will be mitigated by careful anaesthetic and aseptic surgical techniques, careful monitoring daily in the post-surgical period and prompt treatment of any infections that might develop. Generally, these procedures are well tolerated by the animals, and do not impede their natural or trained behaviours or their health. At the end of the experiment the animals will be put to sleep with anaesthetic gas and then killed with an overdose of a sedative drug.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Because the processes under investigation are cognitive, the use of awake freely moving and behaving animals is essential. We will endeavour to use non-animal alternatives such as computational modelling where possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Numbers of animals are minimized by collecting as much data as possible from a single animal, by means of multi- electrode implants and by advances in recording technology. Because each animal is studied over several weeks/months, the total numbers that we use are small by the standards of behavioural neuroscience.
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 are proposed because the rat navigation circuitry is well characterised and has many resemblances to human brain circuits. In addition, rats exhibit high levels of three- dimensional behaviour in their natural lives, and are thus a good model with which to study the encoding of complex space. Mice are proposed because they are similar to rats, and new genetic technologies enable exploration of the underlying molecular processes.

Project 64	**Mechanisms contributing to analgesic use & misuse**	
Key Words (max. 5 words)	Opioid, GABA, neurotransmission, stress, nociception	
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)	The opioid drugs are widely prescribed for treating severe pain, but have well-known problems of tolerance (increasing doses are required to achieve the same effect) and dependence (which can lead to addiction). These drugs have their action by binding to specific opioid receptor molecules in the nervous system. The benzodiazepine drugs (e.g., Valium) also offer pain relief and sedation, but they too have problems with abuse and addiction. They work by modulating the activity of another receptor system, which is normally triggered by gamma-amino butyric acid (GABA). More recently, steroid molecules have been identified in the nervous system ("neurosteroids"), which also modulate the GABA receptor pathway. They may be candidates for a third type of pain-killing medicine. We will investigate in detail the connections between pain relief (the desired effect of all these drugs) and the development of tolerance and dependence. For example, we have already discovered that medicines that are prescribed for other reasons can influence the development of tolerance to opioids and we need to understand how this works at a molecular level. We also wish to understand better how the two receptor signalling pathways implicated in pain perception and relief interact.	

	There is a growing appreciation that other life events, particularly stressful ones, influence the development of drug dependence and addiction in people. Using standard stress models in mice, we have already seen that they influence the activity of neurosteroids. We therefore suspect that they will also influence the pain-relieving properties of opioid drugs and the development of tolerance and dependence.
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)?	If we understand better how tolerance and dependence arise at the molecular level, it may be possible to develop new effective treatments of pain in which these serious side-effects can be reduced or avoided altogether. If we can learn more about how stress, particularly when it occurs early in life, can influence the later development of dependence and addiction, it may be possible to devise treatments that will break this linkage and make it less likely that people will encounter serious problems with illicit drug-taking.
What species and approximate numbers of animals do you expect to use over what period of time?	The study will use mice and rats. We will work with a maximum of 10000 mice and 1400 rats over 5 years. We use specifically genetically modified mice to understand the contributions of different genes and these animals will have to be bred in our facilities, hence their larger number. For example, we use mice that have altered levels of opioid receptors. The GABA receptor system is very complex, but again we have access to a wide range of mouse genetic models in which we can discriminate between the contributions of different subtypes of these receptors.
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?	Much of the work involves detection of a painful stimulus. We use the standard 'tail-flick' assay, in which the animal's tail is immersed in warm water and the time taken for it to flick its tail away is measured. In mice in which no pain relief has been given, the length of exposure to the warm water is not sufficient for any lasting harm to be caused. In animals that have received pain-relieving drugs, there could be a risk that they do not flick their tails away in time to avoid any harm. We always apply a maximum time to these experiments, after which the water is removed, to prevent this risk. The standard stress model we use involves perturbations to the environment while young animals are developing and before they are weaned (e.g., removing them from their mother for short periods of time). These produce very few outward signs of

	lasting harm; there may be a short-term slowing in weight gain, but this reverses as the animals develop further. However, the subtle behavioural tests that we commonly use can detect long-lasting changes caused by this early-life stress (ELS). In some cases, it is necessary to administer agents to very specific areas of the brain, to be sure where they are having their effects. Mice and rats will have "guide cannulae" (extremely fine tubes) inserted under precise control in a surgical procedure under general anaesthesia. Animals are expected to make a rapid and complete recovery from the operation. Thereafter the experimental substance can be administered via the cannula, with a minimum of disturbance to the animal.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are currently no non-animal alternatives or simulated models that can faithfully reproduce the overall response of an individual to stressful challenges or the potential of pain and reward. The use of mice and rats is appropriate as the processes of brain cells communication underlying an appropriate response to stressful challenges and the acquisition of rewards are well characterised in these species. They are, also very similar to the processes in the human brain (to the extent that these can be measured).
2. Reduction Explain how you will assure the use of minimum numbers of animals	Breeding programmes will be carefully managed to accurately generate the required number of animals. From previous experiments, we can predict the number of animals that we need for each study to be robust. Tissues harvested post mortem can be used in several different types of laboratory investigation, thus maximising the amount of data that can be obtained from a single 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 general measures you will take to minimise welfare costs (harms) to the animals.	We use models that have been carefully refined to cause the least outward harm while generating molecular and more subtle behavioural changes consistent with the human states being modelled. All animals are closely monitored and, if any unexpected event occurs, the animal will be referred to our veterinary surgeon or immediately withdrawn from the study and killed humanely.

Project 65	**Functions of the murine Trappc9 gene.**	
Key Words (max. 5 words)	Brain development, stem cells, microcephaly	
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)	Several clinical reports describe patients with an inherited genetic disorder that includes symptoms of a small brain (microcephaly), intellectual disability, seizures/epilepsy and impaired developmental milestones (e.g. learning to speak). These patients carry a defective gene called *TRAPPC9*, which is active in brain as well as a few other organs. Very little research has been done on this gene. We only know that it might be involved in transport processes inside cells and/or that it might be required for maintenance of brain stem cells or specialisation into functional brain cells (neurons). We will investigate how and why a lack of *TRAPPC9* results in defective brain development. We are undertaking a combination of *in vitro* (non-animal) cell culture and molecular biology experiments. But we will also analyse a recently established genetically modified mouse model with defects in the *Trappc9* gene that are similar to humans. Nobody has done this before. We will analyse the brain development in the mouse model via advanced imaging (magnetic resonance imaging MRI) and histological techniques and compare it to the human disease. We will also examine whether brain stem cells are affected or	

	whether they cannot specialise into normal neurons anymore. Furthermore, we will undertake basic behavioural tests with the 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)?	We are carrying out basic research to extend the knowledge about the roles of the Trappc9 molecule. Our work will contribute to the field of research into abnormal brain development related to human disorders. This project will benefit scientific advancement in the field by exploring the disease mechanism, i.e. how Trappc9 interacts with other molecules, what its specific functions are, and what roles it has in the wider context of brain development. Trappc9 might be involved in the maintenance of brain stem cells or their appropriate specialisation into functional brain cells (neurons). New information about such a role of Trappc9 might impact on translational or regenerative medicine. Knowing more about Trappc9 might benefit research into potential treatments of brain disorders with stem cells or gene therapy approaches. This might not only be relevant brain development disorders, but also for neurodegenerative diseases like Alzheimer's or Parkinson's disease, in which neurons are lost and treatments with stem cells to replace them attempted. We have made contacts with an expert research group in the field, to investigate stem cell functions.
What species and approximate numbers of animals do you expect to use over what period of time?	We are using mice, including genetically modified mice. We estimate that a maximum of 3500 mice might be used over 5 years for maintenance breeding as well as experimental cohorts. The majority of mice will be required for the maintenance breeding and intercrossing of the genetically modified mouse strains.
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 is classed as up to 'moderate' severity, since the *Trappc9*-deficient mice might show similar brain abnormalities as human patients, which could affect their postnatal growth, development and/or adult behaviour. Increased mortality as a symptom of the genetic modification is unlikely. Many experimental procedures in this project are non-invasive, e.g. imaging techniques under transient anaesthesia (magnetic resonance imaging), or basic behavioural tests. The examination of brain stem cell functions *in vivo* is

	classed as ‘moderate’ severity. Such experiments involve well-established surgical procedures to inject substances into the ventricles of the brain, where they can reach the stem cells. This will involve the use of anaesthetics and pain killers, and the surgery will be conducted aseptically. Eventually, the animals will be humanely killed in accordance with the Home Office regulations at the registered animal research facility, and tissues will be collected for histological and molecular biological analyses.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Some functions of Trappc9 within cells can be analysed to some extent *in vitro* in cultured cells, and we are undertaking such experiments in parallel to the animal work. For example, we are testing whether cells multiply normally or specialise into brain cells (neurons) correctly in culture. These experiments can provide information about molecular functions inside cells, but it will not provide information on how the brain development is impaired in humans with *TRAPPC9* defects. The best mammalian system to model such human gene defects is the mouse, which can be appropriately engineered genetically. Its brain is still similar enough to the human brain in many aspects, to enable conclusive insights. We considered the worm *C. elegans* as an alternative model, but found out that lack of *Trappc9* in worms results in lethality. This would not allow us to study brain development.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will closely match production of mice to forecasted needs, thus reducing the number of surplus stock. Before any experiments are carried out, the appropriate experimental designs and associated statistical tests will be discussed with a biostatistician, to obtain the most conclusive datasets from a minimal number of mice.
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	As mentioned under ‘Replacement’, the mouse is the most suitable animal model, to investigate a human brain disorder caused by deficiency of *TRAPPC9*. We have obtained a novel and sophisticated genetically modified mouse model as part of an effort by an international consortium of scientists and

measures you will take to minimise welfare costs (harms) to the animals.	institutes, who aim to generate a mouse line for every gene and ultimately analyse the phenotypes of these mice in a standardised way and deposit such data into a public database. These mouse lines are also made available to the scientific community in general, to enable more specialised studies in labs with specific expertise. So far, no other *Trappc9* mouse model has been published in the scientific literature. The experimental equipment used in this project, especially the non-invasive imaging equipment (e.g. MRI), is at the forefront of currently available instruments and will enable us to obtain the most detailed and advanced data. Also, we have established collaborations with international experts in the fields of non-invasive imaging, brain stem cell analysis and behavioural testing, to coordinate and carry out optimised experimental protocols.

Project 66	**Mechanism-based targets for new analgesics**	
Key Words (max. 5 words)	Analgesia, molecular targets, somatosensory, sensitisation	
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)	Pain is one of the commonest reasons for seeking medical attention, accounting for around 20% of all GP appointments in the UK. In this project we are aiming to find novel molecular targets that can be used to design new, more effective, drugs for pain relief in patients suffering from pain that is resistant to current treatments. We are investigating the chemical and functional changes that take place in the nervous system following minor damage to tissue or nerves and underlie increased or altered responsiveness to sensory stimuli. Some forms of pain, such as that triggered by nerve damage in degenerative diseases are extremely poorly treated by current analgesics and represent a major unmet need. Only by understanding the mechanisms within the nervous system underlying pain can we hope to find valid targets for improved therapy that delivers real clinical benefit. Parts of our work have already led to a drug development programme and its early application in the clinic. The specific aims of this project build on leading new observations we have made indicating that different forms of pain hypersensitivity may rely on: 1) particular subtypes of a receptor for an important neurotransmitter in the nervous system, 2) a particular growth factor receptor	

	and 3) functional deficits in neuronal mitochondria (the structures that convert nutrients into energy within cells). Our work will elucidate the mechanisms involved in each case and help to find new targets that could enable the development of novel analgesics to reverse ongoing 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)?	The overall aim of this work is to benefit medical and veterinary pain patients by providing routes to the development of new analgesics that will reduce their pain and improve their quality of life. In the immediate term the work will provide rigorous scientific data on several leading (and previously uninvestigated) aspects of the mechanisms underlying pain hypersensitivity. This will contribute significantly to fundamental neurobiological understanding. Academic and pharmaceutical industry researchers will be the short term beneficiaries. The work can potentially lead to the development of novel, more effective, analgesic drugs to deliver improved pain relief and quality of life to clinical and veterinary pain patients. Long term, benefits should include reduced pressure on healthcare systems and a reduction in the economic burden of treatment costs and lost working hours.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice. We expect to use up to a maximum of 1925 rats and 1450 mice over a 5 year period, although the numbers actually used may be significantly less than this.
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 project involves rats and mice in widely accepted research models that provide good representation of changes in human disease. The models generally involve discrete, localised injuries to peripheral nerves or to tissues, generally of one limb, using specific, well-characterised chemical or surgical procedures. The animals are otherwise entirely healthy, with no impairment of general well-being and no signs of distress. We measure changes in sensory reflexes, for example paw withdrawal from a stimulus provided by a single fine flexible nylon fibre. The protocols used are the mildest possible tests that can be employed to answer these questions (classified as of mild to moderate severity), are widely adopted in the research field and consistently produce no overt signs of distress or suffering. In addition, electrophysiological experiments are carried out in fully anaesthetised animals and at the end of studies, the animals are killed and tissue samples are taken

	for an extensive range of biochemical measurements.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to gain valid understanding of the processes of pain hypersensitivity as targets for potential new analgesics without some use of animals such as rodents. This is because processes closely resembling those that occur in man do not occur in simpler organisms with a less highly developed nervous system. Neither can such processes be adequately studied in cultured cells. We use the minimum possible number of animals by carefully planning the experiments and co-ordinating our studies to utilize tissue for corroborative biochemical measurements. For this, we developed new protocols using extremely small samples of tissue, thereby helping to minimise animal use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments are carefully designed on the basis of our extensive experience using statistical methods that are widely used to predict the number of animals likely to be needed to achieve clear and significant results. Further, we ensure that whenever possible all animal tissues are also utilised for sensitive biochemical assays. As a general aim to reduce animal usage we have worked to miniaturise many of our biochemical assays so that only tiny samples of tissue are needed and a range of different questions can be answered using the tissue from a single 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 general measures you will take to minimise welfare costs (harms) to the animals.	We use rats and mice as these have provided the vast majority of research information in the field and simpler species do not have equivalent pathways that can reflect how pain messages are processed in humans. The literature suggests that rodents provide faithful models of human pain states. We take all possible opportunities to minimise intensity and duration of such models in our work, which generally seeks to validate new improved strategies for relief of pain. All of the tests we use to measure sensory responses are brief threshold-level tests that do not distress the animals. According to 3Rs principles, we have successfully worked to move as many of our assessments as possible to recording responses of tiny tissue samples in biochemical assays.

Project 67	**Encoding Behaviour From Synapses To Circuits**	
Key Words (max. 5 words)	Behaviour, Neurons, Communication, Brain, Experience	
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)	The brain is made up of millions of neurons that are connected to each other in incredibly specific ways that allow them to communicate effectively. Everything we do is encoded by these communications, and the majority of diseases associated with the brain affect how different parts of the brain communicate. The overall aim of this project is to investigate how this specific communication between different types of cells in the brain is achieved, and how it is used to drive behaviours that result from feelings such as safety or reward. We will then look at how this circuitry is altered by experiences and disease, to understand how these alterations affect behaviour, and hopefully lead to new treatments.	
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)?	While there have been large advances in our understanding of how neurons in the brain communicate, we really do not know how this communication results in the generation of a behaviour. Both experiences and disease markedly affect our behaviour, and also how our neurons communicate with each other. Therefore, understanding how alterations to neuronal communication after an experience such as a particularly stressful event or in	

	disease result in changes to behaviour is an important goal in understanding and treating a number of debilitating diseases including Alzheimer's, autism, addiction, schizophrenia and depression. The research outlined here will utilise state of the art technology to investigate how communication between different neurons results in behaviour and how this is modulated by experience. This advance in our knowledge will shed light on how experience and disease can result in changes in behaviour. By understanding the neurons underlying these changes in more detail, we will be able to design more specific and effective treatments in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use roughly 1800 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?	Most of our protocols involve procedures of moderate severity. Expected adverse effects might include post-operative stress or discomfort (but this will be minimised with appropriate analgesia). Animals may very infrequently undergo drug-induced seizures and weight loss, but these will be quickly identified to minimise suffering. In all these cases or when unexpected clinical signs appears we will consult our NACWO and NVS. At the end of each procedure animals will be euthanised according to an appropriate humane method and tissues will be isolated for further studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	During this project we will investigate how the brain is connected to carry out specific functions, and how this wiring is altered by experience. There are no computer models or equivalent that can accurately and effectively model these phenomena, and so experiments on living tissue are required. No cultured cell lines are available to study the mechanisms that control synaptic connectivity, and so acute tissue must be used.
2. Reduction Explain how you will assure the use of minimum numbers	We intend to use the minimum number of animals consistent with achieving our experimental aims. At each stage the same animals will be tested in multiple paradigms to minimise numbers used, and to

of animals	maximise the data collected. For example, multiple electrophysiology recordings can be achieved from one 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 general measures you will take to minimise welfare costs (harms) to the animals.	Mice will be used for this project as they represent the least sentient species appropriate for this type of work. On top of this, decades of research has resulted in highly advanced and efficient techniques developed for investigating neuroscience questions in the mouse. Importantly, the mouse is also high genetically tractable, allowing transgenic identification of specific cell types crucial to the fulfilment of the project. To minimise stress wherever possible, mice will receive environmental enrichment and be group housed and appropriate anaesthetic/analgesic regimens will be used to minimise pain.

Project 68	**Understanding successful brain repair in zebrafish**	
Key Words (max. 5 words)	traumatic brain injury, tissue repair	
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)	Traumatic brain injury (TBI) occurs when a person's brain is damaged through an external force. Many patients with TBI will remain disabled for the rest of their lives. This is because the human brain has only very limited abilities to heal itself after injury. In contrast to humans, zebrafish can repair their brains after injury completely so they suffer no long-term consequences of brain injury. The recovery from brain injury is especially quick in very young zebrafish, which are called larvae and which can repair brain damage within 1-2 days. The current project aims to understand how zebrafish larvae achieve this quick and complete healing. We are particularly interested in the processes that happen in the brain very shortly after injury, in the seconds, minutes and hours after the damage occurs. These early tissue reactions to injury very likely play an important role in starting the healing process but they have not yet been investigated. Zebrafish larvae are transparent, and therefore we will be able to watch the tissue reactions to brain injury in real time in a live and intact organism. This opens up new possibilities to study how the zebrafish can heal its brain.	
What are the potential benefits likely to derive from this project (how science could be	We expect that the findings from this programme of work will help us to understand the biological processes that enable the zebrafish to successfully	

advanced or humans or animals could benefit from the project)?	repair its brain after injury. This knowledge will provide the basis for further research into the development of novel therapeutic approaches to TBI in human patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use the zebrafish (*Danio rerio*) for our studies. Adult fish will only be kept for breeding. All experiments will be performed on larval fish, the majority of which will be younger than 5 days of age and therefore not protected under the Animals (Scientific Procedures) Act. Some larvae will be used at 5-10 days of age. Over the five year duration of the project we estimate that we will use approximately 12,000 adult fish and 6,000 larval fish at 5-10 days of age.
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 adult fish kept for breeding will be transgenic. Generally, the introduction of a transgene has no deleterious effect on fish health and wellbeing. Thus keeping transgenic zebrafish and generating transgenic lines is considered to be a mild procedure. No line will be kept in which the transgene negatively affects fish health. Pilot data from larval zebrafish younger than 5 dpf when they are not protected under the Animals (Scientific Procedures) Act show that the larvae survive very well after undergoing brain injury (<5% mortality). Very rarely, blood vessels can be damaged in the brain during the injury (<5% of animals), or the animals may develop swellings around their heart (<5% of animals). Infections at the wound site are very rare (<5% of animals). After injury, larvae continue to develop normally and only a very small proportion of animals shows defective swimming behaviour (<5% of animals). Therefore, brain injury in larval zebrafish and microscopic observation of recovery is considered to be a moderate procedure. The larvae will be monitored very closely during and after injury, and if blood vessel damage, swellings around the heart or infection are observed they will immediately killed by a humane method. All other larvae will be killed by a humane method at the end of the observation period.
Application of the 3Rs	
1. Replacement State why you need to use	We aim to study the reaction of the brain to injury. The brain is a very complex organ with many different cell types arranged in intricate three-dimensional

animals and why you cannot use non-animal alternatives	structures. This tissue context cannot be modelled in cell culture. However, the majority of our experiments will be performed in larval zebrafish younger than 5 days of age, which are not protected under the Animals (Scientific Procedures) Act and will replace the use of adult fish. Furthermore, before using new reagents in older zebrafish we will first test their functionality in larvae younger than 5 days of age. Only reagents that look promising in these larvae will be used in older animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimise the number of animals in each experiment, we will use statistical tools to determine the smallest number of animals needed per experiment, while still ensuring that the results we obtain are statistically meaningful and therefore scientifically robust. In addition, in many cases it is possible to investigate several biological processes within the same animal and we will adopt this approach whenever possible to further reduce the number of animals. We will also repeatedly measure cellular processes within the same animal, instead of using several different animals, allowing us to further reduce 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 transparent zebrafish is an excellent model system for studying the reaction of the brain to injury in real time in a live and intact animal. In combination with the genetic and pharmacological tools available in zebrafish, this establishes the zebrafish as the model of choice for our studies. Importantly, the genes of humans and zebrafish are very similar, which makes us confident that the findings from our studies will ultimately be relevant to human patients. To minimise suffering all animals undergoing brain injury will be anaesthetised during the wounding procedure. After brain injury we will keep the animals in fish water with suitable antibacterial and antifungal agents. These measures have been shown to be very effective at preventing infection. In addition, animals will be single-housed after brain injury to allow them to recover undisturbed by other fish. This will also allow us to identify individual animals and therefore circumvents the need for more invasive tagging strategies. When feeding the animals after injury, we

	will deliver all food directly in front of the mouth to prevent excessive movement. All animals will be closely monitored during the course of experiments and strict humane endpoints will be applied.

Project 69	**Ion channel function and epileptogenesis**	
Key Words (max. 5 words)	Ion channels, epilepsy, 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
		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 affects I % of the population in the UK. It is currently uncontrolled by medication in about 30 % of patients. Thus, there is a need to understand better the mechanisms underpinning the disorder to obtain better treatment. Ion channels are specialised proteins that are located on the surface of the neurons (brain cells). We have previously shown that particular subtypes of ion channels that are present in two brain regions, the cortex and hippocampus, have altered expression and function following the onset of epilepsy in animal models. We now wish to further investigate their function under normal conditions and also following the induction of epilepsy. We also wish to understand whether overcoming the consequences of changes in the activity of these particular ion channels can alter the progress of the 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)?	Epilepsy affects 1% of the population in the UK. It is currently uncontrolled by medication in about 30 % of patients. Thus, there is a need to understand better the mechanisms underpinning the disorder to obtain better treatment. Ion channels are specialised proteins that are located on the surface of the neurons (brain cells). We have	

	previously shown that particular subtypes of ion channels that are present in two brain regions, the cortex and hippocampus, have altered expression and function following the onset of epilepsy in animal models. We now wish to further investigate their function under normal conditions and also following the induction of epilepsy. We also wish to understand whether overcoming the consequences of changes in the activity of these particular ion channels can alter the progress of the disease.
What species and approximate numbers of animals do you expect to use over what period of time?	At present we still do not fully understand how alterations in ion channels affect the excitability of cortical and hippocampal neurons. Our work significantly contributes to advancing our knowledge on how neurons function in the cortex and hippocampus. In addition, our work is likely to uncover new information on how changes in neuronal activity during epilepsy might be overcome. This work is likely to lead to the identification of new targets for the treatment of certain forms of epilepsy.
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 plan on using normal as well as genetically modified rodents. We would attempt to use as few animals as possible. At present we expect to use at least five thousand animals over a period of 5 years. A large proportion of these are for breeding purposes only.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal alternatives include computer modelling. At present we do not have sufficient knowledge on how ion channels contribute to neuronal function or about ion channel properties per se to be able to use this method. Hence, we need to do animal studies to advance our knowledge significantly about how neurons function.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will perform data analysis on a regular basis and stop doing experiments as soon as we have sufficient data for us to perform statistical tests.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	Rodents will be used because (1) many of the experimental results found using these species have been reproducible in humans; (2) rodents breed well and are easy to maintain and as such sufficient numbers can be generated for our experiments.

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	In most experiments we will be obtaining tissue from animals to make electrical recordings from neurons. This causes little distress to animals. In some experiments, animals may be subjected to convulsant agents. These convulsant agents themselves do not cause any harm. Furthermore, seizures themselves do cause any distress. Moreover seizure activity will be terminated within a short period of time with the use of anticonvulsants. Animals, though, will be monitored continuously and treated with strong anticonvulsants if the seizures become life-threatening. In some experiments, surgery will be performed on animals to administer substances or to put in place EEG electrodes for recording seizure activity. Surgery will be carried out under general anaesthesia and post-operative pain will be minimised by administration of analgesics. Aseptic techniques will be used to prevent infections. Further, animals will be kept warm throughout the procedure and ensured that they remain hydrated to speed up recovery following surgery.

Project 70	**Neuroprotective treatments for traumatic injury**	
Key Words (max. 5 words)	neuroprotection, spinal cord injury, regeneration, nerve injury, repair	
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		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)	Traumatic injury of the nervous system, in particular of the spinal cord or of specific important peripheral nerves, is a complex condition without a satisfactory treatment at present. It affects large numbers of patients worldwide and leads to consequences which are dramatic and life-changing, for patients and also for their families. There is a need for therapies which can be administered from the acute period and into the chronic phase following the traumatic event, and which can help limit the injury, restore function as much as possible and thus improve the quality of life of 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)?	Treatments which could be developed under this project could be used to protect the nervous system against the considerable and often irreversible consequences of traumatic injury of the spinal cord or of peripheral nerves. This would constitute a major new development in therapeutics, as such treatments do not exist at present. If such injuries develop in animals, consequences can also be traumatic, as seen in humans. Because of the models we choose for the testing of our treatments, we expect that neuroprotective and neurorestorative treatments that we validate and characterize in detail could also be used later on in a veterinary context, and could greatly benefit injured animals.	

What species and approximate numbers of animals do you expect to use over what period of time?	The project will use mice and rats, as these animals have a similar nervous system to humans and have been shown to have very similar responses to traumatic injury. During the duration of the project, the maximum number of mice and rats used per year would be 2400, and 800, respectively.
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?	Following traumatic injury of the spinal cord or of specific nerves, animals are expected to develop a temporary significant impairment in the ability to move and also some disturbances in their ability to sense the environment, and also in some cases have abnormal pain perception. This type of impairment can be intense especially in the immediate period following the injury. Therefore, continuous and sustained specialist care will be provided, to make sure that in spite of their impairment, animals can feed and drink and are relieved from bodily discomforts which follow the injury, such as temporary poor bladder control or increased sensitivity to touch. At the end of the studies, the animals will be killed using the humane methods stipulated by the regulatory bodies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Trauma in the central nervous system triggers complex events in the nervous tissue but also in the whole body, which cannot be mimicked using just cells in a dish. Thus, there are no suitable simple non-animal alternatives to study neuroprotection and neurorepair, because such models can never reproduce the whole context of the injury.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers used will be decided after careful assessment of the requirements of adequate statistical power and also on the basis of our previous experience and the experience of other specialist researchers in the rest of the world, using similar models and working under stringent ethical controls and with rigorous scientific standards. Calculations will be carried out to ensure appropriate experimental sample size, with sufficient statistical power. It has been recognized that adequate numbers must be used, which means enough animals for robust conclusions, because too small group sizes may lead to unreliable data, and therefore ultimately this would lead to a waste of animals. Multiple information and outcomes will be obtained from each animal, so that use of each animal is maximized. Animals would be followed in a

	manner which is very individual, similar to patients, so that we also understand factors of variability in the response to injury, which are equally important in patients and animals, for future therapeutic application.
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 and mice are used in the experimental models of spinal cord and nerve injury described in this proposal, as their nervous system resembles anatomically the human nervous system, and most of the literature published so far in neurotrauma research has been generated in these species, thus providing a very detailed supportive scientific background. Mice, and more recently rats, can also be modified genetically, thus providing an additional possibility to obtain more information concerning the critical factors which influence the response to injury and also help identify specific targets in cells for future treatments. An intensive post-operative care protocol will support the animals after injury, including regular clinical and general welfare assessments, appropriate pain monitoring and relief, support of feeding and drinking, bladder function management and facilitation of rehabilitation in animals with impaired locomotion. Such a detailed post-surgery program of care will promote animal good health and welfare and will add value to the conclusions drawn in the specific studies carried out Parallel to the work carried out in the project as described, there will be constant observation, consideration and discussion with the specialist care and veterinary staff, of new care and assessment modalities, which would help further refine in future animal care and management, especially in animals with impaired motility.

<table>
<tr><td>Project 71</td><td>Repairing the damaged peripheral nerve</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>sciatic nerve, axon regeneration, neuroprotection, scarring, nerve conduits</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 yrs</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The objectives of this project are to determine changes that occur after injury to the sciatic nerve that provides the information from the hindlimb to the brain and back. We are particularly interested in learning how neurons deal with the injury that makes them vulnerable to death, the development of nerve conduits to bridge the gaps that are left by such injuries and the lack of nerve regrowth that follows nerve injuries of this type.

This will allow for a better understanding of the mechanisms of nerve injury and will help us to identify therapeutic drugs/agents that will be used to protect nerve cells from death, replace the use of healthy nerves from a donor site and promote nerves to re-grow, thus regaining lost function.</td></tr>
<tr><td>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)?</td><td>The project will provide important data that will improve our understanding of the changes that occur after nerve injury and provide an insight into what is required to promote nerve cell survival and regeneration.

This will underpin the discovery of novel therapeutic drugs/agents that will be used to promote nerve cell survival and re-growth.

Currently, the incidence rate of peripheral nerve injuries is 15 for every 100,000 person-years. In the EU 300,000 peripheral nerve injuries occur each year with the majority of injuries in the upper</td></tr>
</table>

	extremity including the hand and wrist. This is a significant burden to the EU economy costing around 18billion EUR/year. Our discoveries could slash these societal burdens dramatically and bring relief to patients who invariably suffer from neuropathic pain and poor functional recovery.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats: 1,500 Mice: 800 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 level of severity? What will happen to the animals at the end?	Potential harm results from sciatic nerve injury, which will be created under general anaesthesia. We will mostly use nerve gap of 15mm to evaluate our potential therapies. We will then select the best compounds for therapies to promote nerve cell survival and regeneration in nerve gaps up to 30mm because this more closely resembles the clinical presentation after peripheral nerve injury in humans, with manifestation of many of the potential complications including hindlimb paralysis. There are clear guidelines in place in our facility to ensure that suffering in animals is minimised by either administration of pain-killers or termination of experiments. Soft mash may be provided on the floor of cages as well as injections of fluids and extensive care within the first three days after injury. We will remain vigilant for any adverse effects and will promptly provide pain relief or treatment if appropriate, or humanely kill the animal. Animals will undergo behavioural/functional tests to maximise data output prior to using Schedule 1 methods to kill animals or animals will be perfused with 4% paraformaldehyde under terminal anaesthesia for histological analyses.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no adequate substitute for using the *in vivo* models described in this application. Establishment of potential clinical relevance of regulatory molecules interacting in a dynamically changing injury site can only be achieved in an animal model. A less sentient animal such as fish cannot be used since they spontaneously regenerate their spinal axons after injury and achieve complete recovery of function. Therefore, rats and mice are our

	prototypic laboratory animals and have been rigorously characterised by ourselves for the sciatic nerve injury paradigms and shown to be representative of the human condition by others. The tools for the project have all been prepared in relation to the models described herein and continuity of the study in these species will be essential for significant progress to be made in a timely and efficient manner.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Some of the end-point measurements (e.g. nerve regrowth, scar formation, angiogenesis etc) may be essentially qualitative and for these we use 3-6 animals per treatment group. In most experiments with quantitative end-points, 6 animals are randomly assigned to each treatment group, a number calculated as the minimum required to provide statistically significant results. This has been determined on the basis of our previous experience with these procedures, the methods of analysis and after consultation with statisticians to calculate power.
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 models selected closely resemble the features seen in humans after peripheral nerve injury. Most therapeutic agents are evaluated and optimised *in vitro* prior to *in vivo* application. We keep our experimental time points in longitudinal studies to a minimum and use archival control results where possible. Multiple analyses are conducted on all harvested tissues. We use the minimum number of interventions and minimal volumes for drug delivery during experiments and continually seek methods to reduce these by studying alternative drug delivery strategies. These refinement steps significantly reduce animal usage and severity.

Project 72	**Cell-specific chromatin profiling in mouse cortex**	
Key Words (max. 5 words)	Cerebral cortex development; Targeted DamID	
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 cerebral cortex, which is the outermost part of the brain, is one of the most complex of all biological structures and is composed of hundreds of different types of highly connected nerve cells. This complexity underlies its ability to perform exceedingly complex neural processes such as consciousness, language or emotion. One of the most important questions when studying brain development is how the brain can be so complex, and at the same time become so well organised. While considerable progress has been made in understanding the various factors that control development of the brain, many questions remain unanswered, in particular regarding the factors that make one nerve cell different from another. Which nerve cells are generated during embryonic development is decided by the neural stem cells. These stem cells can turn genes on or off, depending on the nerve cell they will give rise to. Our main objective therefore is to understand how the expression of genes is regulated by specific factors that bind to the genome of the neural stem cells and the nerve cells. To this purpose, we developed a powerful new technique in the laboratory called Targeted DamID	

	(TaDa), which enables us to study where specific factors bind to the genome in a living, developing organism, without having to isolate the cells from the animal beforehand. This will enable us to assess the following questions in more detail: 1. Which genes are active in the different subsets of neural stem cells and the various nerve cells they give rise to? 2. How is the activity of these genes regulated?
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 project will first of all advance our understanding of normal brain development, in particular how various factors are able to control which types of nerve cells are generated by neural stem cells. Although the implications for understanding and treatment of congenital brain defects are most obvious, the major impact of our project probably lies beyond these developmental disorders. Many insights into human diseases and therapy have indeed come from studying normal development, of which cancer is probably the best example. Studying how genes are switched on and off during normal cell fate decisions, will advance our understanding of pathology in humans and potentially lead to novel therapies. All forms of data will also be made freely available, with unrestricted access once published. Finally, extending the Targeted DamID technique to a mammalian model system will open up many research opportunities for a wide range of research fields.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, 1700 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 majority of animals that are routinely bred in the context of this project are not expected to suffer or show signs of adverse effects that impact materially on their general well-being. Since our scientific question concerns the developing brain, we need to target the cells in the cerebral cortex of the mouse embryo, while the embryo is growing in the uterus. We therefore plan to use a well-established technique called ‘in utero injection and electroporation’, whereby DNA is injected into the brain cavities of embryos

	while they develop in the uterus. The neural stem cells of the brain will then take up the injected DNA, which will allow us to study them in more detail. Those animals that undergo surgery are expected to recover uneventfully from surgery. Pre-, peri- and post-operative distress will be kept to a minimum by good surgical practice and the use of analgesics. Animals exhibiting any unexpected suffering or adverse effects will be killed humanely. At the end of the study, all animals will be killed humanely and their tissues will be used for scientific analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although there are currently no reliable methods that allow us to answer our key questions without use of animals, we will fully exploit other possible resources. In the past, we have indeed been heavily involved in the development of other non-vertebrate or cell-based model systems that strikingly recapitulate many events that occur during mammalian brain development. As stated before, wherever possible, we rely on study of fruit flies as a model system for brain development. Moreover, we regularly collaborate with research groups both within and outside our institute that are experienced in embryonic stem cell-derived models of brain development. Nevertheless, *in vivo* validation of these findings, either in genetically modified animals or through *in utero* expression studies, remains central to the study of cortical development.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The principles of Targeted DamID have been validated in fruit flies as well as in mouse embryonic stem cells. The technique is therefore mature enough to be confidently employed *in vivo* in mice, without requiring many experiments for optimisation. Importantly, the strength of our technique relies on the need for very little biological material, in contrast to many of the other currently employed techniques that generate the same types of data. With the approaches described above, which combine experiments in flies and mice, we are able to design the best and most efficient experiments and use only a minimum number of animals, but with the prospect to generate information of the highest quality.
3. Refinement Explain the choice of species	When studying development of the brain, mice are currently the most heavily studied and best

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.	understood model system that closely resembles human development. In addition, they offer the possibility for genetic manipulation. The procedures we use are overall in the mild/moderate category. All animals, and particularly those that have undergone surgery, are monitored to ensure that they receive appropriate care and to minimize suffering. This applies to mutant animals as well. In the future, we hope to obtain genetically altered mice that would allow Targeted DamID without the need for intra-uterine manipulation of embryos through the surgical route. This will significantly limit suffering of both mothers and offspring.

Project 73	**The Role of Central Sympathetic Control Neurones**	
Key Words (max. 5 words)	Cardiovascular control, paraventricular nucleus	
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)	We and other scientists have identified a family of nerves in the brain that can control the heart and blood vessels. When these nerves are active they increase the blood pressure and heart rate. Evidence suggests that excessive activity of these nerves could contribute to cardiovascular disease. We do not know what normal function these nerves have though. Scientists have several ideas including the suggestion that they could be the nerves that cause sharp and dangerous increases in heart rate during a sudden shock or fright. The central objectives of this project are to find out what the function of these nerves really is in a normal animal, and how we can modify their activity with drugs to prevent death and disease in people and domestic pets.	
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 to answering our objectives are; firstly that it will provide new avenues for targeting cardiovascular disease and secondly, it will teach us more about how the cardiovascular system is controlled and allow improvements to computer models of this which may, ultimately, reduce the need for animal experiments.	
What species and approximate numbers of animals do you expect to use over what period of time?	Rats 500 and Mice 500 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?	In order to conduct this study, a central portion requires us to make very accurate recordings of animal blood pressure, heart rate and temperature whilst animals are left undisturbed in their cages. This means we need to implant radio transmitters that transmit this type of data. We can then receive this with a suitably adapter receiver device connected to a computer nearby. The surgical implantation of transmitters will be done using anaesthetic, aseptic technique and medicines to control pain. Next we will inject drugs which will activate and inhibit some of the cardiovascular control nerves and measure the impact this has on their cardiovascular system, at rest, or throughout the day night cycle (when blood pressure naturally changes). We will also investigate if cardiovascular responses to stress, exercise or temperature are changed when these nerves are activated or inhibited. In some cases, genetically altered animals will be used. These animals will have alterations bred into their cardiovascular control nerves. This will serve two purposes. Firstly it will allow us to label, specifically, just the cardiovascular control nerves with a fluorescent marker so we can identify them visually later, once removed from the animal and grown in tissue culture. Secondly, we can engineer mice with cardiovascular nerves that can be switched on and off (without affecting other neighbouring nerves) in a way not possible with ordinary mice. This method is technically more difficult than simply injecting drugs, but will provide more specific and valuable results. We do not anticipate any of the injected substances will harm the subjects. We have strict humane endpoints in place in case they have unexpected negative impacts on the health of the animal. At the end of the study, animals will be humanly euthanized and their tissues harvested and used for tissue culture experiments. In these studies, we will be able to identify the detailed workings of these particular nerves and develop computer simulations of their activity in a way not possible in entire animals. This in turn may reduce the need for some animal experiments in the future, but the primary aim is to find novel treatments for cardiovascular disease.

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 cardiovascular control nerves and areas of the brain conserved between mammalian species, whilst we could measure cardiovascular status in people, we cannot ethically modulate their cardiovascular control processes because this would involve application of substances which are not approved for humans and could potentially be dangerous. Non-sentient animal alternatives are not a viable alternative, because the system we are studying is an interaction between the forebrain and the heart and blood vessels. This system is well conserved between mammals, but lower animals are too different to be useful examples from which one could safely draw conclusions about how the cardiovascular system is controlled in a human. Tissue culture systems can provide useful information on how the nerves work alone and we will use these where possible. Also computer simulations do not currently include the type of nerves we are studying in this project and are not useful, but one end-point for this project will be progress towards producing the type of computer simulations which could replace animal experiments in the future.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By using implanted telemeters and non-invasive cardiovascular recording where possible, we will be able to extract more data per animal and thus reduce numbers. We have used sophisticated statistical analysis to ensure we use the minimum number of animals possible, which still output useful 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.	Mice and rats will be used because their physiology is well known and the elements under investigation are very similar to those of humans. We use the same modern analgesics (pain killers), anti-bacterials and anaesthetics that would be used in any purely veterinary context. We will use telemeters and non-invasive apparatus wherever possible and have a modern and well-maintained animal unit with many support staff and a veterinary practitioner to assist us monitor the health and welfare of the animals whilst they are under our care.

Project 74	**The molecular and cellular mechanisms that underpin CNS plasticity**	
Key Words (max. 5 words)	Stem cells, myelin, 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
	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)	It is clear that, rather than being a fixed structure once growth and development is complete, the brain has a tremendous capacity for changing the numbers of cells in specific areas and for altering the connectivity between them. These changes are essential for our ability to learn from experience by, for example, creating new or faster circuits that speed up specific tasks used repeatedly. They also provide the ability, in some regions of the brain, to repair damage following disease or trauma by the generation of new cells and/or connections. These changes can result from both the production of new cells by stem cells within the brain and changes in the shape of those cells that already exist within the brain. Together these changes are termed plasticity. At present our understanding of this process is rudimentary. My research project examines this property of plasticity using two exemplar cell types – stem cells to examine the generation of new cells and the oligodendrocytes that form the myelin sheaths that surround axons in the CNS to examine the effects of changing cell shape on connectivity. Our goal is to define the molecules and signalling pathways that control plasticity in these two cell types and so understand this remarkable ability of the brain.	
What are the potential benefits likely to derive from this	My research will benefit three areas of science. First, basic science by generating knowledge as to the	

project (how science could be advanced or humans or animals could benefit from the project)?	fundamental property of the brain – the ability to adapt from experience and so provide humans with the ability to learn. Second, translational science by defining the signalling pathways that control the behaviour of stem cells in the brain – cells that are essential for any repair process that requires the generation of new cells. Third, drug discovery leading from our work on the pathways examining oligodendrocyte plasticity. Damage to the myelin sheaths these cells form occurs in the common human disease Multiple Sclerosis and leads to progressive disability. Drugs to repair this damage by the formation of new sheaths can only be developed once we have a more complete understanding of the key molecules that promote plasticity. Then, these drugs can be used to increase the ability of the brain to repair itself and so slow, stop or even reverse the effects of multiple sclerosis.
What species and approximate numbers of animals do you expect to use over what period of time?	The work will be performed entirely using rodents-rats and mice. My team of 10 scientists anticipate using a total of 35,000 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?	The great majority of experiments will involve procedures designed to test the ability of the brain to change either the rate of generation of new cells or the shape of existing cells i.e. to show plasticity. We will, to test plasticity, expose the animals to drugs and other treatments or models of injury that mimic human diseases. The animals will also often be bred with a genetic change that will enable us to identify and manipulate the cells or molecules we wish to study. Expected adverse effects can be divided into three: • First, those resulting from stem cell manipulation. These will be mild, as the loss of the ability to generate new cells does not in itself cause a harmful phenotype. • Second, those resulting from oligodendrocyte manipulation. These will be moderate, as damage to myelin can cause a shivering phenotype or stiffness of gait as seen in patients with Multiple Sclerosis. • Third, those resulting from the surgical procedures used in the manipulation or the analysis. These (administration of drugs into the brain via a cannulae and the creation of a window in the skull to image cells within the brain) are however very well tolerated and so

	the expected severity level is only moderate. All animals will be humanely killed at the end of each procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex nature of the brain makes animal studies the only realistic way at the present time to study plasticity. Rodents allow diseases found in the human brain to be studied more easily than in non-mammalian species such as fruit flies or zebrafish which, with their very different brains, make extrapolation of any findings to the human more difficult. Complementary work in our own lab and that of others has and will use human-derived stem cells and oligodendrocytes to test the effects of specific signalling pathways we discover in our animal work at the level of the single cell, but obviously cannot be used to model the full complexity of the brain.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise the number of animals we use by making extensive use of cell culture techniques-my lab over the last decade has pioneered a number of these for looking at multiple sclerosis and we will continue to develop still more sophisticated culture models such as, for example, the use of artificial fibres around which oligodendrocytes can form myelin sheaths to replace the need for axons in cultures examining myelin plasticity. We are also developing new methods for analysing the images we obtain by microscopic examination so as to reduce the number of experiments we need before we can be sure of the answer we seek.
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 take great care to minimise the amount of injury caused in our experiments on disease models by restricting the consequences of any experimental manipulation to a small part of the brain. Also, our genetic manipulations will be designed so as to cause minimal suffering; indeed in the great majority of the animals we use the changes will have no adverse effects whatsoever. As a result, most animals will show little or no effects of the manipulations we will use whilst we, given the sophisticated analysis techniques we propose to use, will be able to obtain the information we need to comprehensively answer the important scientific questions we are addressing.

Project 75	**Recovery of peripheral nerve function**
Key Words (max. 5 words)	Nerve, bladder, physiology
Expected duration of the project (yrs)	5 year(s) 0 month(s)

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

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

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Loss of mobility and control of bladder or gut are all major concerns for patients with nerve injury. One solution could be peripheral nerve interfaces, devices that are implanted into cut or surgically teased nerves so that activity in the nerve fibres can be recorded or stimulated. In this project interfaces are being developed for bladder control, for restoring movement in amputees and for controlling visceral functions via the vagus nerve. This project aims to develop interfaces suitable for human use, to prevent the scarring reaction that

	currently limits their useful life, and to develop wireless communication from interfaces to receivers outside the body.
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 aim ultimately is to restore function to human and canine patients that have lost bladder control after spinal cord injury, or who have lost limb function through nerve injury or amputation, although only rat and mouse models will be used in this early study. A nerve interface is used to pick up signals for limb or bladder control, and this is used to drive stimulators or nerve block to prevent unwanted bladder empyting, empty on command, drive robotic limbs, or control muscle contraction in a paralyzed limb. In addition vagal nerve recording and stimulation is being developed for control of the immune system, gut and other organs.
What types and approximate numbers of animals do you expect to use and over what period of time?	800 adult rats 450 adult mice Over five 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 procedures are of moderate severity or, where possible, carried out exclusively under humane, terminal anaesthesia. The main adverse effects from nerve surgery are sensory changes that on very rare occasions lead to animals biting their toes. This can be minimized by choosing strains that do not do this and by humanely killing the animal if it is observed. Animals will be humanely killed at the end of the procedures, and most will be examined by histology.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-protected animal alternatives	The aim of the licence is to develop nerve interfaces for limb, bladder and visceral control. This can only be done by implanting prostheses into nerves in which there is electrical impulse activity, driving muscle contractions and bladder control. Tissue culture experiments would be meaningless because there would be no nerves to interface with, and no activity to record. In addition we need to develop methods to stop the scarring reaction that currently limits the useful life of prostheses. This only happens in animals with a working inflammatory system and a scarring reaction.

2. Reduction Explain how you will ensure the use of minimum numbers of animals	The main method for minimising numbers is careful preparatory work in pilot experiments with small numbers of animals, or terminal experiments on a few animals. By using fully developed methods, and behavioural experiments that we have fully validated in previous work we can get highly repeatable results, so the groups of experimental animals can be small.
3. 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.	Rats are the best experimental animal for nerve interface development, because the nerves are reasonably large, there is sufficient space in the body or under the skin for connectors and interfaces, and the patterns of nerve impulses in response to movement are similar to those in humans. Mice are used when it is important to use genetically modified animals, particularly in experiments in which we are working out which mechanisms in the immune system are responsible for scarring in response to prostheses. All experiments are done on one side of the animal only, which means that there is little disability and no loss of bladder control. By choosing the correct strains of animals we can minimize the tendency of animals to bite their toes after nerve surgery.