Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 20

Projects with a primary purpose of Basic Research into the Nervous System

Project Titles and Keywords

1. **Structure and function of cerebellar modules**
    - Cerebellum, networks, movement, cognition, behaviour

2. **Studies of Synaptic Plasticity in Health and Disease**
    - Neurological diseases, synaptic plasticity, neuronal dysfunction, signalling molecules

3. **Mechanisms that drive development of brain circuits**
    - brain, neonatal, neuron, sensory experience

4. **In vivo analysis of basal ganglia function**
    - Parkinson's disease, deep brain stimulation, Motor cortex, neuronal network oscillations

5. **Modulating BDNF levels and role in brain growth**
    - Growth factor and brain volume

6. **Sex-linked effects on brain and behaviour**
    - Behaviour; brain; psychiatric illness; sex chromosomes; sex differences

7. **Animal Models of Migraine**
    - Genetic alteration, stem cell graft, migraine, neurology

8. **Restoring normal sensory and motor function following neural injury**
    - Nerve injury, motor function, neuropathy, neuropathic pain, inflammation

9. **The acute physiology and pharmacology of acute and chronic pain**
    - afferents, reflexes, pain models, sensitization

10. **Understanding osteoarthritis pain in dogs**
    - Pain, Dog, Osteoarthritis, Phenotype, Sensory

11. **Functional Organisation of the Basal Ganglia in Health and Disease**
    - Neuroscience, Parkinson's disease, neuronal networks

12. **Characterising the Neural Basis of Learning and Memory**
    - Memory, schizophrenia, depression, monoamines

13. **Effect of environment and drugs on brain function**
    - Neural stem cells; drugs, cognition

14. **Tolerance and addiction to drugs of abuse**
    - Tolerance, addiction, reward, learning, memory

15. **Opioid receptors in depression and anxiety**
    - Depression, anxiety, opioid, behaviour, stress

16. **Neurobiology of hearing and deafness**
    - Brain, auditory, plasticity, deafness, tinnitus

17. **Development of epilepsy in mammals**
    - Seizures, drug-resistance, epileptogenesis

18. **A murine model of nystagmus to study potential treatments**
    - Mice Nystagmus Eye Brain Treatments

19. **Tolerance and addiction to drugs of abuse**
    - Tolerance, addiction, reward, learning, memory

20. **Understanding neural control of urinary bladder**
    - Bladder, urination, incontinence

21. **Genetics of Rhythms, Sleep and Behaviour**
    - Behaviour, circadian, sleep, cognition, mouse

22. **Neuronal Networks for Social Behaviour**
    - Social disabilities, psychiatric disorders, neuronal circuits

23. **Neural coding and perception of sound**
    - hearing, neural coding, perception, auditory processing

24. **Maintenance of genetically altered zebrafish lines**
    - Fish, neurodegeneration, disease, ageing, animal model

25. **Neuronal circuits of cortical plasticity**
    - Mouse, visual, imaging, two-photon, spine

26. **Signalling in sensory processing and drug effects**
    - Pain, rodents, signal transduction, opioid tolerance, mood

27. **Nociception and analgesia in zebrafish**
    - Zebrafish, pain relief

28. **Control of midbrain dopaminergic neuronal subtype specification and function**
    - Dopamine neurons, Parkinson's disease, subtype specification, neurological disorders

29. **Ion channel expression and function in the nervous system**
    - ion channel; receptor; G-protein; signalling; action potential

30. **The cellular basis of sleep and circadian timekeeping**
    - Sleep, body clock, circadian, cellular

31. **Sensory Processing in the Nervous System**
    - Nociception, Pain, Depression, Anxiety, Epigenetics

32. **Protein misfolding disease: pathogenesis and intervention**
    - Protein misfolding diseases. Cell death

33. **Functional improvement post nerve injury**
    - Spinal cord injury, scaffold, peripheral nerve injury, microglia, neuropathic pain

34. **Neural Mechanisms and Neuropharmacology of Cognition**
    - Cognition, attention, mental disorders, neuropharmacology

35. **Investigations into Disorders of Movement**
    - Parkinson's, Dystonia, neurodegeneration, movement, dyskinesia

36. **Characterisation and Modulation of Traumatic Brain Injury**
    - Trauma Brain Injury

37. **Primary visual pathways in the rat**
    - Rat brain vision

38. **Investigating ADHD and common co-morbid conditions**
    - Attention Deficit Hyperactivity Disorder

39. **Development and plasticity of sensory pathways**
    - Development, Plasticity, Sensory, Pathways

40. **Primary headaches and associated conditions**
    - Headache, Pain, Electrophysiology, Imaging, Translational Research

41. **Studies on the neurobiology of sensation**

- Touch, pain, itch

42. Neurodegeneration and autophagy in zebrafish

- Zebrafish, neurodegeneration, autophagy

43. Metabolic regulation in health and disease

- Metabolism; obesity; diabetes

44. Biophysical models of neural activity and neurovascular coupling

- Excitation, inhibition, models, LFP, neurovascular

45. Understanding and influencing neural responses in the rodent visual system

- Vision, cortex, rodent

46. Neurorestoration following nervous system injury

- Spinal cord injury, peripheral nerve injury, sensory, motor

47. Prion disease in ruminants

- Prion, scrapie, BSE, pathology

48. MRI and IHC in a preclinical migraine model

- MRI, immunohistochemistry, migraine, sumatriptan, CNS

49. Cortical and sub-cortical motor control

- Cortex; spinal cord; reticular formation

50. Screening for aggression therapeutics in zebrafish

- Aggression, zebrafish, drug screen, behaviour

51. Nervous system development and function in zebrafish

- Vision, CNS, zebrafish, autism, epilepsy

52. Develop and treat fish models of neurological disease.

- Fish, neurodegeneration, disease, ageing, animal model

53. Gene function in neuronal circuit formation and maintenance

- Wnt, synapse, neurodegeneration

54. Neural basis of motivated behaviour

- Obesity, food, dopamine, addiction, nutrition

55. The behavioural neuroscience of adaptive behaviour

- Reward, habit, learning, addiction, risk-taking

56. Plasticity in spinal cord networks: development, disuse and activity

- Locomotion, spinal, injury, exercise

57. Molecular neurobiology of circadian rhythms and sleep

- Sleep, clock, circadian, neurodegeneration, brain

58. Assessment of the pathophysiology of brain dysfunction in diabetic mice

- Brain, Diabetes, Brain compounds, receptors

59. Inflammation and phagocytosis in CNS disease

- Inflammation Phagocytosis Neurodegeneration

60. The function of neuronal networks underlying sensory processing

- Brain, neurological disorders, neurons, information processing

61. Leptin regulation of hippocampal synaptic function

- Leptin; hippocampus, synaptic transmission, ageing, Alzheimer's disease

62. Understanding the pathophysiology of pain

- Pain, inflammation, diabetes, obesity

63. Stress, the brain and pregnancy outcomes

- Preterm birth, stress, offspring, transgenerational

64. Role of AMPA receptors in synaptic plasticity

- AMPA receptors, neurotransmission, plasticity, memory

65. Mechanisms of synapse function and disease

- Synapse function and disease

66. The unfolded protein response in neurodegeneration

- Neurodegeneration, Alzheimer's, prion, dementia, neuroprotection

67. Molecular studies of calcium channel function and their role in disease

- calcium channel; ataxia; epilepsy; chronic pain

68. $GABA_AR$, neurosteroids and stress in brain function

- GABA, neurotransmission, stress, depression, addiction

69. Brain circuits controlling visual behaviours

- Zebrafish, brain, vision, neurons, circuit

70. Spinal cord injury and potential therapies

- Spinal cord injury, stem cells, regeneration, pain, spasticity

71. Neuronal connectivity in development and disease

- Connectivity, neural development, developmental disorders

72. Production of fertilized *Xenopus* oocytes

- Hormone assisted breeding of toads

73. Improving outcomes following nerve injury/repair

- Nerve injury, conduits, regeneration enhancing

74. Effects of exendin-4 in a novel model of early stage PD

- Early Stage Parkinsons's Disease, Pre-motor symptoms, exendin-4, neuroprotection, hyposmia

75. Zebrafish as a model of inherited renal disease

- Cystic kidney, cilial, treatment, pronephros

76. In vivo imaging in normal subjects

- Imaging, diagnosis, therapy

77. Early-life and psychological impact on food choice

- Obesity, reward, mood, stress, early-life

78. Synaptic integration and plasticity in neural circuits

- In vivo, mouse, motor cortex

79. Anatomy and physiology of midbrain dopamine system

- Neurons, synapses, basal ganglia, plasticity

80. Zebrafish models of neurological disease

- Zebrafish, neurodegeneration, translational research, genetics

81. Breeding of genetically altered mice

- Transgenic, breeding, GA mice

82. Neural regulation of circadian rhythms

- Circadian, Eye, Neuroscience, behaviour, light

83. Neural circuit assembly

- Brain, synapse, cortex, development, GABA, neurodevelopmental disorders

84. Brain development and function

- Mouse, development, cerebral cortex, interneuron, brain function

85. Tail docking in pigs

- Pig, pain, tail docking, tail biting

86. The molecular pathology of axon death

- Axon, membrane traffic, microtubule

87. Vitamin A and retinoids in the central nervous system

- Retinoic acid, hormones, receptor, season, circadian

88. Zebrafish Models for Neuromuscular Diseases

- Neuromuscular, Diseases, Genetics, Molecular, Pathology

89. Mechanisms controlling axonal transport in neurons

- Axonal transport, neurodegeneration, neuron

<table>
<tr><td>Project 1</td><td>Structure and function of cerebellar modules</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cerebellum, networks, movement, cognition, behaviour</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></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>
</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 cerebellum is a major region of the brain vital for the coordination of movements. When in mammals (including humans) it is damaged – e.g. due to stroke, genetic disorders, or tumours – voluntary movements such as reaching to grasp an object become inaccurate and poorly timed; balance is severely disrupted; and our ability to learn new motor skills is impaired. Recent studies have also indicated that the role of the cerebellum may extend beyond movement control to include mental processes such as decision making as well as emotion (including fear and anxiety). A fundamental gap in our understanding of emotional behaviours (e.g. defensive behaviours triggered by threatening or fearful events) is how the initiation, adaptation or maintenance of appropriate co-ordinated motor responses are generated. The cerebellum may provide this link given its role in motor coordination.</td></tr>
</table>

	Studies have also established the hypothesis that the cerebellum is subdivided into numerous anatomical/functional ‘modules’. This modular organization provides an important framework in which to investigate cerebellar function and to compare data obtained in different preparations and species. One vital area in which our current understanding is deficient concerns precisely how different modules in the cerebellum differ in regard to the importance of their various input and output paths in relation to their connections with brain structures involved in cognition and emotion. One objective is to provide fresh information in this area in rodents in relation to the neural circuits that underlie emotional behaviours by using anatomical tract tracing – which defines the 'postal address'; neuronal recording – which determines the nature of the 'message' being transmitted; and also behavioural studies – which determine the effect the message has. The cerebellum is the largest motor controller in the brain and it is likely to play a key role in this postal system. How the internal circuitry of cerebellar modules ensures that movements are performed smoothly and accurately also remains unknown. Neurones called Purkinje cells are central to this function because they form the only output of the cerebellar cortex. They influence activity in the cerebellar nuclei, which, in turn, provide the output of the cerebellum, so the way in which cerebellar nuclear activity is modified by Purkinje cells is central to understanding how the cerebellum exerts its control over movement and other functions. Purkinje cells are exceptional in the mammalian brain in that they discharge two very different types of electrical impulse - simple spikes and complex spikes. The latter are thought to hold the key to cerebellar operation but their role in movement control is not understood. Complex spikes can be further categorised into two types: ‘spiking’ and ‘non-spiking’. This has important implications for

	cerebellar information processing because the two types of complex spike are likely to produce distinct patterns of activity in the cerebellar nuclei, providing powerful timing signals that could underlie cerebellar contributions to coordinating movement. Using neuronal recording techniques in anaesthetised and decerebrate rodents, the first objective will be to determine how the two different types of complex spike: i) modify simple spike activity, and ii) influence cerebellar nuclear activity. Because the cerebellum plays a vital role in the control of voluntary limb movements, it is also important to examine the natural patterns of Purkinje cells and cerebellar nuclei in the awake behaving animal. This will be achieved in a small number of felines trained using only positive food rewards to perform a skilled reach-to-grasp movement and when the same movement is perturbed under carefully controlled conditions. The neural circuits involved in relaying sensory information to the cerebellum during movement are not ‘hard wired’ but instead the ability to transmit information can be selectively attenuated or enhanced at different times during voluntary movement. The origin and functional significance of this ability to transmit information only at particular times during behaviour (a phenomenon known as gating) is unknown. Our third objective is to identify the origin(s) of the gating as well as to test the hypothesis that gating during voluntary movement reflects the times when signals are most useful because they are unexpected and are therefore behaviourally useful and need to be forwarded to the cerebellum to update activity and thereby modify on-going or future movement. This objective will be studied using non recovery and recovery electrophysiological and behavioural methods in rodents. Our final objective will use a combination of non-recovery, decerebrate and recovery electrophysiological recordings and behavioural methods in rodents to determine whether age-

	related changes in motor coordination are related to corresponding changes in the ability of neural pathways to forward information to the cerebellum. Given the importance of Purkinje cells in cerebellar function we will also investigate whether their ability to process information is affected with age.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	An increased understanding of the major role that the cerebellum plays in the regulation of voluntary movements should in the longer term assist with the devising of rehabilitation methods for patients with movement disabilities of cerebellar origin, while our studies of cerebellar interactions with brain structures involved in emotional behaviours will also provide new avenues to explore in the treatment of certain psychiatric disorders such as phobias, generalized anxiety disorder and post traumatic stress. The findings will also assist the interpretation of functional imaging studies of the human cerebellum, by providing information about where and at what time during carefully controlled behaviours, specific cell types in the cerebellum are active. Changes in gating and Purkinje cell firing patterns may explain age-related difficulties with motor coordination, which contribute to falling in the elderly, with a consequent increase in mortality and morbidity from traumatic injuries.
What species and approximate numbers of animals do you expect to use over what period of time?	Based on extensive prior experience of experiments of a similar type it is estimated that the total number of animals used over the 5 year duration of the project will be 980 rats, 50 mice and 10 felines. Wherever possible we will use rats or mice to meet our objectives. Rodents are the animals of lowest neurophysiological sensitivity on which studies of this type can be performed to provide reliable and applicable data. Most of the experimental procedures in rodents will be carried out under terminal anaesthesia. However to meet our objectives we will need to obtain high quality recordings from single cerebellar Purkinje cells during natural behaviour. Currently this is only possible in felines.

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?	Wherever possible the experiments will be carried out on terminally anaesthetized animals. In the remainder, the animals will be allowed to recover for at least part of the experiment. The anatomical pathway tracing experiments, and chronic recording studies, are classified as moderate procedures. Based on considerable prior experience adverse effects are likely to be infrequent (<10%) and include premature death under anaesthesia; postsurgery infection and behavioural abnormalities (e.g. subdued behaviour or loss of appetite). Most electrophysiological studies will be non recovery experiments carried out under general anaesthesia so the animals will suffer no adverse effects. For recovery experiments all surgical procedures will be carried out using aseptic techniques and with general anaesthesia throughout. All animals will receive painkillers following recovery surgery. All animals in recovery experiments will undergo terminal anaesthesia to obtain either acute electrophysiological data or to obtain brain tissue that will be processed histologically to chart the chain of neural connections that link the output of different cerebellar modules with key brain structures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no non-sentient systems that model the mammalian sensorimotor system and can replace the use of animals. Other non-animal methods (e.g. cell culture, computer modelling) are incapable of providing the new information required. Therefore, our objectives are attainable only through invasive studies of the intact functioning central nervous system, only possible in animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Numbers of all species will be kept to a minimum by a range of strategies, including (i) studying wherever possible both sides of the brain in the same animal (and thereby effectively doubling the results obtained from each animal); (ii) by developing and using the latest multiple pathway tracer and multiple neuronal recording techniques

	(increasing the yield of results obtained from each animal); and (iii) by recording neural activity at the terminal anaesthetic stage of 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.	Wherever possible we will use rats or mice to meet our objectives. Rodents are the animals of lowest neurophysiological sensitivity on which studies of this type can be performed to provide reliable and applicable data. Rats are the scientific animal of choice for all of our anatomical and most of our electrophysiological studies because the relevant background literature is most complete in this species. Mice are the animal of choice for studies involving genetic manipulations. Most of the experimental procedures in rodents will be carried out under terminal anaesthesia. All of the anatomical and electrophysiological techniques we propose to use have been used successfully in rats. A small number of felines are required for one of our objectives as the detailed and stable neural recordings that we require to answer our objective is not yet feasible in rodents. The reasons for this include: 1) Individual cerebellar modules are larger and more accessible in felines than in rodents (3mm wide in cats versus ~0.5mm in rats) and therefore can be more readily targeted. 2) Increased body size also means that implants for cerebellar recording can be more securely fixed on a cat's skull than is the case in a rat or mouse; 3) In rodents, the cerebellum is hidden below the occipital bone at the back of the skull, making headpiece fixation difficult. A secure head implant means that cerebellar recordings in felines are more stable, artefacts are minimised (e.g. from neck muscle activity contaminating recordings), and generally there is less movement of the cerebellum in the cranium so a much higher quality of recording can be achieved. 4) Increased body size also means that in cats more than one multielectrode recording implant can be accommodated on the head (necessary for simultaneous recording of cerebellar cortical and nuclear activity). This is not currently possible in rodents. Also, simultaneous monitoring of a greater number of other biological

	signals such as EMG activity from different limb muscles together with neurograms from peripheral nerves is possible in felines This is essential in experiments where a full understanding of the neural control of complex movements is sought. 5) For some electrophysiological studies in awake animals access to the surface of the anterior lobe of the cerebellum is essential. In rodents this surface is buried or obscured by a large transverse venous sinus. 6) Our main concern is with visually guided movement so a species is required in which vision is a more dominant sense than in rats. The only other group of animals in which visually-guided target reaching is likely to be achievable would be non-human primates. And 7) a major objective is to understand the importance of spiking and non-spiking complex spike activity in different cerebellar modules in the control of skilled voluntary movements. The high resolution and stable neural recording required to do this is currently only possible in felines.

<table>
<tr><td>Project 2</td><td>Studies of Synaptic Plasticity in Health and Disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neurological diseases, synaptic plasticity, neuronal dysfunction, signalling molecules</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></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>Using in vitro preparation (brain slices or cell cultures) we have recently identified synaptic proteins known to be involved in many neurological diseases and also to play a pivotal role in synaptic plasticity. In parallel we developed new pharmacological tools, classified as Positive or Negative Modulators of the brain's major excitatory neurotransmission system. In principle this new family of drugs have the potential to improve and reverse cognitive decline or enhance learning and memory.

This research project aims to advance our current understanding of cellular, sub-cellular and molecular mechanisms underlying synaptic dysfunction, such as in Alzheimer’s disease (AD) and epilepsy. It also aims to investigate whether or not our findings, from in vitro preparations, can be translated to whole animal and provide meaningful data to advance treatment of neurological diseases in general.</td></tr>
<tr><td>What are the potential benefits</td><td>We expect that this work will lead to the</td></tr>
</table>

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	development of clinical strategies for rescuing synaptic dysfunctions associated with neurological diseases. We also expect the results to assist in the development of new medicines to restore and to enhance synaptic plasticity in pathological processes observed in different forms of neurological diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	The research will be conducted in rats and mice and in the five year period, of this license, we may use up to 16.300 animals (total, rats and mice together).
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?	A significant number of the procedures will be conducted under non-recovery general anaesthesia and the animals are not allowed to recover from the anaesthetic. In a minority of the studies, animals will have recording electrodes implanted to monitor long-term neuronal activity within specific region of the brain. Therefore, as some experiments will involve surgical procedures, there is a possibility that some animals may react to the anaesthesia, or develop pain or infection. Although there is a possibility, our experience has allowed us to reduce the incidence to less than 1% with fairly low severity by the use of peri-operative analgesia and high standard level of aseptic conditions. For some studies epileptic activity will be induced under controlled conditions by electrical or chemical stimulation of specific regions of the brain. At any stage of the experimental procedures, any animal showing signs of distress or ill health that can compromise its welfare will be killed immediately using a an overdose of anaesthetic. At the end of the normal experimental protocol the animals will be killed under general anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use	Due to obvious ethical and practical reasons (such as: long-term electrophysiological recordings or the need of brain tissue for biochemistry analysis)

animals and why you cannot use non-animal alternatives	these studies cannot be developed humans patients. Alternatively animal models have been used in studies to investigate some features of AD, like learning and memory. For example, insects such as Drosophila melanogaster provide restricted models for many molecular aspects of human disease but are unsuitable for the long-term electrophysiological studies needed to meet the complexities of the mammalian brain and the aims of our studies. Therefore we will use both mice and rats as models in our studies. Clearly mice or rats are not people; however the same can be said that apples are not planets. Yet, because apples and planets both have mass, Isaac Newton was able to elucidate the forces ruling planet's movements, using falling apples as models. In a similar way, mice / rats and people share several features that make them viable models for studying human diseases. They represent the lowest sentient mammalian species appropriate to the proposed neural and neurochemical research due to their size and amenability to behavioural training and testing. Their brain morphology and neurotransmitters systems are comparable with that seen in primates including humans. The **advances in genome sequencing and research have been exponential during the last decade with** rats and mice becoming the species of choice for studies of diseases; especially those of neurological origin. Rats and mice are also the species of choice for behavioural experiments since extensive literature relating to their behaviour is already available.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For each experimental group we will use the statistical "Power calculation ANOVA" to estimate the number of animals. Overall in our studies the number of animals will be minimised by: • Defining effective concentration of the drugs on brain slice preparation prior to the *in vivo*

	experiments. • Using independent inputs (stimulating and recording electrodes) will allow a pathway to be used as control and the second as the experimental pathway, therefore reducing animals used by approximately 50%. • Long-term studies in animals allows the animal to act as its own control, with recordings being made before, during and then after treatment. • Making use of historical data to minimise the number of control animals used. • Interleaving experimental groups to enable the control group to serve more than one set of experiments. • Using cell cultures and cell lines for the basic studies of neurotransmitters receptors properties.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodents are the animals of lowest sentience that allow quantification of synaptic function from neural networks during physiological activity, and allow a combination of electrophysiological and behavioural techniques to be deployed together with pharmacological treatment. Furthermore, the use of genetically altered mice allows us to determine the role of genes in the development and function of neural networks, and provides models of human disease. In these studies we will use rat or mouse models that are well characterised and have the typical neurological symptoms of the disease. For example, the Tg2576 mouse model of AD is well characterised and has several features of the disease; including: 1- declining in brain functions associated to synaptic plasticity and learning and memory, 2 - alterations of synaptic proteins and 3 – alterations in signalling pathway molecules that are essential for learning and memory. Therefore the model above is one of the most appropriate to address the objectives of our project. We will apply electrophysiological techniques to study two forms of synaptic plasticity; Long-term depression and Long-term potentiation (LTD and

	LTP) in the hippocampus of these animals. They are reliable methods for inducing LTD and LTP in the hippocampus of rats and mice and were established and refined in the last 5 years under the scope of the previous licence. The electrophysiological methods, we will use, are the most accepted and used worldwide for investigation of synaptic and cellular decline observed in neurological diseases. The quality of the data will be judged in real time as to its validity, based upon our experience with these techniques and of the available literature.

<table>
<tr><td>Project 3</td><td>Mechanisms that drive development of brain circuits</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>brain, neonatal, neuron, sensory experience,</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></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>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>We do not understand how the newborn brain matures, or how this maturation goes wrong in neurodevelopmental diseases like autism. Our overall objective is to generate new understanding of the neuronal mechanisms which underpin brain maturation early in life. Ultimately, these mechanisms can be identified in young children and manipulated to promote healthy brain maturation.</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>Achieving the aims of this study will advance our understanding of the neuronal mechanisms that drive the early life maturation of the brain. In particular, we will identify the changes that are driven by real world sensory experience during neonatal development. As such, the outcomes of this work will be of great value to future research and will drive the development of targeted molecular or environmental strategies for clinical assessment and manipulation of neonatal brain development</td></tr>
</table>

What species and approximate numbers of animals do you expect to use over what period of time?	We use rodents (mice and rats) because they are the lowest species which have the same basic structure of sensory pathways as other mammals, including humans. Indeed, the basic structure, function (as we understand it) of the cerebral cortex is preserved from rodents to humans. We also know that there are parallels in the way that brain circuits develop in an experience-dependent way in the neonatal rodent and in human babies. This is not true for other models such as invertebrates. Furthermore, rodents are amenable to genetic intervention that is crucial to many of our experiments. As such, rodents are the most appropriate model system for our experimental objectives. We envisage the use of upto 1000 mice and 200 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?	To generate appropriate experimental animal models, we will breed transgenic 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 also induce premature labour in pregnant rodents to model the effects of premature birth in humans. This procedure is designed to maximise the survival of the prematurely-born pups, although some may have difficulty breathing/feeding when born very premature. To will use expression of genetic material in the brain of some animals to enable measurements of brain function and to manipulate brain development. This involves injection of genetic material into the brain under anaesthesia and its subsequent expression. There are small possibilities of adverse effects of surgery (used for injections) but, otherwise, 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 and a window in the skull to allow us to use microscopes to image the brain underneath. 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, although it is possible that some animals will experience adverse effects on skull or brain growth. Recording is made under anaesthesia so as not to cause stress. To understand the impact of sensory experience, we will make manipulations to sensory receptors, such as trimming of rodents' whiskers or, occasionally, removal of single digit from the paw. Although the procedures make cause mild pain and stress, such manipulations do not cause any long-lasting pain or suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complexity of the brain, with its myriad of regions and connections, cannot be mimicked. In relation to our research, our key aim is to understand how experience transmitted from the outside world into the brain through a complex set of neuronal connections is responsible for adjusting its own properties. There is no non-animal alternative to that real-world experience and the activity it generates. As such, we need to use animals with working brains to be able to measure the properties of those brain circuits.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise the number of animals bred for our experiments by using the most efficient breeding strategy based on genetics and female receptivity. We will check the genetic status of young pups so that we can ensure that the most appropriate animals are used in further experiments. Whenever possible, we will use a longitudinal study design where the same animal is assessed over time. This design sidesteps the great variability that is found between the brains of different individuals. This generates greater statistical power and hence

	reduces 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 minimise welfare costs (harms) to the animals.	We will use the whisker-sensory system of the rodent brain as a well-established experimental model of how sensory experience can alter brain development. This system is powerful because it can be manipulated simply and non-invasively (for example, by stroking or trimming whiskers); it develops in an experience-dependent way early in life; it mimics many anatomical and functional features of the human sensory system; and it has abnormal development in models of disease. These characteristics will specifically enable us to address our objectives, which relate to generating understanding of the mechanisms that underlie how early life experiences control brain growth, development and function. To minimise harm to experimental animals, highly trained personnel will use the least invasive and most appropriate anaesthesia and surgical methods. In all experiments except those requiring behaviour, the experimental recording will take place under general anaesthesia to ensure there is no pain or stress associated with them. All animals will receive pain-alleviating medication when appropriate and we will ensure that no animals suffer prolonged discomfort or stress by careful monitoring of behaviour after experimental procedures.

<table>
<tr><td>Project 4</td><td colspan="3">In vivo analysis of basal ganglia function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Parkinson’s disease, deep brain stimulation, Motor cortex, neuronal network oscillations</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">Objective 1 To determine the mechanisms by which the brain chemical dopamine modulates brain activity in the motor cortex.

Objective 2 To determine the changes in neuronal activity in the control and the dopamine-depleted state and how this is related to deep brain stimulation and future non-invasive therapies.</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">To identify new non-invasive stimulation strategies and new targets for drug therapy in Parkinson’s disease.</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">Adult rats - 750 over 5 years duration of the project</td></tr>
<tr><td>In the context of what you propose to do to the animals,</td><td colspan="3">Lesioning experiments are of moderate severity.</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?	Behavioural changes are expected but no adverse effects. Humane end points for this protocol will be determined by a Distress Scoring System. All Animals will be killed by a Schedule 1 method or perfused under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The work outlined will be carried by recording electrical activity from single cells and networks of cells in brain slices (*in vitro*) and anaesthetised animals (*in vivo*). This type of work offers considerable technical advantages as the architecture and neuronal circuitry of the tissue in the vicinity of recording is largely left intact. There are no other alternative methodologies available (including cell culture or computer modelling), which deal with the objectives stated. However results from studies of this nature do inform future modelling studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Data collected from the *in vivo* experiments will guide future *in vitro* work i.e. stimulation protocols. Multiple recordings will also allow much more data can be collected from a single animal than with traditional electrophysiological (e.g. single-electrode) preparations. The consequence is that fewer numbers of animals are used as compared to traditional preparations. The marked reduction in animal numbers is demonstrated by the fact that in rats, the majority of past studies using traditional (1-8 channels) preparations required up to 20-30 animals, whereas most of the recent studies using multi-channel (64-128) recording approaches have fewer than six animals. With regard to brain slices experiments four slices containing the relevant brain nuclei of thickness 300 μm can be obtained from any one animal. Thus, a single animal can satisfy more than one researcher's requirements on any one day.
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	The focus of the work is the understanding the structure and function of brain circuitry involved in voluntary movement, what happens when this goes wrong i.e. in PD, thus leading new non-invasive stimulation strategies and new targets for drug therapy. The use of rats will take advantage of the wealth of anatomical, behavioural and physiological information obtained and makes the

measures you will take to minimise welfare costs (harms) to the animals.	interpretation of results easier as well as the integration with existing knowledge. In many respects these species appear good models of human function applicable to the human situation. All available evidence suggests that the unilateral injection of 6-hydroxydopamine is a good model of PD that closely mimics the disease and has fewer behavioural side effects than bilateral lesions; however we will continually keep abreast of the literature to assess any new models that arise. In order to minimise animal suffering all experiments will be conducted using general anaesthesia. In addition *in vivo* experiments will use appropriate local anaesthetics and analgesics following surgery. A homeothermic blanket will be used in all cases to maintain body temperature and prevent hypothermia. Fluid replacement will also be given in all cases as appropriate. The welfare of each animal will be subject to strict monitoring and humane end points for this protocol are determined by a Distress Scoring System.

Project 5	**Modulating BDNF levels and role in brain growth**	
Key Words (max. 5 words)	Growth factor and brain volume	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Yes	Basic research
	Yes	Translational and applied research
	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)	The central objective is to determine whether or not variation of the levels of a single growth factor in the adult mouse brain is sufficient to modulate the volume of the brain. These results will contribute to our understanding of human conditions such as Rett syndrome, Alzheimer's, Parkinson's, Huntington's diseases as well as chronic forms of multiple sclerosis where decrease in brain volume either globally or of restricted areas is a significant manifestation of the disease process.	
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)?	Loss of brain volume is a central characteristic of neurodegenerative diseases including Alzheimer's and Huntington' disease. One of the questions asked in the proposed project is whether the administration of a drug already used in the treatment of multiple sclerosis can modulate brain volume in the adult. The project is also about understanding the role of blood-derived BDNF in humans, including the potential benefit of physical exercise known to increase BDNF levels in humans.	

What species and approximate numbers of animals do you expect to use over what period of time?	The species to be used are mice as well as rats and we expect to use over the next 5 years up to about two thousand animals in our experiments to ensure reproducibility and significance.
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?	Except for the *Mecp2* animals, a near perfect model of Rett syndrome in humans, we don't expect negative effects on the health of the animals, not least because the experiments will be limited to a period of 2 months after birth, before the onset of any possible adverse effects. The animals will be 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 vitro* experiments have been used extensively in our projects based on a novel culture system developed in our laboratory. This system will also be used in our project as it significantly reduces the number of animals required and also allows precise, quantitative investigations. However, nothing can replace *in vivo* experiments in Neuroscience because of the complex question of connectivity that characterizes the brain.
2. Reduction Explain how you will assure the use of minimum numbers of animals.	*In vitro* differentiation of embryonic stem cells carrying specific mutations have been and will be used by our laboratory to minimize the number of in 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.	There are currently no alternatives to the use of mouse and rats as the species of choice for our investigations as our work centres on animal carrying various modifications of their genome. The *Mecp2* mouse mutant is widely recognized as a near-perfect model of the second most frequent cause of mental retardation in young girls, i.e. Rett syndrome. Careful monitoring of the locomotor behaviour of these animals will prevent health deterioration resulting from reduced food access. All other mouse mutants to be used will carry inducible gene deletion allowing the acute removal of genes for short period of times, which for example avoid problems that may occur during development. All animals will be humanely

	killed before any possible adverse effects of the genetic changes appear. In case of doubts NAWCO will be consulted.

<table>
<tr><td>Project 6</td><td colspan="3">Sex-linked effects on brain and behaviour</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Behaviour, brain, psychiatric illness, sex chromosomes, sex differences</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>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 colspan="3">Men and women differ substantially in their behaviour, and the two sexes are differentially vulnerable to a number of common and disabling psychiatric illnesses. Both normal and pathological behaviour are substantially underpinned by genetics, and in the case of sex differences, genes on the sex chromosomes are likely to be particularly important. Currently there is very little information on how sex-linked genetic mechanisms influence brain function, and on how they might contribute towards sex differences in normal and abnormal behaviours. This project aims to increase our understanding in this area, with a view to identifying potential new therapeutic targets for sexually dimorphic brain disorders.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or</td><td colspan="3">Achieving our objectives will add a new dimension to the understanding of sex differences in normal behaviour and in mental disorders. These studies will provide important insights into how sex-linked (or</td></tr>
</table>

humans or animals could benefit from the project)?	autosomal candidate psychiatric genes) might influence neurobiology, and therefore sex-specific behaviour and sex-specific vulnerability to common neuropsychiatric disorders (e.g. autism, schizophrenia, ADHD). These disorders show a sex bias in their incidence, age-at-onset, course, underlying neurobiology and/or response to treatment; our work could therefore characterise protective/risk factors for psychiatric phenotypes, and hence suggest potential new treatment avenues.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use wild type and genetically altered rats and mice in this work; these species, in which genes can be modified, show considerable genetic and neural homology with humans, key attributes in terms of modelling psychiatric processes dependent upon altered gene function. Moreover, elegant behavioural tasks with established translational utility are already available for these species. We anticipate using ~2,800 mice and 900 rats over the course of the licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Novel genetically altered animals may exhibit unanticipated health issues. Administering drugs to pregnant mothers may have mild, adverse effects on the mother's health, or on rates of pup death/malformation. Transferring litters from mutant to wild type mothers to test genetic mutation effects on mothering may lead to increased rates of pup-killing. In order to motivate performance in some behavioural tasks, a degree of food/water restriction will be necessary leading to potential weight loss. A minority of behavioural tasks will be aversively motivated (e.g. by mild foot shocks, or by immersion in water) resulting in transient pain or distress. Neuroactive substances may be locally or systemically administered to some animals; these drugs may have mild, transient effects on behaviour. Surgical procedures may result in mild pain. At the end of the experiments, animals will be transferred to collaborators for further analysis, or culled by an appropriate method. Animals showing severe health/welfare issues resistant to treatment will be

	culled by an appropriate method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals in our research for several reasons. First, it is not yet possible to model the complexities of behaviour in isolated tissue systems, or by using computer simulations. Second, our rodent lines will, in many cases, serve as direct models for human disease situations. Where our *in vivo* work suggests underlying molecular/cellular mechanisms for behavioural abnormalities, these will be further explored in *in vitro* systems; the results of these studies will help inform our *in vivo* work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise animal usage through performing power calculations once initial pilot data are available to ensure that we use the minimum number of animals for obtaining a reliable experimental result; we will also use as many animals from each litter as possible (either as experimental subjects or controls). We will continue to improve our behavioural methods such that fewer animals are lost through attrition e.g. failure to learn the task.
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.	Existing genetically altered rodents to be used exhibit no adverse effects on health/wellbeing; any new mutants created will be monitored closely in consultation with a vet and experienced animal technicians, and if necessary will be treated/culled. The majority of behavioural procedures to be used will either be non-regulated, or appetitively motivated with palatable foodstuffs; the restriction protocols to be used do not result in adverse effects on general health or well-being. Where aversive procedures are used, these will be mild and will not result in long-term adverse effects; particular attention will be paid to animals undergoing such procedures. Any drugs given will be non-toxic, and will be given in suitable and minimal volumes of vehicle. All surgery will be performed under aseptic conditions under general anaesthesia, and all efforts will be made to minimise potential associated pain using an appropriate analgesic regime.

<table>
<tr><td>Project 7</td><td colspan="3">Animal Models of Migraine</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Genetic alteration, stem cell graft, migraine, neurology</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>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></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 colspan="3">Migraine is a common, debilitating and costly headache disorder. Treatments are often absent or inadequate stemming from incomplete understanding of the causes and the lack of good experimental models. Through this project we therefore aim to better understand how the brain and other parts of the nervous system are dysregulated. We will investigate how migraine predisposing genes alter the way particular part of the nervous system respond to stimulation. We will aim to get a better understanding of how dysfunctional nervous system leads to behavioural changes such as aversion to light.

In order to achieve these major objectives, which are current scientific unknowns, we will generate animal models which have been genetically altered to express migraine predisposing gene variants. Stem cells can be obtained from patients with migraine and these cells have the capacity to</td></tr>
</table>

	become any cell type of the body. If stem cell are implanted into the brain, they will become integrated into the animal and mature into nerve cells. This allows the investigation of grafted cells harbouring human migraine predisposing genes and their responses in a network of nerve cells.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	A key primary benefit is the development of better disease models for migraine, which will then benefit the millions of people suffering migraine worldwide. The work will lead to a better understanding disease mechanism, provide better models for preclinical drug discovery and eventually lead to newer and more effective treatments for migraine. It will also demonstrate the value of stem cell models derived from human patients for investigating disease mechanism and drug discovery and could therefore lead to reduced animal experimentation. Through publication of findings in academic journals, the information is likely to be of interest other researchers with an interest in the function of the nervous system in health and disease.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use mice and rats. Over 5 years we expect to use 9500 mice and 7000 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?	We expect the level of severity to be mild to moderate. We do not expect genetically altered or stem cell grafted animals bred for these experiments will have detrimental phenotypes. If harmful effects are seen, the animals will be killed by an approved method to prevent ongoing suffering. Animals undergoing invasive procedures such as blood sampling, administration of drugs or surgical procedures will have analgesia and anaesthetic as required. Complications from such procedures such as infection, swelling or bleeding will be carefully monitored for and appropriate action taken if seen. Animals will be routinely examined for their appearance (including weight

	loss, breathing patterns, coat condition and discharges), posture and behaviour (including abnormal movement, aggression and vocalisation). This will ensure animals are healthy before, during and after experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The demonstration that abnormalities identified in cultured cells are relevant to a given disease requires investigation in whole organisms. Animals are being replaced in certain aspects such as in a number of areas of drug discovery. This includes work on stem cells cultured from patients with a disease of interest. An aspect of this project is to demonstrate that stem cells are a suitable means of replacement. cellular abnormality is relevant to a given disease. Nevertheless, the full assessment of a disease process and a putative new drug requires animal studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimize animal numbers our experiments are always preceded by preliminary studies using cells in culture followed by pilot studies in animals. We will use optimized techniques to reduce experimental error. We will ensure our experiments have sufficient animals to detect an effect and will use the appropriate statistical tests. Where possible we will try to undertake control experiments in the same animal, or look at multiple outcomes in the same animal or test multiple hypotheses in the same experiment. We will ensure however that animal suffering is minimized and does not exceed the moderate severity limit.
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 sufficiently close to humans to be of relevance for human neurobiology and neurological disease. Genetic alteration is well established in rodents, particular mice and stem cell grafting has been show to be facile with low risk of adverse effects in rats. The evaluation of rodents through anatomical, behavioural and neurophysiological studies has been refined over several decades. Stem cell grafting is used as a complement to genetic alteration as it allows the modelling of human neurons with the full set of human genes in whole animals. A continuous process of refinement of procedures

	allows the use of more specific and minimal invasive methodologies, use of minimally invasive and least stressful behavioural and physiological assessments. Suffering will also be minimized through good and appropriate use of analgesics. We will use neonatal stem cell grafting where possible as the most refined since we do then need to suppress the immune system to prevent rejection. Any grafts in adults, will be on animals that have been tolerized to foreign material during the neonatal period. We will use the most refined route and mode of anaesthesia for the developmental stage of the animal. For example, in pups less than 5 years old, deep hypothermia is the most refined method with quicker recovery allowing the pup to be returned to its mother more quickly and to resume suckling.

<table>
<tr><td>Project 8</td><td colspan="3">Restoring normal sensory and motor function following neural injury</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Nerve injury, motor function, neuropathy, neuropathic pain, inflammation</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>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 colspan="3">Following nerve injury (for instance following trauma to a nerve) repair is usually incomplete and significant deficits such weakness and pain persist. There are currently no effective clinical therapies available and patients can therefore spend many years suffering from severe disability. We will determine the consequences of injury to the peripheral nerves, spinal cord and brain and determine the detailed inflammatory processes and the mechanisms of inhibition of regeneration. We will also test potential treatments to enhance neural repair.

A possible consequence of injury to the nervous system is the development of chronic pain.. Despite recent advances we still have a limited understanding of the sensory nervous system and insufficient knowledge of pathophysiological processes triggered by diseases such as nerve</td></tr>
</table>

	trauma. We propose to study injuries to the peripheral and central nervous system in order to identify important factors in the physiological and pathophysiological production of pain in order to develop 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)?	Currently there is no treatment to promote neural repair following injury and treatment of persistent pain is inadequate however both of these conditions are common problems in the population with a major impact on quality of life. 6% of the elderly population have peripheral neuropathy and one in six of the general population suffer from pain. The ultimate aim of this project is to develop treatments for these conditions which would be of major benefit to humans and animals.
What species and approximate numbers of animals do you expect to use over what period of time?	The rodent has many similarities in its nervous system compared to man. We estimate that we will use up to 2750 rats and 8250 mice with the majority of the latter being generated through the breeding of transgenic mice. Transgenic technology means that genes can be manipulated in mice to study very specifically the role of individual genes. Careful experimental and statistical design will be employed to minimise the number of animals used to generate robust results. For instance the minimum number of animals will be used in each group to give robust statistical result.
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?	Under general anaesthesia animals will undergo surgery in which a peripheral nerve or the spinal cord will be partially injured in a controlled fashion. This will produce focal weakness for instance of a hind limb and altered sensation such as numbness and pain. To test the response of animals to sensory stimuli mechanical stimuli e.g. bendy hairs (von Frey hairs), thermal stimuli (e.g. warming or cooling) or chemicals (e.g. capsaicin an extract of chilli peppers or formalin) will be applied to the paw. These are predicted to evoke a brief sensation of pain – through using stimuli from which animals can withdraw suffering is minimised. If following administration of a chemical such as formalin there

	is evidence of continuous ongoing pain behaviour at 90 minutes post administration animals will be immediately killed. The electrical activity within the nervous system will be used to assess repair and function of the sensory and motor nerves. To do this, animals will undergo general anaesthesia. 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	Techniques using cultured neurons are not yet sufficiently advanced that they can fully model the integrated actions of the nervous system. This is due to the complex connectivity of the nervous system and the multiple cell types involved. Behavioural analysis of gait and sensory function requires the use of awake animals. We are attempting to differentiate human induced pleuripotent stem cells into sensory neurons. These will enable the investigation of molecular interactions and electrical properties of these neurons hence ultimately reducing the use of animals. Wherever possible we do test normal sensory function in humans however some models for example creation of experimental nerve injury can't be performed in humans
2. Reduction Explain how you will assure the use of minimum numbers of animals.	Throughout this project we will reduce the number of animals used by using rigorous experimental design in each experimental paradigm to use the minimum number of animals to generate a statistically meaningful 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.	Rodents are the most commonly used animals for the study of neural injury and persistent pain because there is vast knowledge of the rodent peripheral nervous system. Sensory and motor neurons in the rodent have comparable features to those seen in primates, including man and both species show a similar response to nerve injury. The models used attempt as far as possible to mimic human neuropathies and persistent pain states. Our intention is for the different animal models to represent different underlying

	mechanisms generated following nerve and tissue injury in patients in order to translate findings. We will minimise the severity of models to reduce suffering. The most common test of sensory function is measurement of reflex withdrawal to threshold stimuli rather than subjecting animals to supra-threshold stimuli. Animals will be closely monitored following surgical procedures which will be performed efficiently by well trained staff and peri-operative analgesia will be administered.

<table>
<tr><td>Project 9</td><td>The acute physiology and pharmacology of acute and chronic pain</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>afferents, reflexes, pain models, sensitization</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>
<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>Chronic pain remains a major cause of human and animal suffering, with nearly half the population experiencing some form of chronic pain within their lifetime. Chronic inflammatory pain (e.g. due to rheumatoid arthritis) affects around 1% of the UK population and along with osteoarthritis (OA), the commonest form of chronic pain, the burden on the NHS is significant. Underlying causes of OA pain in particular remain incompletely understood and current treatments are inadequate. In addition, common drug treatments (e.g. non-steroidal anti-inflammatory drugs such as ibuprofen) have potentially serious side effects when taken for a long period of time. Pain due to nerve damage is particularly difficult to treat as standard pain killers for chronic pain (e.g. opioids such as morphine) can have no effect. Therefore the mechanisms underlying chronic pain states need to be better understood so that new drug treatments can be developed.</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>These studies will advance understanding of mechanisms behind acute and chronic pain states by investigating functional changes in the nervous system in whole animals subject to clinically relevant models of chronic pain. Currently a lack of consistently effective pharmacological therapies means many patients suffering from chronic pain (e.g. due to arthritis) are in desperate need of relief, so basic research findings are often rapidly translated into clinical experiments. The research</td></tr>
</table>

	has the capacity to provide information towards development of new drugs or new approaches to using existing licensed compounds. Our work in clinically relevant pain models also enables further characterization and validation of the models themselves.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use around 4200 rats, 2200 mice and 440 rabbits 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 intended pain models are mild compared with some in use, in that animals do not lose weight, continue to eat, groom and show normal exploratory behaviour. Under general anaesthesia, some animals will receive either an injection into one hind limb or knee joint, or a surgical intervention on the joint to induce inflammation or arthritis. Others will be subjected to a surgical procedure that causes injury to the peripheral nervous system. The resulting conditions produce joint pathology and pain behaviour responses that mimic key features of human arthritic and neuropathic pain states. In models in which it is necessary to incise the skin but this incision does not constitute part of the model per se, we will administer local anaesthetic prior to incision to reduce discomfort post-surgery. Some swelling may be present in the test limb and lameness or impaired movement is likely. Some pain and discomfort may also be present but none of these symptoms will be allowed to exceed moderate severity. Pain behavioural responses of these animals will be measured by well-established tests. The animals are unrestrained and are able to move away from the applied stimulus at any point. At the end of a study, electrophysiological recordings will be performed in terminally anaesthetized animals or animals will be euthanized by a Schedule 1 method or method approved under licence authority.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The research will study how different parts of the nervous system interact and how this changes in different pain conditions. This requires an intact nervous system therefore could not be carried out on cultured tissues. While *in vitro* preparations have been developed in which the majority of the central nervous system (CNS; brain stem and spinal cord)

	remains intact, these are almost invariably confined to neonates/juvenile animals and do not usually retain any contact between the CNS and the rest of the body. Hence studies on spinal reflex organization, for instance, which rely on interconnections between peripheral and central nervous systems, must be studied in whole animals. In inflammation, osteoarthritis and nerve injury, dissociated neurones can yield some relevant information, but without the interstitial milieu, adjacent inflammatory cells and associated target tissues, they cannot be assumed to exhibit properties that reflect those seen in whole animals. Drugs have multiple sites of action in the body which may be strongly influenced by pharmacokinetic factors that cannot be modelled *in vitro* therefore need to be studied in the whole animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A consideration of the eventual statistical analysis of the data is the first factor in experimental design such that group sizes are used which are the minimum necessary to achieve a statistically and biologically meaningful outcome. To ensure this, power calculations will be performed in advance of experiments using, wherever possible, historic data in order to determine appropriate group sizes. In addition, in order to ensure that high quality, reliable and valid data is extracted from the minimum number of experiments, the ARRIVE guidelines will be followed, and where necessary, advice on experimental design and analysis will be sought from the in house biostatistician. *Ex vivo* studies to identify key targets prior to their investigation *in vivo* will also complement *in vivo* work and in these experiments animal numbers will be minimized by examining expression of as many targets as possible in the collected tissue; in addition tissue will often be taken from euthanized animals following electrophysiological and/or behavioural 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.	Rodents are the least sentient species on which studies of this nature are performed and almost all pain research in animals is conducted in rats (very good subjects for behavioural and acute invasive electrophysiological experiments) or mice (facility for transgenic models) hence there is a wealth of historic data to compare our findings to. Due to their well developed hindlimb anatomy, rabbits are

	excellent subjects for the electrophysiological study of reflexes as multiple responses of large amplitude can be recorded in the same animal thereby potentially limiting the number of animals required, and again, there is a good body of previous data in this species. The models of chronic pain have been chosen for the different types of clinical pain they represent and where possible for being the least detrimental to animal welfare. In all cases, following model induction, animals will be monitored for behavioural and weight changes, and any other signs of illness or discomfort. With respect to OA pain models where severe lameness is not an inevitable feature of the model, we will monitor lameness using a scoring system, and no OA model animal will remain in the study if exhibiting signs of severe lameness. Our experience with other models of chronic pain (CFA, SNL, mSNA) tell us that less severe levels of lameness are well tolerated by the animal and are not associated with weight loss or other signs of illness or discomfort. Due to the nature of the research, post-operative analgesia will not be possible following model induction however animals will receive the highest possible standard of post-operative care from a dedicated animal husbandry and technical support team. Animals will be closely monitored and veterinary advice promptly sought if needed. Behavioural threshold tests will be used where animals are unrestrained and free to move away from the noxious stimulus at any time, thus preventing any long lasting discomfort or tissue damage.

<table>
<tr><td>Project 10</td><td>Understanding osteoarthritis pain in dogs</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Pain, Dog, Osteoarthritis, Phenotype, Sensory</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></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></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>Osteoarthritis (OA) is very common cause of chronic pain in dogs. We currently assume that all dogs with OA suffer similarly from pain and show similar altered sensitivity to sensory stimuli such as heat and pressure. However, in people suffering from OA, different types of pain associated with different sensory sensitivities are recognized, and these distinct pain patterns are likely associated with different underlying changes in the sensory nervous system. Furthermore, these distinct pain patterns are likely to predict response to different analgesic drugs. We predict, given the similarity between the disease of OA in dogs and people, that we will be able to identify similar distinct pain patterns in dogs suffering from osteoarthritis.

We will study pet dogs with OA, recruited through liaison with veterinary surgeons. We will use a simple, validated experimental paradigm to determine underlying pain mechanisms in individual dogs and subsequently map the individual pain</td></tr>
</table>

	pattern or pain phenotype to allow us to link pain mechanism with clinical pain expression.
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)?	Ultimately we hope that new knowledge generated by this proposal will improve chronic pain management in dogs and therefore benefit dog welfare.
What species and approximate numbers of animals do you expect to use over what period of time?	We will study approximately 100 pet dogs with OA and for comparison, will also recruit 35 normal pain-free dogs that are client owned. The programme of work will take place over 3 years, but each individual dog will be studied over a total time period of 5 weeks. Within this time window the dog will be studied 3 times (week 0, week 1 and week 5). Dogs with OA and normal control dogs will undergo the same experimental paradigm.
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?	Dogs will be anaesthetised for radiography to confirm the presence of OA (or that they are normal) and for a simple neurophysiological test that will allow us to determine underlying pain mechanisms in the individual animal. Anaesthesia carries a risk of adverse events (hypothermia, low blood pressure), but with appropriate anaesthesia care and support the severity of these physiological disturbances is limited. There is also a low risk of death resulting from anaesthesia (1 in 2000 dogs). We do not expect any adverse events to result from anaesthesia in any dogs in this study. The neurophysiological test requires delivery of low intensity electrical and mechanical stimuli to the limbs of the dog, however these will not be of sufficient intensity or duration to cause tissue damage and pain on recovery from anaesthesia. Application of thermal and mechanical stimuli to awake dogs during sensory testing also has the potential to cause tissue damage and therefore longer lasting rather than transient pain. However due to the experimental paradigm and imposed limits on the magnitude of these stimuli any adverse

	events are expected to be mild. All dogs will be returned to their owners between each test session (week 0,1, 5). After the final measurement time point (week 5) the dog will be discharge from the Act and returned to the owner.
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 investigate the relationship between pain mechanisms and behavioural expression of pain in dogs. Pain is a conscious experience and therefore cannot be modelled using non-animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have preliminary data collected by one of the researchers relating to sensory testing in dogs with OA compared with healthy control animals. These pilot data enabled us to carry out a power analysis to calculate the total number of animals required for the study.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The aim of the study is to investigate pain mechanisms in dogs with OA and therefore dogs will be studied under this programme of work. A number of other refinements will be implemented to limit any discomfort or harm to the dogs as a result of the study protocols: 1) Anaesthesia of dogs will be carried out under the supervision of a European Diplomate in Anaesthesia and Analgesia. This will ensure that all dogs receive a very high standard of care, reducing the likelihood of any adverse events during anaesthesia. 2) During sensory testing all dogs will be freely able to move away from the stimulus or make a withdrawal response to indicate that the stimulus is perceived as painful; at which point the stimulus will be terminated. 3) The maximum duration of exposure to sensory stimuli is limited to a level below that which necessary to cause tissue damage.

	4) If any dog shows signs of fear or anxiety, or is not responsive to positive encouragement (from people) or reward (e.g. food treats or play) during testing, the procedures will be stopped and the dog will be returned to their owner.

<table>
<tr><td>Project 11</td><td>Functional Organisation of the Basal Ganglia in Health and Disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neuroscience, Parkinson’s disease, neuronal networks</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><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></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><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td><s>No</s></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 object of this project is to increase our understanding of how nerve cells are connected and understand their electrical activity in a region of the brain called the basal ganglia. In doing so we will gain insight as to what the basal ganglia do and how they do it. As the basal ganglia is the seat of many neurological diseases, most notably Parkinson’s disease, we will apply the knowledge gained in normal animals to Parkinsonian animals to increase our understanding of what goes wrong in 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>The benefits of the project will be a better understanding of how the brain is put together and how the brain works. We will also gain a better understanding of what goes wrong in Parkinson’s disease (and other diseases of the basal ganglia, e.g. Huntington’s disease, OCD, ADHD) which will aid in the development of new therapeutic</td></tr>
</table>

	approaches to treat the disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice, some of which will be genetically altered. Over 5 years we expect to breed in the region of 10,000 mice and about 2000 rats. A maximum of 5000 mice and 5000 rats will be subjected to protocols.
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 principal adverse effects are likely to be the consequences of the systemic administration of substances and the surgical procedures for the injection of substances into the brain or attachment of 'head-stages' to hold wires and probes in place. Lesions designed to mimic a neurological disorder may also lead to adverse effects but usually these will be made on one side of the brain only. This minimizes adverse effects as they are able to compensate for deficits of movement on one side of the body. We will also use some genetically altered animals that mimic genetic forms (or some aspects) of neurological disorders. In our experience so far, these lead to only mild adverse effects in the form of minor disturbances in movement. All animals will be killed by a Schedule 1 method or deeply anaesthetised and killed.
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 form 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 basal ganglia in health and disease since the principles of organisation and operation of the basal ganglia seem to be similar throughout all mammals, they are thus a good model of the human basal ganglia. The main model of Parkinson's disease that we use is a toxin model that kills those neurons that die in Parkinson's disease, i.e. the dopamine neurons. It is the loss of these nerve cells that underlies most of the symptoms of Parkinson's disease. This is the best characterised model of Parkinson's and we make the lesion only on one side of the brain so that the animals can function with only minimal problems with movement. 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.

<table>
<tr><td>Project 12</td><td>Characterising the Neural Basis of Learning and Memory</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Memory, schizophrenia, depression, monoamines</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></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>This project will determine the influence in rodents of exposure of the dam to infection or drugs of abuse, the resultant pups to maternal neglect or separation, or genetic manipulation on neuronal development, function and resultant behaviour of the adolescent and adult offspring to attempt to mirror factors that are known to enhance the risk of developing neurodevelopmental disorders with learning and memory dysfunction in man.

In complimentary pharmacological studies it will investigate the role of specific neuronal pathways, neurotransmitters and their receptors (in particular monoamine, acetylcholine, glutamate, GABA and cannabinoid neurones) in the regulation of learning and memory and potential novel therapeutic test drugs to reverse learning and memory dysfunction in these animal models.

Finally it will develop improved animal models of cognitive dysfunction such as seen in schizophrenia, depression, Autistic Spectrum Disorder, Attention Deficit Hyperactivity Disorder and Alzheimer’s disease and assess the potential</td></tr>
</table>

	of novel therapies to reverse these behavioural and neurochemical abnormalities. This work will improve our understanding of the neurobiological basis of learning and memory dysfunction in mammals and may lead to the identification of novel therapeutic targets to restore memory impairment in common CNS disorders, such as schizophrenia, depression Autistic Spectrum Disorder and ADHD.
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 use and improve the translational relevance of animal models for learning and memory in part supported by collaborative funding from pharmaceutical industry and a tangible outcome is development of improved therapy for such disorders. However, the development of any new therapy through to phase III clinical trials and eventual use in patients is likely to require at least 15 years.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice at all developmental stages 1600/year
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most experiments performed under this licence will involve acute drug administration and monitoring of behavioural, some will occur with indwelling intracerebral cannula or intraperitoneal implants previously implanted under anaesthesia. In most cases the drugs administered will produce mild transient (typically up to 4h) changes in behaviour with no long-lasting effect. In some cases pregnant dams will be administered drugs to modify neuronal development of offspring without causing any gross developmental abnormalities of other organs. None of the behavioural paradigms involve exposure to long lasting painful stimuli, some involve exposure to a transient load noise to induce a startle or to a transient mild aversive foot shock. Each procedure has been evaluated for severity. The drug studies combined with behaviour in the absence of any surgical intervention are likely to be associated with only mild clinical signs but several can be graded as moderate due to, for instance, the use of electric shock or desired and expected drug-induced changes in behaviour, so these, like all procedures

	involving surgery, have been rated as moderate. It is anticipated that no more than 30% of the experiments will involve surgery in some form, so an overall grading of moderate has been given. Most animals will be humanely killed by a schedule 1 procedure but some will be killed under terminal anaesthesia particularly if essential to enable collection of brain tissues for further analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Due to the nature of the project there is no suitable *ex vivo* alterative to assess complex behavioural functions of the CNS. *In vitro* slices and cell preparations may be used to examine molecular mechanisms underlying specific components involved in learning and memory such as long term potentiation, but this cannot be extrapolated to human behaviour. As the underlying causes of cognitive dysfunction are unknown, computer modelling techniques are not suitable. It is also not ethical or possible to use patients to test novel experimental agents used in the current animal studies. In rodent models it is possible to perform invasive procedures that will establish specific neuronal circuits, neurotransmitters and molecular mechanisms underlying learning and memory which is not possible in man.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For each study undertaken many parameters will typically be measured and careful consideration is given to incorporating suitable controls, often both for drug treatment and experimental condition. Almost all data is analysed by ANOVA, often with two or three independent factors. Personal licence holders undertaking this work attend statistical workshops as part of their graduate training which cover experimental design and data analysis. Where required, advanced statistical knowledge will be obtained from Statisticians within the University or specialists employed by sponsors from the pharmaceutical industry. Typically a power analysis has been performed on the primary outcome measure for each behaviour, to ensure that sufficient animals are included in the design and that there is a robust likelihood of achieving significance ($P<0.05$ with a power of 0.8). For each experiment, as part of good laboratory practice and the ARRIVE guidelines, we will write

	an experimental protocol which includes: a statement of the objective(s) a description of the experiment, covering such matters as the experimental treatments, the size of the experiment (number of groups, number of animals/group), and the experimental material an outline of the method of analysis of the results (which may include some account of the tests of significance to be made and the treatment differences that are to be estimated).
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.	This project utilises rats and mice because many aspects of rodent learning and memory are similar to humans. Behavioural tasks assess multiple learning and memory domains relevant to human psychiatric disorders. A wealth of data is available on mouse and rat behaviour and excellent stereotaxic atlases enable CNS implantation/recording. For genetic studies mice are the most appropriate species but the larger size of rats makes them more suitable for lesion, microdialysis and MRI studies. Rodents were chosen over less sentient vertebrates, such as zebra fish, because it is not possible to perform complex learning and memory tasks in these animals, and tasks do not have recognised translational predictive validity to the cognitive domains affected in human CNS disorders. However, the well characterised learning and memory paradigms to be utilised in rodents have proven relevance to cognitive dysfunction seen in man, which means that a higher species of animal is not required to meet the objectives of this study. The most severe procedures used in this project involve surgery which is carried out under anaesthesia, using aseptic surgical techniques practised by experienced licence holders and followed by high standards of post-operative care including analgesia as advised by the NVS. Refinement will advance and improve electrophysiological and neurochemical measurements and further develop MRI and biosensor probes to improve measurement of GABA and glutamate both in terms of sensitivity and temporal resolution. Mathematical modelling will be used for electrophysiological data to attempt to establish a predictive model for selected neuronal circuits.

	Improvement in the predictive validity of animal models of common neurodevelopmental learning and memory disorders is also being actively pursued under this licence, by combining early-life adversity with chemical or genetic manipulations which may produce models with better translational relevance to schizophrenia and depression. The project uses rodents at all stages of development including exposure of the dam and/or neonatal pups to environmental and/or drug treatment to produce a programmed change in neuronal development with consequent alteration in adolescent or adult behaviour required to replicate the human CNS disorder. It also uses adult rodents to examine drug-induced alterations in behaviour and relate these to the underlying neurotransmitter or anatomical pathways involved. Pilot Studies will be used where needed to develop a new technique. The recently published Guiding Principles for Behavioural Laboratory Animal Sciences will be used to inform and refine the proposed programme of work. http://www.lasa.co.uk/LASA_BAP_BNA_ESSWAP_GP_Behavioural_LAS_Nov13.pdf

<table>
<tr><td>Project 13</td><td colspan="3">Effect of environment and drugs on brain function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Neural stem cells; drugs, cognition</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>~~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 colspan="3">To understand why certain drug regimes cause memory and concentration problems in patients.

To understand why this cognitive decline persists for a number of years after the end of treatment.

To develop treatments which could protect the brain or compensate for cognitive decline.</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">Large numbers of individuals suffer from prolonged memory and concentration decline after drug treatments (e.g. chemotherapy; antiepileptic treatment). Understanding the causes of this and developing treatments would benefit significant numbers of patients.</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">Rats and mice will be used. The project will last for 5 years and use approximately 1500 animals.</td></tr>
<tr><td>In the context of what you</td><td colspan="3">Animals will be treated with drugs used in treating</td></tr>
</table>

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?	patients. Some of these substances have toxic side effects (e.g. chemotherapy) and animals will suffer from these effects (e.g. moderate weight loss, loss of appetite, cognitive impairment). Most drugs will be administered by injection which requires restraint and will cause discomfort. Some drugs will be administered directly into the brain after surgery under general anaesthetic. Animals may suffer pain and discomfort due to surgery. All animals will be put down at the end of the procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The memory and cognitive effects we are studying require an intact central nervous system and so require the use of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have taken statistical advice in order to use the minimum number of animals consistent with obtaining scientifically valid results. We are currently working with neural stem cells in culture to understand the impact of drugs on these cells. This understanding will reduce the numbers of animals which need to be 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.	Spatial memory can be tested in rats and mice and the impact of environment and drugs on this aspect of their behaviour can be correlated with the impact of these factors on human performance. Where possible experiments will use in vitro tissue and cells to determine the cellular response to drugs showing cognitive changes in vivo. In procedures involving injection of drugs, animals will be handled by investigators who have completed an animal handling course and have been trained in administration of substances by these routes. The LASA guidelines for these procedures will be followed.

	All surgical procedures will be carried out under full anaesthesia with appropriate aseptic techniques followed by high standards of post operative care including appropriate analgesia. Systemic analgesia will be administered pre and post operatively as well as local analgesia to the wound. Animals will be monitored until they come round from anaesthesia and at regular time points post operatively.

<table>
<tr><td>Project 14</td><td>Tolerance and addiction to drugs of abuse</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Tolerance, addiction, reward, learning, memory</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>6 months</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>OVERALL AIM: investigate mechanisms underlying tolerance and addiction to drugs of abuse.

Objective 1: To investigate the cellular mechanism(s) underlying opioid receptor desensitization in brain regions involved in the rewarding properties of drugs of abuse.

Objective 2: To investigate the molecular mechanisms underlying the synaptic plasticity events involved in the induction, maintenance or extinction of drug-related memory.</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>Objective 1

The work is expected to provide novel information concerning desensitization of mu-opioid receptors (MORs). Agents that enhance opioid tolerance may be useful in drug rehabilitation programs to prevent relapse.

Furthermore, detailed information on the</td></tr>
</table>

	timecourse of the decline of tolerance to the euphoric effects will reveal information leading to the risk, and potential treatment, or opioid overdose. **Objective 2** The work is expected to provide fundamental insight into the specific synaptic plasticity events involved in the development of drug-seeking behaviour. There is a clear need for therapeutic agents that will prevent relapse (approx. 70-80% of recovering addicts relapse within a year) and identifying novel strategies in this area would represent a major advance in the treatment of drug addicts. Affecting learning and memory and synaptic plasticity mechanisms involved in addiction is a novel potential treatment target.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice: 190
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All procedures are either mild or non-recovery. At the end of procedures animals will be killed either while under anaesthesia or by Schedule 1 methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	**Objective 1** For electrophysiological recordings, in order to investigate the mechanism(s) of opioid receptor desensitization in brain regions relevant to the rewarding properties of opioids it is essential to study the process in relevant brain regions of the mammalian brain. **Objective 2**

	To study synaptic plasticity processes relevant to drug-seeking behaviour it is necessary to record from intact neuronal networks.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Usually, brain slices will be prepared from animals killed by decapitation while under general anaesthesia, reducing animal use by optimising slice health. Generally one animal per day is used permitting ongoing data analysis, sample sizes can be determined in an ongoing fashion to minimise the number of animals used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	With a colleague at the University of Bath, we are exploring whether *in vitro* brain slice cultures can be developed to answer some of the key scientific questions to be answered in this Project, without the need for procedures on whole animals.

<table>
<tr><td>Project 15</td><td>Opioid receptors in depression and anxiety</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Depression, anxiety, opioid, behaviour, stress</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>
<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>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 treatments for depression, for example the selective serotonin-reuptake inhibitors (SSRIs e.g. Prozac), are limited in that they have a slow onset of therapeutic effect, are associated with side effects and may have significant withdrawal syndromes. Furthermore, fewer than 50% of all patients with depression show full remission indicating that there is an unmet clinical need. Major depressive disorder in adolescence tends to recur, is a major risk factor for completed suicide and is comorbid with anxiety disorders, eating disorders and substance abuse. 40% of adolescents with major depression remain symptomatic on first-line treatment (SSRI fluoxetine), and inadequate treatment of depression increases the risk of suicide. The role of the opioid</td></tr>
</table>

	neuropeptide system in controlling pain, reward and addiction is well established, however, its role in regulating mood disorders is an area of growing research. Targeting opioid receptors has the potential to provide a new generation of antidepressant drugs. In this project we will (1) examine the role of opioid-receptor signalling in the regulation of mood (2) test novel ligands which act on opioid receptors for their anxiolytic and antidepressant potential.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This work is expected to provide novel information on the role of opioid receptors in depression and anxiety-related behaviours. It will allow the contribution of different opioid receptor types to the regulation of mood to be more clearly delineated. The results will also help the development of novel opioid receptor ligands with potential as antidepressant and anxiolytic drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate that we will use 800 mice per year, 4000 over 5 years. A smaller number of rats will be used for diuretic studies, estimate 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?	Most of the procedures in this project are mild. Moderate steps in the protocol relate to surgical procedures for implantation of depot devices for drug delivery, the repeated stress protocol to develop a model of juvenile depression and repeated blood sampling from an individual animal. Identifying the role of opioid receptor signalling in depression/anxiety-related behaviours uses non-invasive behavioural tasks with little risk of adverse effects. Severity of stress-induced models of depression-related behaviour in mice will be limited by monitoring home-cage behaviours. Severity of repeated blood sampling is limited by the maximum volume that can be sampled and refined technique. Testing novel compounds in vivo for the first time can cause toxicity and death. Severity is limited by using a minimal numbers step-wise approach. At the end of the protocol all animals will be killed.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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 contribution of specific opioid receptors could be achieved using human brain imaging studies, however there is a lack of suitable ligands. It is not possible to trial novel opioid receptor ligands directly in humans without establishing potential therapeutic effectiveness in an in vivo model. In vitro pharmacological studies with novel compounds, using isolated tissues and cultured cells, will inform in vivo studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In this programme of work we will use a multidisciplinary approach to maximize the data obtained from each animal i.e. we will identify functional (electrophysiological analysis of brain slices, hormonal measurements), molecular (ex vivo tissues for gene analysis, morphological studies) and behavioural effects of opioid receptor signalling in the same animal. A power calculation, based on prior experience with the behavioural measures, will be conducted 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.	Rats and mice are the lowest vertebrate animals in which well characterised, pharmacologically validated models of depression- and anxiety-related behaviours have been established. A wealth of literature on the anatomical circuits, neurophysiology and pharmacology of anxiety- and depression-related behaviours in mice and rats exists. These rodents also express all of the opioid receptors of interest. The majority of the procedures in protocol 19b1 are mild. The behavioural tests are non-invasive and exploit normal rodent behaviours e.g. exploration, escape from mildly stressful test environments. The preparation of brain slices for electrophysiology is conducted under terminal anaesthesia and can therefore be considered mild. Moderate steps in the protocol relate to (1) surgical

	procedures for implantation of osmotic minipumps: the 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, is well described in rats. In pilot studies we will adapt this paradigm for mice using the minimal effective stimuli to obtain a depression-related phenotype. Welfare will be monitored using a modified general distress scoring sheet (after Lloyd & Wolfensohn, 1998) to identify humane endpoints. (3) Repeated blood sampling: in juvenile mice this may be moderate severity as their blood volume is estimated to be very small (< 1ml). Experiments are designed such that an individual animal may have blood samples taken at 3 time points but not all animals will be sampled at the same time points. The blood volume collected is limited to no more than 10% on each occasion and no more than 25% in any 28 day period. Repeated sampling from the same mouse minimizes the number of animals used overall.

<table>
<tr><td>Project 16</td><td>Neurobiology of hearing and deafness</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Brain, auditory, plasticity, deafness, tinnitus</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>
<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>We aim to improve our understanding of the processes that take place in the brain when we listen to speech and other sounds. In particular, we wish to find out how nerve cells in the hearing centres of the brain represent these sounds in a manner that is robust to changes in the soundscape – as we move, for example, from a noisy bar to an empty street. We also wish to find out how these representations are altered when auditory perception improves as a result of learning or when inputs to the brain are reduced due to impairments in the function of the ear. A final objective is to identify the changes in the brain that give rise to tinnitus – persistent ringing in the ears – and whether we can reverse those changes as a potential cure for this debilitating condition.</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>Improving our understanding of the neural basis for auditory perception is in the first instance a matter of considerable, fundamental scientific interest. In the longer term, this may also open up a variety of</td></tr>
</table>

animals could benefit from the project)?	practical applications, from improved diagnosis and treatment of patients with hearing deficits to the development of better artificial sound processing systems. Hearing loss is the commonest sensory disability, which will undoubtedly become more prevalent as we live longer. If we can understand the factors that enable the brain to compensate for a partial hearing loss and what triggers tinnitus, we should be able to improve the treatment and rehabilitation of people with these conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year period of the project, we will expect to use 2300 mice, 140 rats and 395 ferrets in procedures other than simple breeding and maintenance. We may breed and/or maintain up to 2000 mice, some of which will be the same ones as in the additional procedures.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Part of the project will involve the raising of genetically altered mice to allow us to investigate the functions of particular molecules in auditory processing. These animals are expected to be no different in the way they behave from wild-type controls. The behavioural testing procedures we will use to measure the animals' hearing abilities are painless. In some cases, it will be necessary to motivate the animals to perform these tasks by rationing their food or water during testing. This may result in a temporary weight loss, but this will always be monitored carefully and extra food or water provided if this occurs. The availability of modern techniques for monitoring or altering neural activity in particular regions of the brain make it possible to carry out almost all of this work in a manner that should cause only temporary pain or discomfort to the animals in the study. For example, surgical operations for implantation of ultrafine microelectrodes or for inserting genes into the brain will be carried out under general anaesthesia, in aseptic conditions, and with appropriate post-operative care. The adverse effects that may occur following surgery include transient pain and bleeding, but their incidence is likely to be low and methods of control (e.g. analgesia) and the most

	refined experimental techniques will always be used to mitigate them. Chronic implants for recording neural activity or for delivering flashes of light for the purpose of altering that activity are small and light-weight, and do not materially affect the animal's quality of life. Animals will be killed humanely 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	Because this project investigates the neural basis for auditory perception and how this is affected by hearing loss, it can only be carried out using *in vivo* approaches. Brain imaging measures in humans lack the sensitivity to observe changes in the response properties of nerve cells caused by hearing loss or training or alterations in the acoustic environment. Moreover, a key aim of this project is to try and account for changes in auditory perception at a microscopic level in terms of the underlying neural circuitry. This requires the use of post-mortem histological measurements, which would not be ethical or practical to carry out in humans. Using animals to measure the effects of precisely controlled forms of hearing loss also avoids the inevitable variations that would be found among a clinical population. Finally, computer modelling does form an important component of our work, but this relies on the information provided by the animal studies and cannot replace them.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Calculations are carried out to determine the necessary number of animals for each experiment, ensuring significance of our results but also minimising the number of animals used. We are additionally able to keep animal numbers to a minimum by using cutting edge methods that yield large amounts of data and experimental designs that allow multiple measurements to be made from each animal.
3. Refinement Explain the choice of species and why the animal model(s)	Mice and rats will be used because they are the lowest vertebrates with an auditory system that is comparable to that in humans. Rodents are unsuitable, however, for some of this work because

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.	they are unable to hear the low tones that enable humans to understand speech, hear melodies or localise certain sounds. In contrast, ferrets have an auditory frequency sensitivity that overlaps with that of humans. They can also be readily trained in behavioural tasks that will capture how well they can detect, distinguish between or localise different sounds. Being larger and stronger than rodents, ferrets can carry implants with ease which would be uncomfortably large on a rat or a mouse. Animal welfare costs will be minimised by carrying our procedures in state-of-the-art facilities and using best practice methods. Operations are carried out very carefully under anaesthesia and the animals are given painkillers and closely monitored until they have fully recovered. The earliest endpoints consistent with the scientific aims are applied. The data obtained from these experiments will be used to refine computer models of the brain that will help to guide subsequent experiments and contribute to a reduction in the number of animals needed.

<table>
<tr><td>Project 17</td><td colspan="3">Development of epilepsy in mammals</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Seizures, drug-resistance, epileptogenesis</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 section 5C(3)</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>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 colspan="3">To understand how epilepsy becomes established in the brain and how this might be prevented. To understand why some people do not respond to any of the antiepileptic drugs through development of a model of drug-resistant epilepsy. To investigate non-invasive surgery for epilepsy using gamma-ray ‘knife’ technology.</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">Identification of key drug targets underlying establishment of epilepsy and assessment of candidate drugs. Identification of key targets mediating drug-resistance in man and animal models. Proof of concept for non-invasive surgical intervention in epilepsy.</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">Rats, about 400.</td></tr>
<tr><td>In the context of what you propose to do to the animals,</td><td colspan="3">Rats will undergo a moderate severity protocol that establishes chronic epilepsy in the temporal lobe.</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?	They are likely to lose a small amount of weight for a short period (48 hours), and they may rarely suffer very severe or difficult to control seizures. At the end of the protocol the animals will be killed so that brain tissue can be analysed using electrophysiology.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Epilepsy is complex, involving multiple brain regions and resulting in complex behavioural changes. This requires a complex brain in which to model the disease and associated behaviour and rat brain is about as low a species as can be considered whilst maintaining enough complexity to be of realistic use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the minimum consistent with our statistical calculations, and maximise the use of each animal through procedures designed to keep brain tissue alive for as long as possible, including tissue culture.
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 model being used was developed with refinement as the primary goal, through NC3Rs funding. The model minimises harms through reduction of time spent having epileptic seizures, and better drug-control of seizures to prevent spread of uncontrollable epilepsy to the brainstem (causing death). The model does not cause gross damage to the brain, unlike many models, and does not cause ocular keratitis or similar issues. Animals undergoing the protocol are kept hydrated, fed sweetcorn and chocolate and mashed rat chow, and usually recover within a few hours and certainly overnight.

Project 18	**A murine model of nystagmus to study potential treatments**
Key Words (max. 5 words)	Mice Nystagmus Eye Brain Treatments
Expected duration of the project (yrs)	5 years
Purpose of the project (as in section 5C(3)	Basic research: Yes Translational and applied research: Yes 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: Yes
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To investigate the cause and possible treatments for a condition called Nystagmus which causes a rapid, uncontrolled, to-and-fro movement of the eye and is associated with poor vision in children and adults. Currently, we do not know how nystagmus is caused for the majority of human patients. For children born with nystagmus and for most adults who acquire it, there is no treatment and in many cases only a descriptive diagnosis. We have access, for the first time, to a mouse strain which has had a gene (Frmd7) knocked out which is known to cause nystagmus in humans. We hope to use this mouse and others to find out what the underlying cause of the nystagmus is, what other genes and pathways are involved, what other genes we should be screening in the 95% of humans without a genetic diagnosis and to develop the mouse into a model so that we can test some suggested treatments which are not used in human

	practice yet due to lack of supporting evidence. We will then create sufficient data and scientific support for us to move to human clinical treatment 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)?	Patients will benefit by the identification of the underlying cause of their disease, the identification of potential treatment targets for future development, the identification of new diagnostic targets and by evaluation of a set of possible treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	We would estimate a total of approximately 600 animals for the project. Exact numbers will depend on the results of breeding and the experimental data. Mice have been chosen as the knockout mouse is the only model of human isolated nystagmus and they are amenable to eye examination in life. The minimum number of animals will be calculated for each part of the project based on power calculations and statistical estimates. Where possible tissue will be used for additional experiments and methods will be used where multiple experiments can be run on a single sample or tissue.
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 record eye movements in some mice it will be necessary to attach a small fixing plate to the head by an adhesive using a surgical technique. This will be done under general anaesthetic with appropriate subsequent pain-killers and a long recovery period. Subsequent eye movement tests will be done by minimal animal contact and are limited in time and frequency. Some animals experience discomfort but in most cases only in the period immediately after surgery. Additional pain-killers will be used as necessary. Eye-drops, contact lenses and eye muscle surgery will be used in very similar techniques to those employed in humans with the same use of local anaesthetic drops, systemic pain killers and general anaesthetic where appropriate. Further examination techniques may include restraint for short periods and pre-injection of

	visualising substances such as Fluorescein as is used in humans. Minimal discomfort is expected from these procedures which will be limited in number and frequency. Close attention will be made to the health and behaviour of the animals throughout all experiments and when at rest. Appropriate action will be taken where an animal is thought to be distressed at any stage.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Humans with this condition have had much study by us and others. Unfortunately, the underlying anatomical and physiological cause has been shown to be to be within the brain or developing visual pathway. Clearly, it is impractical and unethical to take samples from the brain of human children or adults. A strain of mice with the Frmd7 gene 'knocked out' is a unique chance to study a large population of animals with the same genetic cause for their nystagmus. To answer the same wide question, human studies are being conducted in parallel in order to identify the genetic cause of nystagmus in more people in addition to being able to study the eye movement anomaly in greater detail. When enough data has been produced by animal work human treatment trials will take over and animal work will be ceased.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals will be used for each experimental protocol. For elements of the proposal we aim to utilise a particular tissue culture techniques called organotypic culture. This culture system will allow us to reduce the number of animals by performing multiple experiments from one preparation.
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	We will reduce suffering by minimising the number and frequency of procedures, minimising the stress and suffering to mice during procedures and reducing the number of animals used. For example, for eye examination, animals will need to be restrained. We have established protocols for

measures you will take to minimise welfare costs (harms) to the animals.	causing minimal stress and minimising the time of restraint. Strict rules will be applied to the number of procedures or examinations per week and per animal and for the duration of each technique. All procedures will be completed with close attention to animal stress signs and general anaesthetics, local anaesthetics and systemic pain-killers will be used as they are when these procedures are carried out in human children. Suffering will also be reduced by accurate statistical calculations allowing us to use the minimum number of animals possible per protocol and by utilising culture techniques which allow multiple experiments per prep thereby reducing animal numbers further per protocol. Additionally, part of the work is to try to develop novel, less invasive methods of eye movement analysis, which we hope will reduce the need for surgical procedures for head stabilisation in our work and future work of others.

<table>
<tr><td>Project 19</td><td>Tolerance and addiction to drugs of abuse</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Tolerance, addiction, reward, learning, memory</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>
<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>OVERALL AIM: To investigate mechanisms underlying tolerance and addiction to drugs of abuse.

Objective 1: To investigate the cellular mechanism(s) underlying opioid receptor desensitization in brain regions involved in the rewarding properties of drugs of abuse, a process thought to underlie tolerance to the rewarding properties of opioid drugs.

Objective 2: To investigate the molecular mechanisms underlying drug-related memory.

Objective 3: To investigate the abilities of novel opioid ligands to affect addiction-related behaviour.

Objective 4: To develop a transdermal patch for drug delivery of anti-addiction agents.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>The work is expected to provide insight into the mechanisms underlying drug addiction, and suggest and test novel targets for the treatment of</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	drug addiction. There is a clear need for therapeutic agents that will prevent relapse. Addicts to all types of drugs of abuse (opioids, cocaine, alcohol and nicotine) experience approx. 70-80% of rates of relapse within a year of quitting. Identifying novel strategies in this area would represent a major advance in the treatment of drug addicts. Three overall approaches will be taken to: A: understanding the mechanisms of tolerance to opioids; agents that enhance opioid tolerance may be useful in drug rehabilitation programs to prevent relapse. Further, detailed information on the time-course of the decline of tolerance to the euphoric effects will reveal information leading to the risk, and potential treatment, or opioid overdose. B: understanding the role of learning and memory in drug addiction. Agents affecting learning and memory have been suggested as novel treatments for drug addiction but a specific, selective drug target needs to still be determined and tested. C: Understanding the role of opioid receptors in addiction to all drugs of abuse. These studies are expected to identify novel opioid ligands and/or novel methods of administration that may prove to be useful in treating drug addicts.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice: 5230 Rats: 1100
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?	An estimated 2400 animals will be wild-type or transgenic animals (of a mild phenotype) that will either be used solely for maintenance of a breeding colony or will only undergo non-recovery (terminal anaesthesia) procedures. The predominant behavioural model to be used (conditioned place preference), is non-surgical and relies on the animal learning a positive associative memory between a rewarding/euphoric stimulus (eg. drug of abuse, sex, palatable food) and a

	novel, non-stressful environment, then volitionally spending more time in the environment with positive associations. The only individual procedures that are of moderate severity are surgical implantation of slow-release devices and single acute stressful stimuli to model stress-induced reinstatement to drug-seeking behaviour. An estimated 1050 animals in total will undergo one of these procedures once only. The remainder of animals will undergo repeated systemic injections which, depending on the specific cumulative amount of injections administered, will mean that the animal will experience overall levels of mild or moderate harm. At the end of procedures animals will be killed either while under anaesthesia or by Schedule 1 methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	**Objective 1** For electrophysiological recordings, to investigate the mechanism(s) of opioid receptor desensitization in brain regions relevant to the rewarding properties of opioids it is essential to study the process in relevant brain regions of the mammalian brain. To study behavioural measures of reward tolerance, there is no alternative to using a whole animal. **Objective 2** The overall ethos of the experiments within objective 2 is to directly combine *in vitro*, *in vivo* and *ex vivo* experiments, often deriving more than one data-set from the same cohort of animals (see reduction below). To study addiction-related behaviour or drug-seeking behaviour there is no alternative to using a whole animal. **Objective 3** To demonstrate that novel ligands are systemically active, and have effects on addiction-related behaviours, there is no alternative to using a whole

	animal. Before this stage, however, extensive non-animal or Schedule 1-only experiments will have been performed to fully characterize the pharmacology of the novel ligands (*in silico* measurements of bioavailability, affinity and efficacy measurements in cultured cells, *in vitro* isolated tissue experiments). **Objective 4** To demonstrate that the compounds can be successfully delivered transdermally, and that they are functionally active in affecting addiction-related behaviours, there is no alternative to using a whole animal. Extensive experimentation has already taken place for the project to reach this stage that did not involve animals or used Schedule 1-derived or abbatoir-derived animal tissue: *in silico* modelling, *in vitro* rat and pig skin experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In Objectives 1 & 2, wherever possible, animals that had undergone chronic opioid treatment or behavioural training will derive 2 data-sets: a behavioural measure of tolerance or addiction, and, electrophysiological or biochemical measurements of receptor desensitization or synaptic plasticity. In Objectives 3 and 4, for initial toxicology studies, or for initial assay optimisation, or for initial transdermal patch tolerability studies a minimal numbers approach will be taken.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	In order to model reward or addiction-related behaviour with translational validity to human reward or addiction, experiments need to be performed in mammals (mice or rats). Conditioned place preference, drug-discrimination and voluntary ethanol drinking procedures are non-invasive methods of assessing the rewarding properties of drugs of abuse and other rewarding stimuli, and cause no pain, suffering or lasting harm. Where stressful stimuli are used they will only be applied once to an individual animal and will result in no lasting harm. Warm water tail withdrawal is a behavioural assay that yields robust data regarding opioid receptor

	activation. Temperature-limits and time-limits are in place in the protocol to ensure no lasting harm. With a colleague, we are exploring whether *in vitro* brain slice cultures can be developed to answer some of the key scientific questions in this Project, without the need for procedures on whole animals.

<table>
<tr><td>Project 20</td><td>Understanding neural control of urinary bladder</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Bladder, urination, incontinence</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></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>
</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 aims of the work are:

a) to understand the mechanisms underlying normal control of bladder function

b) to understand how the system malfunctions in pathological states

c) to identify factors such as diet or lifestyle that may lead to malfunction of bladder control mechanisms

d) to identify new therapeutic approaches to manipulate the functional activity of the bladder control system, which could be developed for the treatment of incontinence in humans.</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>Urinary incontinence (UI) is a enormous clinical problem worldwide which impacts significantly on quality of life and has significant socio-economic consequences in terms of costs to health services, costs to individuals (both financially and in terms of lifestyle), worktime lost etc. One of the difficulties</td></tr>
</table>

project)?	encountered in developing new and effective treatments is that the mechanisms underlying the way in which the brain controls bladder function are not understood. This project will investigate brain control of bladder function in an animal model. The work will provide basic information on brain control of bladder function, which can then be utlised in the development of more effective treatment strategies for incontinence in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice – numbers used will depend on the availability of funding streams for the project. It is anticipated that up to 300 mice and 200 rats will be used over the 5 year period of the licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All animals will be humanely killed at the end of the experiment. Many of the experiments can be carried out in unconscious animals under general anaesthesia. Many experiments to measure urinary voiding in conscious animals do not require any surgical intervention. However, some animals will be required to undergo surgery under general anaesthetic to implant electrodes or inject substances into parts of the brain that control bladder function. Small pressure sensing devices will also be implanted into the bladder to measure bladder activity during urinary voiding in the conscious animal after recovery from the surgery. In the post–operative period animals may be expected to experience some transient discomfort. However, this will be minimised by the use of post operative pain killers and careful post operative care. Some animals may develop abnormal urinary voiding patterns e.g. frequent urination, which is not overtly aversive. Alternatively urinary retention (inability to urinate) might develop, which could become painful. If this is detected and does not resolve, the animals would be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	Urination is a complex event, which requires co-ordination of the bladder, its nerves and the brain. There is no non-animal alternative that can be used

use non-animal alternatives	to study this process.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments have been carefully designed to obtain the maximum amount of information from each animal. We will initially carry out pilot experiments using small numbers of animals to obtain preliminary data; then we will use power calculations (a statistical analytical tool) to predict the minimum number of animals that will be needed to give statistically valid 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.	Rats and mice will be used for this study because show sufficient similarities in their control of bladder function to act as a model for humans. Lower animals e.g. reptiles, birds or invertebrates do not void urine in the same way as humans. The study has been designed so that a large proportion of experiments can be carried out using non-invasive, non-painful procedures in conscious animals or can be carried out under general anaesthesia. The remaining animals that have undergone procedures will be closely monitored for signs of distress, remedial action such as administration of pain killers will be taken and if this does not alleviate discomfort, the animals will be killed.

<table>
<tr><td>Project 21</td><td>Genetics of Rhythms, Sleep and Behaviour</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Behaviour, circadian, sleep, cognition, mouse</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></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>The primary experimental work in this project will be to study genes that affect specific behaviours in mice. The behaviours to be studied include measures of cycles of rest/activity, sleep and learning/cognition. Mice carrying alterations in their genes will be screened for deficits in these behaviours. Additional matings will be established to identify the abnormal genes responsible for the behaviours. Finally, mice carrying these defective genes will be further characterised using series of behavioural tests followed by extensive molecular investigations.</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>Using these tests, we aim to identify new genes that underlie or contribute to neurological and psychiatric diseases in humans. This could increase our understanding of human diseases and identify new routes for disease intervention.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The mouse is the only species to be used in our studies. Over the course of 5 years, we plan to breed approximately 40,000 mice. However, the majority of these numbers will be used to generate the correct combinations of different gene alterations for further study. Less than 20% of these will be used in further experimental procedures.</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</td><td>The majority of work to be carried out in this project will be on mouse mutants with subtle behavioural abnormalities. In cases where abnormalities are more evident, we will strive to modify the testing regime to minimise welfare issues. The majority of</td></tr>
</table>

happen to the animals at the end?	procedures used in this study are non-invasive and consist of moving animals from one apparatus (chamber) to another or on animal observation. Some of tests involve mild, temporary and reversible periods of discomfort such as exposure to bright light, constant darkness, restricted access to food. We keep these periods to an absolute minimum and remove any animals from our study that show signs of discomfort. In a small number of cases we use surgical techniques to implant telemetric recording devices and record brain activity over a number of days or weeks. The procedure is not expected to result in prolonged pain or discomfort but we will closely monitor animals and, where any is evident, we will remove animals from the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Whole animal work is necessary for this study as there is a requirement to follow the effects of DNA mutations on whole animal behaviour over time. Aspects of this work can be followed in cell culture alone but, in order to study the effects of gene mutations on behaviour, whole animals must be used.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For breeding purposes, we pay close attention to the number of animals required for each of our studies and plan the appropriate number of matings in advance. To employ this, we use conventional statistical methodology. We also hold regular meetings with animal facility staff to maintain efficient colony management. In experimental testing we ensure that appropriate numbers are being used using statistical calculations and by comparing planned experiments with studies carried out previously. Wherever possible, we use the same animals in multiple tests with the assurance that there are no additive adverse effects in animal cohorts.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is the lowest mammalian species that we can use to examine the full complement of parameters that are measurable in behavioural and physiological changes associated with sleep and cognition. Many of the protocols being used in the study have been developed or modified by us with the distinct priority being minimising welfare costs to animals. For example, in previous projects we have been able to detect early changes associated with neurodegenerative disease in mice before major deterioration in welfare is evident. We plan to

	continue with these refinements over the coming years by, for example, monitoring as many animal behaviours in the home cage as possible (social interactions, activity, sleep etc.) using automated tracking and software technologies.

<table>
<tr><td>Project 22</td><td colspan="3">Neuronal Networks for Social Behaviour</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Social disabilities, psychiatric disorders, neuronal circuits</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>Yes</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">The purpose of this project is to use different genetic mouse models for social disability to characterise the biological underpinnings of specific social skills, and their breakdown in pathological conditions.
Specifically my project will be developed along three aims: 1) identify general pharmacological strategies for specific social disability, 2) identify neuronal networks associated with specific social skills, discrimination and 3) define the neuronal network that underpins social skills.
This will lead to both increased biological understanding of social disabilities and the development and precise characterisation of model systems for the testing of novel therapeutic approaches.</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 colspan="3">Our work will help to better understand social behaviour in mice, from molecular mechanisms to neuronal circuits. This will advance our knowledge on the pathophysiology of mouse models for</td></tr>
</table>

animals could benefit from the project)?	psychiatric disorders and, we anticipate, will lead to the development of novel approaches for the treatment of these disabling conditions in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Species: Mice Numbers: 2500 mice Time period: 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 we will use are not anticipated to produce serious adverse effects in the animals studied. We will use behavioural methods and molecular studies on post-mortem tissues. If any animal shows undue distress during the study, we will consult with The NACWOs and the NVS and where appropriate humanely euthanize the animal.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is necessary to use animal models to study the molecular and cellular mechanisms underlying social behaviour, and their breakdown in pathological condition, because: (i) we cannot directly access the relevant tissue in patients (ie the brain) AND (ii) cellular models and more basic systems cannot fully recapitulate the complexity and function of brain circuits.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use appropriate statistical and experimental approaches to optimise the number of animals used in each study. We will only generate animals when needed and will avoid excess breeding. The experience of the investigators in the techniques used in this licence, and the advices of collaborators in Cardiff University, will also help minimise the number of animals required.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	Mice are chosen as they provide a combination of genetic flexibility, a sufficient and established behavioural repertoire and suitability for physiological/molecular investigation. No severe severity protocols or procedures will be used in this licence. Data will be collected in vitro and in vivo on small cohort of animals to limit consequences to due

(harms) to the animals.	toxicity of compounds. To minimize consequences of aggressive behaviour on the animal welfare, mice will be chosen at an age of low aggressive behaviour. For every behavioural test a rapid escape will be provided at any time if necessary. Animal welfare will be maximised by close liaison with animal support and veterinary staff. In addition the experience of the investigators in these approaches will minimise harms.

<table>
<tr><td>Project 23</td><td colspan="3">Neural coding and perception of sound</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">hearing, neural coding, perception, auditory processing</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">The main objective of this project to understand how processing by neurons in the brain leads to the conscious perception of sound, and how this impacts on hearing impairment. We are interested in how we hear and process sounds in complex listening situations (e.g. in a crowd of people). We know a lot about the responses of the brain and our perception of simple sounds, but much less about how the brain processes complex ‘auditory scenes’. Whilst we normally measure hearing loss in terms of simple sounds, the main handicap in people is that of communicating in complex social environments. Thus an important component of this work is how processing of complex scenes changes with hearing loss.</td></tr>
<tr><td>What are the potential benefits likely to derive from this</td><td colspan="3">The primary outcome from this work is fundamental scientific knowledge about hearing. This work</td></tr>
</table>

project (how science could be advanced or humans or animals could benefit from the project)?	impacts not only on hearing but also more general theories of how the brain work. However, understanding how we hear is important if we hope to effectively treat hearing disorders. Nine million people in the UK alone are hearing impaired or deaf. Current treatments for hearing impairment help people hear sound. However, in noisy circumstances people with hearing aids or cochlear implants often struggle to understand speech. Thus, a fundamental challenge is how to improve communication for the hearing impaired in complex situations, which is central to our main objectives.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use around eight ferrets each year. It is possible to use such small numbers because of the rich nature of the data, each animal yields many observations, and results are generally consistent across individuals.
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 are trained to perform 'listening' tasks, and we measure their ability to hear and identify sounds, for which they get water rewards (overall consumption is as close as possible to normal). This training and testing does not cause any pain (the sounds are not very loud). Many recordings from neurons are made under non-recovery anaesthesia, and depth of anaesthesia is monitored carefully so they remain unconscious and cannot feel pain. Some animals will undergo surgery under general anaesthesia to implant recording electrodes (similar electrodes have been implanted in humans for medical reasons), so we can measure the responses of neurons to sound when animals are awake and actively listening. This is important, as the way the brain processes sound depends on whether we are listening or not. The implants themselves are not painful, but animals will feel some pain after surgery which is reduced by treating them with pain killers. Some animals will be exposed to loud-sounds whilst deeply anaesthetised in order to give them some moderate hearing-loss (they will not be profoundly deaf) in order to study how their ability to understand and identify sounds, and how the brain processes

	sounds, is affected by hearing impairment. The maximum level of severity expected is moderate, but this would only be for short periods. The highest standards of animal welfare will be maintained throughout these experiments as minimizing pain and distress is required not only for ethical reasons but also to ensure that the data obtained is consistent and reliable. The general habits, behaviours and state of health of animals undergoing testing are difficult to distinguish from untested animals. At the end of the experiments all of the animals will be humanely culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where an alternative exists we use this in preference to animal. In some instances we are able to use computer models to simulate the behaviour of neurons. However, in most instances these models are not currently accurate enough. When computer models fail, the responses of neurons to sound can only be adequately studied in an intact functioning brain connected to intact ears. Tissue culture cannot reproduce the same information flow or input from the ears, and brain imaging in humans can only reveal gross patterns of activity that does not tell you with which neurons are firing or when with sufficient detail to understand how they function.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where an alternative exists we use this in preference to animals and we strive to improve our methods in animals in order to decrease the number required. Whenever possible, experiments are conducted in humans using imaging and behavioural techniques instead. Computer simulations of brain activity form an intrinsic part of the scientific process, of the analysis, and how we form clear hypotheses and formulate experimental designs. Over time, advances in experimental technology, improving computer models of how the ear or brain process sound, and improvements in neuroimaging are reducing the number of animals

	required for these 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.	The species we use has good low frequency hearing (similar to humans) and is well suited to testing how they perceive sound. Not all animals are readily trainable to respond to sound. Some species (e.g. rats, mice) also have poorer low frequency hearing – essential for relating to human hearing. The physiology of amphibians, fish and even birds are much less readily related to humans. All involved in this program of research place great emphasis on the welfare of the animals. We carefully monitor the health of our animals. They live in a state of environmental enrichment, of social interaction, exercise and stimulation. The procedures themselves are developed specifically to minimise suffer pain, distress or discomfort. This ensures both high standards of welfare and valid experimental results that can contribute to the understanding of auditory perception and improve treatment of hearing problems in human patients.

<table>
<tr><td>Project 24</td><td>Maintenance of genetically altered zebrafish lines</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fish, neurodegeneration, disease, ageing, animal model</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>3 Months</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>The long-term goal of the project is to develop fish models of human neurological diseases such as motor neuron disease. The specific purpose of this interim project is to maintain established lines of genetically altered zebrafish pending grant of further project licence authority and continuation of the full programme of work.</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>We hope that the fish model would complement rodent models and thus provide a more rapid and high 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.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>We plan to use Danio rerio (zebrafish) and to test the disease models and plan to use approximately 1,000 zebrafish in our studies during the 3 month interim period. Many of the animals generated for</td></tr>
</table>

over what period of time?	the study will be utilized prior to the age when they are protected (<5.25dpf) or just past protection (6-10 dpf) and a smaller fraction would be used for older age 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?	As we are primarily studying ageing related diseases, many of the phenotype we observe would mimic ageing and thus of marginal consequence to the health and welfare of the animals. As these animals are living sedentary lifes (as compared to living in turbulent river flows), the impact of their disability on their living conditions are minimised. Thus most of the animals in these studies will have no visible symptoms with a fraction of older animals showing moderate symptoms with minimal impact on their ability to obtain feed and move around. When studies are completed on the animals, they are humanely sacrificed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Fish are lower vertebrates and thus similar to mice and humans. Thus, they are reasonable replacement for studies on mice and we have shown that fish can mimic many aspects of human neurological disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our goal in using fish in addition to studying disease process is to uncover early disease specific changes that occur prior to the onset of symptoms and hence, we try to identify early disease biomarkers that can be analysed before they become protected (5.25dpf). This will allow great reduction in use of animals in research. Additionally, we power our studies to utilize the minimal number of animals to obtain robust statistics on the data collected and thus reduce the number of animals used and the number of times the studies need to be repeated.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	As mentioned earlier, our goal is to identify early disease biomarkers for disease. When such markers are identified, we can utilize these markers in our drug screening and therapeutic development program to refine the screening to utilize earlier

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	readouts of disease to test efficacy of novel therapies. These allow refinement of our protocols to reduce the use of animal in research.

Project 25	**Neuronal circuits of cortical plasticity**	
Key Words (max. 5 words)	Mouse, visual, imaging, two-photon, spine	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 perceptions, thoughts and actions depend on the coordinated activity of billions of neurons in our brain. These electrically excitable cells are wired up into networks and communicate with each other through synapses. These networks are established during the development of the brain but some of their properties can be modified throughout our lives, Indeed, our ability to learn relies on the potential of these neuronal circuits to change through experience. These changes are mediated by the loss, the formation or the regulation of the communication pathways between neurons, the synapses. Revealing the nature of these modifications is essential to understand how our brain adapts to new circumstances and allows us to learn from our experiences. The objective of this proposal is to understand how neuronal circuits involved in visual perception are modified by visual experience. The brain areas receiving visual information from our eyes have	

	become a popular system for studying how neuronal circuits encode sensory information and how they can be modified by experience. Due to recent ground-breaking developments in imaging and genetic tools, it is now possible to use optical methods to image the activity of individual synapses in the living brain, In the context of this proposal, I will use an imaging approach to determine how visual experience modifies the activity of synapses between specific classes of neurons. I will distinguish the activity of so-called excitatory neurons from that of inhibitory neurons, which are respectively responsible for enhancing or decreasing brain activity. Finally, J will apply this knowledge to find which neuronal types and which synaptic functions are disrupted in neuronal circuits of mouse models of autistic spectrum disorders and in particular, mouse models of Fragile X, the most widespread single-gene cause of autism. Individuals with autistic spectrum disorders experience hypersensitivity to sensory stimuli and perceptual deficits including well-studied visual deficits in face recognition and motion perception. Several studies suggest that abnormal primary visual networks would impair higher-order sensory processing and, consequently, contribute to social and communicative deficits in autism. I will test whether and how the activity of specific classes of interneurons is disrupted in a mouse model of Fragile X. This is essential to link recent findings of the genetic basis of these disorders to their behavioural and cognitive symptoms, with the ultimate goal of identifying potential targets for future 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)?	The results of this proposal will reveal key mechanisms underlying synaptic plasticity in the adult visual cortex and will increase our general knowledge about how the brain stores information and adapts to new environments. In addition, these results have direct impact into research about pathologies affecting the visual system originating both in adulthood and during development. In adults, pathologies affecting the primary visual cortex include strokes or brain trauma. Knowing how synaptic connections are strengthened by experience will bring about new potential strategies to augment plasticity and promote recovery of function after brain injury.

	During development, it is known that inhibition plays a key role in the refinement of cortical circuitry in response to sensory experience, If plasticity does not happen in a proper fashion during developmental critical periods, lifelong loss or impairment of cortical function can occur. A well-known example is the development of amblyopia. If not treated early, there is a loss of cortical binocularity and a significant reduction in visual acuity that cannot be improved by corrective lenses. Being able to enhance plasticity in the visual cortex could for example increase the impact of perceptual learning for the recovery of visual function in amblyopics. In addition to pathologies of the visual system, the deregulation of cortical plasticity during development underlies many brain disorders, such as intellectual disability and autism spectrum disorders, In this proposal, I will study impairments of synaptic plasticity in mouse models of these diseases. Increasing evidence indicate that inhibition is altered in autism spectrum disorders. A better understanding of the role of inhibition in synaptic plasticity will contribute to the search for treatments and is likely to benefit public health in the long term. During the period of this project, staff involved in the project will acquire valuable skills. They shall learn how to work independently on long-term projects in an international team while being exposed to state-of-the- art imaging techniques. The skills they learn will be valuable in several employment sectors including the pharmaceutical industry and companies specialized in biotechnology, microscopy, optics, and laser devices. Many members of the public are fascinated by neuroscience and want to know more about how the brain works. The public will benefit from this research through dissemination during open days or public events. Two-photon imaging produces beautiful images of neurons that can be used for exhibitions to increase public awareness of neuroscience and medical research. Families affected by neurodevelopmental disorders will benefit, in particular from the knowledge that work is being done to better understand and ultimately alleviate these conditions.
What species and approximate numbers of animals do you expect to use	Mouse, I will use approximately 5 animals per week, 50 weeks per year for a total of 1250 mice over 5 years

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?	Some mice will be used for breeding only. The highest severity will be moderate. The experiments proposed in this study require surgery, for stereotaxic delivery of viruses to specific brain regions and for the placement of a recording chamber on the skull of the animal. For these experiments, pain will be controlled during surgery by general anaesthesia and post-surgery by analgesics. Deaths resulting from anaesthesia or surgical complications are uncommon (<1%) and will be minimised by correct dosing of anaesthetics, by accurate weighing and by maintenance of body temperature during and post-surgery e.g. use of heat pads. Risk of infection will be minimised by good surgical and aseptic techniques. At the end of each protocol, animals will be killed by using approved humane methods and tissues from these animals may be analysed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The experiments in this proposal are designed to improve our understanding of synaptic plasticity and neuronal circuit function in the visual cortex of the mammalian brain. In order to record neuronal activity evoked by visual stimulation, these experiments require studying intact neuronal circuits in living animals. It is therefore impossible to avoid the use of animals for addressing these questions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals will be minimised wherever possible, and animals and brain tissue will be shared across experiments as much as possible. Experimental work will be complemented with theoretical modelling to further minimise the number of experiments and animal use. I will use computational modelling to establish the consistency and sufficiency of hypotheses, and to make predictions that can be used to guide the design of future experiments. I will also ensure that experiments are effective at testing hypotheses and therefore reduce the probability of unnecessary or unhelpful experiments being carried out.
3. Refinement Explain the choice of species and why the animal model(s)	Mice are the most appropriate animals for these experiments because: - Basic mechanisms of synaptic plasticity and neuronal circuit functions are likely to be preserved in

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 mammals including humans. - Essential knowledge has been accumulated over years of research about the anatomy, the physiology and the plasticity mechanisms in the visual cortex of this species. - State-of-the-art imaging techniques allowing recordings of neuronal activity in the living brain have also been developed in mice and will be used in this study. - Transgenic mice offer the unique possibility to study specific neuronal populations, such as inhibitory neurons, in the mammalian living brain. Since this proposal aims at investigating the role of specific classes of interneurons these transgenic mice are highly valuable and necessary for this project. -, Mice have emerged as valuable models of human genetic disorders, offering the opportunity to understand how brain circuits can be altered in genetic disorders and, hopefully, lead to ways in which these disorders could be treated. I will use mouse models of autism spectrum disorders in order to understand how neuronal circuit functions are altered by these diseases. Pain will be controlled during surgery by general anaesthesia and post-surgery by analgesics. Deaths resulting from anaesthesia or surgical complications will be minimised by correct dosing of anaesthetics, by accurate weighing and by maintenance of body temperature during and post-surgery e.g. use of heat pads. Risk of infection will be minimised by good surgical and aseptic techniques. Surgical sites will be monitored for signs of inflammation and infection. Appropriate effective treatment e.g. antibiotics will be administered under the advice of the Named Veterinary Surgeon if required. In addition, principles for good surgical practice will be followed throughout.

<table>
<tr><td>Project 26</td><td colspan="3">Signalling in sensory processing and drug effects</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Pain, rodents, signal transduction, opioid tolerance, mood</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">Pain is a complex medical and social issue with a poorly defined relationship between injury and the subsequent pain state. Currently available pain therapies, particularly in chronic pain that can result from back injury or nerve damage, are highly unsatisfactory. This has a major social and economic impact in term of lost employment and medical costs. Thus, better understanding of sensory processing mechanisms may directly lead to improved pain control in human patients. This project will employ well-established models of acute and chronic pain, including nerve section to produce clinically relevant symptoms of neuropathic pain or injection of inflammatory agents to simulate inflammation state. Identification and characterization of novel drug targets and novel therapeutic strategies may directly improve pain</td></tr>
</table>

	control in the clinic, also through improvement of currently available but not satisfactorily pain therapies. Also, better understanding of correlation between mood and pain suffering may lead to improvement pain treatment. Thus, this project aims to address fundamental problems of pain, drug effects and emotional processing. These all are areas of medical concern that are related to pain suffering.
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)?	All my experimental work with animals will be carried out with a view to translating obtained results into new treatments for humans. In this project I will look how cellular molecular mechanisms regulate perception of chronic pain and itch, increase or decrease analgesic effectiveness of traditional (e.g. opioids) and newly synthesized medications and finally, if the intensity of pain is related to emotional response like anxiety or depression and *vice versa*. In summary, the results from these studies will provide us with important information relating to how the body processes information about pain and may lead to the identification and characterization of targets for new drugs to control chronic pain such as found after nerve damage in patients. New therapies will benefit people, especially since different forms of chronic pain due to e.g. ageing affect the increasing percentage of human population and currently available therapies fail to provide sufficient pain control.
What species and approximate numbers of animals do you expect to use over what period of time?	It is expected that no more than 3,750 mice and 3,000 rats will be used during the 5 year-course of this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the	Experiments will be carried out in rats and mice under anaesthesia when required by employed procedure to minimize pain and suffering. In summary, only a small proportion of the animals will be under terminal anaesthesia and the majority will reach moderate severity. In order to study changes that occur during the development of chronic pain,

end?	in some cases, it will be necessary to induce ongoing pain by inflammation or by inducing nerve injury to mimic states of neuropathic pain. It should be noted that these animals will not be in constant pain but will have an increased sensitivity to mild mechanical and thermal stimuli. Only a model of loose ligation of the sciatic nerve (CCI - Chronic Constriction Injury) may cause more profound motor impairment observed by dragging of the affected limb and if there is any noticeable distress (loss of mobility around the cage or weight loss) the animals will be immediately terminated with a schedule 1 procedure. EMLA cream may be used to reduce local oedema at the site of incision or injury.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In all of these projects it is essential to use animals rather than cultured cells in dishes, as complex diseases require looking at the behaviour of the whole animal. In addition, previous research in rodents has shown that these animals can shed considerable light on human diseases and indeed as we have shown, even lead to new therapeutic approaches. Itch has always been studied in mice but not rats.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal responses and pain thresholds are generally recorded manually by the investigator. However scratching bouts will be recorded with a digital camera. Once preliminary results (from pilot studies) have been obtained, I shall use the Resource Equation routinely to estimate the appropriate final sample size. The results will be regarded as satisfactory when a clear conclusion emerges: either a statistically significant difference or a sample size (guided by the resource equation) that should have revealed a difference should one exist. Based on extensive experience and the validated nature of the tests, n=6-8 animals are sufficient for determining significance for the behaviour 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 will use rodents such as rats and mice for these studies since they have been proved to be very useful in predicting the sorts of changes that occur in patients with chronic pain. Moreover, there is substantial background information about the structure and function of the brain and spinal cord in this species and obtained experimental results will be relevant to my previous work and to other laboratories using these animals in studies of pain. I will generate animal models with increased mechanical and thermal sensitivity on the plantar surface of the hind paw but not in continuous pain.

Project 27	**Nociception and analgesia in zebrafish**	
Key Words (max. 5 words)	Zebrafish, pain relief	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 zebrafish is becoming increasingly popular in biomedical research (more than 400.000 individuals were used in 2011 according to the Home Office), as it is relatively small and still shares many features with other back-boned animals (vertebrates). This makes scientific findings from the zebrafish translatable for medical research. Adult and larval fish are being used in diverse fields, including research in cancer and neurodegenerative diseases and undergo potentially painful procedures, yet most procedures are done without proper analgesia (pain relief). This is mainly because not much is known about how fish process potentially harmful input. Efforts are underway to look, for example, at changes in behaviour after potentially painful experiences and how specific drugs (analgesics) mitigate these, in order to develop protocols for effective analgesia (pain relief) in fish. However, the central nervous system, which receives these stimuli, processes them and directs the behavioural changes, is not being analysed. We believe that analysing changes in the central nervous system is indispensable to directly demonstrate that harmful stimuli affect the brain, and that analgesic drugs are effective at the level of the	

	brain. In this project, we aim to support efforts analysing behaviour and other parameters by finding changes in the activity of different genes in areas of the spinal cord and brain after potentially painful experiences. This will give direct information on the processing of such input. We will also determine from which age these changes occur in the fish brain, since a lot of experiments are done on fish that are only a few days old. Moreover, by trying a number of potential analgesics we will determine which of these are most effective by directly measuring gene activity in the brain. Our preliminary analyses show that application of mustard oil to muscle tissue leads to increased activity of genes in the spinal cord and brain that are often activated in the context of harmful stimuli. Ideally, we will find analgesics that block this reaction already at the level of the spinal cord, where stimuli are first processed. In subsequent research, our findings from the brain will be compared to data from behaviour and other non-invasive measures to determine optimal protocols for analgesia in fishes and derive non-invasive indicators of successful analgesia in future zebrafish research.
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 data will thus help to refine experiments in fishes in the sense of Refinement as defined in the 3Rs, by devising protocols for analgesia that are supported by neurobiological observations and thus inform scientists and policy makers on best practice in fish experiments.
What species and approximate numbers of animals do you expect to use over what period of time?	*Danio rerlo* (Zebrafish) of all ages will be used. We will breed about 1500 fish in house and will use about 900 fish for experiments over the coming two 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 aim of the project is to find out what changes potentially painful stimuli cause in the brains and spinal cords of the zebrafish which could indicate pain perception, and to find drugs that can prevent these changes, indicating that they could provide pain relief. Most experiments will be of moderate severity. Fish will be killed at the end of the experiments to investigate gene expression in their brains and spinal cords.
Application of the 3Rs	
1. Replacement	In the present project, we are investigating the central nervous system reactions to potentially noxious

State why you need to use animals and why you cannot use non-animal alternatives	stimuli, which is an in vivo phenomenon.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will consult with statisticians to ensure that we will use the least number of fish possible to obtain reliable results. Whenever possible, we will use larval fish, under the age of 5 days, before the become protected under the A(SP)A.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	In this project, we aim to elucidate the cellular and anatomical mechanisms of nociception in the zebrafish *Danlo rerlo* and test whether potential analgesics can suppress central nervous system reactions (like alterations in gene expression) to a potentially noxious stimulus. Nociception is an in vivo phenomenon and the results from this project will ultimately be applied to refinement through the determination of effective analgesia after invasive procedures in the zebrafish. Most experiments will be performed under protocol 3 of moderate severity and effective analgesics and concentrations of analgesics will be determined after peripheral noxious stimuli. Since the ultimate aim is to provide analgesia after spinal cord injury in our and other laboratories, analgesia regimes established under protocol 3 will then be tested after spinal cord injury (protocol 4). Several measures are in place to minimise suffering, as determined from previous experience: After spinal cord lesion, animals are single-housed to eliminate intraspecific aggression and kept in the dark to minimise agitation and the water is conditioned with elevated salinity and anti-fungal agents.

Project 28	**Control of midbrain dopaminergic neuronal subtype specification and function**	
Key Words (max. 5 words)	Dopamine neurons, Parkinson's disease, subtype specification, neurological disorders	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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

- We would like to understand how defects in dopamine neurons contribute to diseases like Parkinson's disease, ADHA and anxiety.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.
  It is well establish that dopaminergic neurons located in the ventral midbrain are involved in controlling several brain processes and that these neurons are implicated in diseases including Parkinson's disease, addiction, ADHD and anxiety. Regulating such a wide variety of processes and having projections to several brain areas, dopamine neurons are thought to be a very heterogeneous cell population consisting of several subpopulations. One of these dopaminergic neuronal subpopulations are substantia nigra neurons, which selectively degenerate in Parkinson's disease. However, very little is known about the genes expressed in these distinct subpopulations of dopaminergic neurons and how these are generated during the development. Our aim is to get more insight into the generation of these subpopulations. Understanding of how these subpopulations like substantia nigra neurons are specified during development will facilitate the development of embryonic stem (ES) cell model system where we can generate these distinct subpopulations *in vitro.* The development of ES cell model systems offer important

opportunities in disease modelling and drug testing, reducing the need for animals in research. In addition, our project aims to understand the role of specific genes in the functioning of distinct dopaminergic subpopulation and will reveal the possible involvement of these genes in the onset of diseases, which will result in the development of novel treatments.

- Outline the general project plan.
- In our lab we recently identified several genes that are expressed in dopaminergic neuronal subpopulations, like Substantia nigra neurons. We will investigate the involvement of genes that we have recently identified in our lab in the specification of dopaminergic neuronal subpopulations. We will do this by the analysis of embryos and adult mice that have mutations in these genes. To assess the functional consequences of ablation of these genes in dopaminergic neurons we will perform behavioural analysis. It has recently been shown that very short RNAs (miRNAs) are also important in the specification and function of dopaminergic neurons. We would like to identify them by making use of genetic mouse lines. After breeding the mice, we will take embryonic and adult brain tissue to analyse the miRNA expression profile. Furthermore, we will use other genetic mouse lines to follow labelled cells over time to determine where in the embryo SNc dopaminergic neurons are being specified.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.
- We will breed various different mouse strains with mutations in particular genes or transgenic mouse strains. We do not expect that this will cause major suffering. If the mice suffer, because they develop certain diseases, we will try to relieve the suffering. We will mainly take tissue from dead animals. Some animals will receive injections with non-toxic substances, like tamoxifin. These injections will not affect the animals. We will also look at the behaviour of the animals by analyzing how they move through a cage or climb down from a pole. These tests will be short and are not very stressful.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.
- This study will be beneficial for other scientists. They will understand more about the genes that are important in dopamine neurons and how different dopamine neuronal subpopulations are developed. This will be advantageous for scientist who would like to generate dopamine neurons from embryonic stem cells. The research will be also an advantage for clinicians and pharmacists. The link that this study will make between the genes and diseases caused by dysfunction of the dopaminergic system will facilitate the development of treatments.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.
- For this study we expect to use about 1000 mice. We will use different mouse strains that are either transgenic or have defects in certain genes. To be able to study the role of genes the mouse is still the best model system. It is the most accessible vertebrate animal for targeted genetic manipulation and the dopamine neurons in the mouse are highly similar to human dopamine neurons. We will try to keep the numbers of the mice at the minimum by using statistical tests to calculate the

number of animals that we would require. Furthermore, we will use our previous experiences with similar type of research and published research to make an estimate of mouse numbers
• Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project. • We need to use animals because we would to be able to study if there will be any effects on the innervation of brain areas by dopamine neurons in mutant animals. The projections that dopamine neurons make to other brain areas can only be studied in animals. In addition, the impact of genes on the behaviour of animals cannot be studied in vitro. Some parts of this project will also involve the use of embryonic stem cell derived dopamine neurons. By the use of the neurons that are cultured *in vitro* we can analyse genome wide the transcriptional changes after ablation of genes expressed in dopamine neurons and analyse the interaction that the proteins encoded by these genes make with DNA, RNA and other proteins.
• Explain why the protocols and the way they are carried out should involve the least suffering. • The protocols we will use in our study will only cause very mild suffering. We will try to keep the chance for adverse effects to a minimum by using as much as possible mouse strains in which genes will only be ablated in dopamine neurons and not elsewhere in the body.

Project 29	**Ion channel expression and function in the nervous system**	
Key Words (max. 5 words)	ion channel; receptor; G-protein; signalling; action potential	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	Nerve cells communicate through electrical signals termed action potentials. These are generated by ion channels, which are specialized protein molecules in cell membranes. Individual ion channels are on/off switches that control the flow of ions across the membrane, thereby producing electrical currents that generate the action potentials and allow encoding and transfer of information. Action potentials in neurons are very brief and constitute the currency of rapid neuronal signalling. The aim of our project is to study how ion channels are distributed and function in the nerve cells at different ages, and how their activity is regulated by neurotransmitters in the brain. This will enable us to understand basic mechanisms of normal function during brain development and ageing.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or	Many neurological disorders are characterized by abnormal neuronal development, connectivity and ageing. If we can understand how neurons and circuits develop and generate signals in the healthy young and mature brain, this knowledge will help us	

animals could benefit from the project)?	decipher the aberrant molecular mechanisms responsible for many cognitive disorders, including mental retardation, autism spectrum disorders, epilepsy, dementia and schizophrenia.
What species and approximate numbers of animals do you expect to use over what period of time?	~500 rats and ~ 1100 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?	Large part of the work proposed can be completed using tissues dissected from animals humanely killed. This would be the least severe procedure possible. Some of the experiments involve dissection procedures for isolating tissues that will be performed on terminally anaesthetised animals that are killed after or during tissue excision and before recovery from the anaesthetic. Similarly, for the preparation of acute brain slices for electrophysiology, animals will be terminally anaesthetised with inhalation anaesthetics to minimise stress preceding rapid cervical dislocation and decapitation. New born pups (0-5-day old) will be killed simply by decapitation. Thus, the animals would not suffer in any of the proposed experimental protocols. Only one out of the seven strains of transgenic mice to be used in this project presents a phenotype of overall moderate severity. Protocol 003 has been assigned a moderate severity limit, but very few animals, if any, are expected to exceed a mild severity, and therefore the overall severity of the project is expected to be mild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The ion channels and receptors we are interested in are present in nerve cells, and our aim is to understand **how they are** expressed, localised and function in neurons at different developmental stages. Neurons are complex cells and have very specific, unique mechanisms, only partly understood, for the regulation of ion channel and receptor expression and their sorting, Additionally, they present unique developmental patterns of migration, differentiation and maturation. The complex functional properties of neurons and neuronal networks, their development, ageing and their modulation by neurotransmitters cannot be mimicked by cell lines. Cells lines will be

	used to optimise experimental tools, but ultimately we'll need to use brain tissue or neurons isolated from brain to address our questions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have calculated animal numbers according to the type of experiment and the sample size that is most likely to be statistically sufficient to produce a meaningful result. This is largely based on our experience and published studies using similar methodological approaches. Whenever possible and justified by the experimental aim, we shall use neuronal cultures that can survive for several days/weeks in vitro in order to maximise the number of experiments obtained from one rat/mouse brain. Finally, some methodological troubleshooting (e.g. testing of DNA/RNA constructs, antibodies) will be performed on immortalised cell lines rather than tissue directly extracted from animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats and mice are proposed throughout this project because all of the antecedent work that has led up to this project has been done on rat and mouse neurons and we need to ensure comparability with previous work, reproducibility, and peer-review acceptance for grant-funding and publication. All procedures are designed to minimise stress and discomfort for the animals: with the exception of immature ones (0-5 day-old rats or mice), all animals will be anaesthetised before killing and tissue extraction. This will prevent them from experiencing any pain.

<table>
<tr><td>Project 30</td><td>The cellular basis of sleep and circadian timekeeping</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Sleep, body clock, circadian, cellular</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></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>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>There is a biological clock, or circadian rhythm, within every cell of the human body that controls major aspects of our physiology, such as when we sleep and how we breakdown food. We know that this body clock is important for health, since when it goes wrong or gets out of synch with the day/night cycle (as in jet lag or shift work), people are more likely to suffer from conditions such as diabetes, cardiovascular disease and various forms of cancer. Similar issues, particularly mental health problems, are more common when people do not get enough sleep, but it is not understood why.

We plan to investigate: first how this circadian timing mechanism works at the level of individual cells; secondly, what are the essential processes that occur in brain cells during sleep; and thirdly, how these two processes interact.</td></tr>
<tr><td>What are the potential benefits likely to derive from this</td><td>Elucidation of the cellular processes regulating when and why we sleep will enhance</td></tr>
</table>

project (how science could be advanced or humans or animals could benefit from the project)?	understanding of a fundamental biological process; in the long term this will help us to understand the complex interaction between lifestyle, our genes and human health. Furthermore we hope our research will reveal potential targets for therapeutic intervention and management of sleep and other clock-related disorders, such as jet-leg and shift-work disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use no more than 12350 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	This project involves using mice carrying genetic manipulations which we already know or have good reason to expect will be non-harmful. The experiments we perform will be designed to ensure that the minimum smallest possible number of animals is used, and that they endure the least possible suffering. The majority of adverse effects are expected to be mild, with a small number having the potential to be moderate in severity, Moderate severity would mean that the mice are experiencing some persistent discomfort, and this is something we can monitor by looking for clinical signs of this discomfort such as reduced body weight and not interacting with other mice. The protocols will involve breeding and generation of genetically altered mice so that we can investigate their biological timing and sleep at the level of individual cells, tissues and in whole animals. Because we are interested in sleep and the internal clock that regulates when sleep occurs, in some cases we will use common, non-toxic drugs to interfere with these processes. Some animals may be subject to the implantation of miniaturised pumps that deliver drugs directly into the brain. At the end all animals will be killed humanely. In order to obtain healthy nerve cells whose behaviour can be studied outside of the body, it will be necessary to kill some young animals (7 days old and less) by decapitation.

	Mouse pup cortex is the preferred tissue since greater numbers of viable neurons can be obtained than from adults. To maximise the yield of healthy nerve cells, decapitation is essential in order to avoid compressing the skull and brain that would occur during cervical dislocation or concussion. Decapitation is less stressful than other non-dislocation methods such as CO_2 or intraperitoneal injection, it does not interfere with neuronal function as in terminal anaesthesia and is more rapid.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no feasible alternative that would entirely replace the use of a living animal, this is because the health-relevant mammalian biology we seek to ultimately understand (behavioural and physiological rhythms, sleep and wakefulness) are processes that only occur in the intact living organism
2. Reduction Explain how you will assure the use of minimum numbers of animals	We significantly reduce our reliance on animal models by performing the majority of our experiments in cell lines grown in the laboratory, and as far as possible, these techniques will always be employed to minimise the use of animals. Where animals are necessary, tight control over breeding programs means that we produce very few animals surplus to needs, whilst robust experimental design enables us to generate statistically valid results from the minimum requirement of experimental stock.
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 least sentient animals that can be used for satisfactory tests of the roles of genes and signalling pathways in the circadian organisation of behaviour, physiology and sleep. Inbred strains will ensure consistency of results, and minimise the variations between individuals, thus allowing us to keep the experimental cohorts relatively small. Moreover, only in mice is the genomic knowledge and technology sufficiently well advanced to develop and apply conditional inactivation/activation of genes by inducing agents. The work will be carried out in dedicated, state-of-the-art facilities by

	highly trained technicians and scientists, all of whom are dedicated to the highest standards of animal welfare. The procedures to produce genetically new types of mouse, to breed them up into viable colonies and to experiment on them by recording cycles of behaviour and physiology have been refined over many years in our facility. The scientists and technicians will work closely with Named Veterinary Surgeons to ensure that animals are exposed to minimal adverse effects.

Project 31	**Sensory Processing in the Nervous System**	
Key Words (max. 5 words)	Nociception, Pain, Depression, Anxiety, Epigenetics	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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
	X	Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	There is a great need for the identification of new drugs for the control of chronic pain, addiction and emotional distress. It has been reported that pain is poorly controlled in up to 4.2 million patients dying with cancer. One of the reasons for this is the clinical fear of producing an addicted state if opiates have been prescribed. Of patients who suffer pain, 33% had pain all or most of the time and 87% of those with pain rated it as moderate to severe. Pain control is still in its infancy. Only 33% of patients with nerve injury gain pain control with existing drugs. 20% of people with cancer have movement-related or neuropathic pain, and perhaps one-third of visits to pain clinics are from patients with similar pain not due to cancer. It has been estimated that about 365 million days are lost per annum in the UK alone through pain-related illness. Pain in particular has proved difficult to control particularly so-called chronic pain that can result from back injury, nerve damage or indeed from unknown causes. It might be thought that pain is easy to prevent by simply cutting off the origin of the pain with drugs or surgery. Unfortunately the pain experience is	

	very much more complicated and we now know that the brain makes a significant contribution to the over all level of pain felt. Some diseases such as fibromyalgia are felt in the periphery as tender joints and a general malaise but there is no peripheral change to account for the pain, leading us to conclude that the brain is generating the pain. We are studying this relationship between brain and signals from pain fibres that arrive from the skin or inside the body. In this project we will look at the way the brain allows pain signals to enter the spinal cord, how pain fibres themselves contribute to the level of pain felt and how the previous history of the organism changes the way pain is felt. It is also known that the presence of a pain state can prevent the development of opiate addiction but it is not known how. If we could work out the molecular basis for this we may be able to develop drugs that prevent or reverse the addictive state 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)?	Our investigations will uncover new targets for the development of drugs that can alleviate pain in human chronic pain sufferers. The research will also investigate the molecular basis for drug addiction and the emotional behaviour that accompanies chronic pain and addiction suggesting new therapeutic routes for dealing with these diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use rats and mice because these species have told us quite accurately about many aspects of pain processing in humans. Genetically modified mice will also allow us to confirm results from molecular analysis on wild-type mice and rats. We will use approximately 4600 rats and 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?	We will generate animal models with increased mechanical and thermal sensitivity but not in continuous pain. While some guarding of the sensitive paw is often seen, the model we generally use is the least severe available. Levels of severity are never more than moderate.
Application of the 3Rs	
1. Replacement State why you need to use	In all of these projects it is essential to use animals rather than cultured cells in dishes. All diseases that we are approaching through animal studies are fundamentally a reflection of how the brain goes

animals and why you cannot use non-animal alternatives	wrong both internally and in the way it interacts with the body. Cultured cells can tell us a lot and if required we use these approaches. But complex diseases require that we look at the behaviour of the whole animal. Fortunately, previous research in rodents has shown that these animals can shed considerable light on human diseases and indeed as we have shown, even lead to new therapeutic approaches.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experience from published experiments has shown that experiments require at least 6-12 animals per group. We consistently use male animals but as these are purchased from an outside vendor or bred by our Facility there is no wastage. Sample size will have been estimated by the resource equation and differences compared statistically using analysis of variance.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We are investigating the neurobiology of pain and rely on testing interactions between experimental interventions and animals' behavioural/neurochemical responses. We will use rats and mice because these species have told us quite accurately about many aspects of pain processing in humans. Genetically modified mice will also allow us to confirm results from molecular analysis on wild-type mice and rats. We will use care in ensuring that rats and mice are well maintained and suffer minimal distress as well as using best practice surgical procedures.

<table>
<tr><td>Project 32</td><td colspan="3">Protein misfolding disease: pathogenesis and intervention</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Protein misfolding diseases, cell death.</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>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 colspan="3">The objective of this work is to understand and ameliorate human diseases that arise when protein quality control fails such as neurodegenerative diseases, diabetes and cancer.</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">The benefit from the work described here is to
improve our understanding of the mechanisms at the origin of important human diseases that arise upon accumulation of misfolded proteins
provide evidence that modifying the natural cellular defence systems against misfolded proteins can slow down human diseases in order to identify pathways that can be exploited therapeutically</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">Mice are required as they provide good models of human diseases. I would expect that 17225 mice may be required.</td></tr>
</table>

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 used in this protocol may exhibit moderate clinical signs, it is expected that at least 80% of animals will show no more than mild clinical signs, less than 20% will show moderate signs and of these up to 80% will require to be killed in order not to exceed the moderate end point. Any animal showing moderate clinical signs such as gradual weight loss approaching 20%, hind limb paralysis, hunched posture and piloerection will be culled (local procedures are in place). It is not anticipated that other adverse effects will occur. However, it is recognised that when aged and/or genetically modified animals are used during treatment with novel molecules, or following surgery, particular care is needed in case these two factors or the new molecule interact leading to unforeseen problems. In view of this possibility, there will be increased vigilance during the conduct of the experiments both in monitoring indices of general health (weight, grooming, reactivity) and additional observations designed to detect more specific problems. All animals will be killed at the end and tissues will be collected for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have discovered novel, powerful and straightforward approaches to rescue cells form failure of protein quality control. These results have a potentially huge impact for human health because a large number of diseases arise when protein quality control fails. Prior to being used in humans, there is a legal requirement for virtually all potential disease modifiers to be tested in animal models for the disease(s) in question. The mouse is best suited for this work since, of all existing models, mouse models are highly relevant to the human diseases. We have done as much as possible, and will continue in the future to carry out preliminary experiments in cell lines or in ex vivo cultures.

	Whilst this will provide some useful information, cultured cells do not provide physiological conditions nor the complex interactions amongst different cell types. The work in mice we propose to carry out is essential to validate our discoveries and may have a big impact on human health.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Mouse breeding will be carefully monitored to ensure that surplus animals are not generated.We will use the minimum number of animals necessary to give a statistically significant result. Cryopreservation of embryos and sperm will be used for long-term storage of genetically altered mouse lines and pedigree lines. Rederivation will be undertaken should the health status of the animals be compromised in a way that would significantly affect the welfare of the animals or where the experimental results might be altered unduly.
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.	Existing animal models of common neurodegenerative diseases exhibit the essential features of the human diseases, a necessary prerequisite for the identification and evaluation of disease modifiers. As such, they are true models of the molecular and cellular features of the human diseases. At present, no valid alternative model exist. Signs of disease and the adverse effects will be limited to the minimum required for a valid scientific outcome and in all cases the general health and condition of an animal will remain the overriding determinant. Only those mice needed for experiments will be kept up to the moderate severity limit. Otherwise only young mice will be kept. When new lines are generated for the first time animal technicians will be specifically informed and the first litters carefully monitored. Any untoward phenotype will be discussed with the NACWO, veterinarian and, if appropriate, the Home Office Inspector.

<table>
<tr><td>Project 33</td><td>Functional improvement post nerve injury</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Spinal cord injury, scaffold, peripheral nerve injury, microglia, neuropathic pain</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>
<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 the project are: 1) to develop a combinatory treatment strategy based on novel biomaterial scaffolds to repair nerve damage following injury to the central or peripheral nervous systems (CNS and PNS), as no effective treatments are currently available to regrow injured nerves in human patients; 2) to develop novel treatments for chronic pain following injury to the CNS and PNS and currently there are no effective and satisfactory treatments available.</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 UK is thought to have about 40,000 spinal cord injury (SCI) patients, 800-1000 new SCI patients per year, with total costs of new patients estimated at £500 million per year. So far there are no established regenerative therapies for SCI highlighting the immense socioeconomic importance of research in this area. The project will help identify novel regenerative strategies that have</td></tr>
</table>

	huge translational potential. The ultimate goal is to help SCI patients improve their motor and sensory functions. The possibility of developing a new treatment for SCI repair permits the quality of life to be restored and removes a financial burden from the individual and society. Chronic pain following SCI has a major impact on daily functioning, affects rehabilitation, and often leads to anxiety, depression and even suicide. Many SCI patients rate pain as one of the most difficult problems to manage and consider eliminating it as important as improving sexual/bladder/bowel dysfunctions. However, there is no effective treatment to relieve such pain. The project will generate new knowledge in, and a greater understanding of, the pathophysiology of chronic pain after SCI. In addition, by using clinically relevant animal models, the project will help identify novel analgesic compounds for chronic pain after SCI with potential to be translated to the clinic to benefit SCI patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 500 rats per year 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?	Depending on the individual study, the operation may result in neurological deficits. For Protocol 1, the expected severity is mild, as we will use most appropriate non-recovery terminal anaesthesia regime throughout the procedure to minimise animal suffering to collect embryos. For Protocols 2-4, the expected severity is severe, as animals may have unilateral (Protocol 2) or bilateral (Protocols 3-4) hindlimb paralysis, loss of sensation in the affected hindlimbs, development of neuropathic pain at injury level and below the injury level, and bladder dysfunction, thus like spinal cord injury patients. However, these animals will be given full support, such as the use of soft bedding, manual bladder emptying, and will be only kept for up to 8 weeks. Of the 300 animals for each Protocol

	(2-4), about one third will receive spinal cord injury, and the rest would be sham control and naïve control animals. For Protocol 5, the expected severity is moderate, as animals may have temporary paralysis of one hindlimb, loss of sensation and neuropathic pain development of the affected hindlimb following peripheral nerve injury. One third of the 300 animals will receive peripheral nerve injury, and the rest will be sham control and naïve control animals. The extent to which the animals will be allowed to develop impairment of movement or other symptoms following the surgery will be clearly defined and, if the clinical signs shown by the animals reach defined endpoints, they will be killed to prevent unnecessary suffering. At the end of each study, animals will be humanly killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Trauma in the nervous system triggers a complex cascade of events, both in the affected organ and in other organs around the body. There is no alternative that would entirely replace the use of living animals for studies of the complex response of the injured nervous system. Most literature in neurotrauma research has been produced in models of injury in rats and mice, as they have a nervous system, which is similar to the human nervous system. However, cell culture systems that mimic certain aspects of spinal cord injury and peripheral nerve injury will be used to identify the best combinational strategies for nerve regeneration, and similarly be used to pre-screen novel analgesic agents for neuropathic pain following spinal and nerve trauma, before testing in vivo. Furthermore, neuropathic pain (NP) can only be assessed by examining the integrated response of the nervous system to nerve damage, which can only be properly examined in intact organisms that have a nervous system comparable to that of humans. It is not possible to use experimental

	human volunteer models of NP, as one cannot deliberately injure nerves in healthy humans, a prerequisite of NP. However, we will use alternative *in vitro* (cells) and *ex vivo* (tissue from animals) techniques (histology, gene expression) where possible to limit the use of live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals will be minimised by careful experimental design and appropriate statistical analysis, using ARRIVE guidelines from the NC3Rs and good laboratory practice methods from published literature, to minimise animal use and thus apply the principles of reduction and refinement in animal use. We will use cell culture systems as pre-screen tools for identifying the best nerve regeneration strategy and the novel potential analgesic agents, therefore reducing the number of animals used. Moreover, anatomical/structural data will be correlated with functional behavioural measures in the same animal, leading to a reduction in the use of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats will be used in preference to other species because they are: • The evolutionary lowest species to display behaviours, which are considered analogous to those of human pain and for which suitable models are available. • The "standard" neuroscience animal, allowing comparison to data from other neurological diseases. • We no longer propose to use genetically modified mice, as advances in techniques such as "gene knock-down" are making this redundant. This has two advantages: it circumvents the need to retain breeding colonies of transgenic mice. The measurement of complex behaviours, such as those associated with pain, are technically easier in rats. Three different models of SCI in rats will be used: partial section, compression, and contusion of the spinal cord, representing different aspects of human SCI. We will also use rat models of sciatic

	nerve injury. These injury models are well established and extensively used in the literature to study regenerative and neuroprotective strategies and NP following injury to the nervous system. All surgery used to model injury will be carried out under general anaesthesia. We will also make use of postoperative analgesics following the recovery from general anaesthesia. After injury we will follow the recovery of the animals and will also analyse their tissue, to assess whether the treatments we test have regenerative or analgesic effects. The stress experienced by animals following nerve injury will be limited to only what is necessary to achieve objectives.

<table>
<tr><td>Project 34</td><td>Neural Mechanisms and Neuropharmacology of Cognition</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cognition, attention, mental disorders, neuropharmacology</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>
<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>This project will study how the brain allows us to perform cognitive control, i.e. to integrate various types of information into an action plan that results in desired (rewarding) behaviour. Specifically it will focus on attention, working memory, and how they affect learning and decision making. Although the words ‘attention’, ‘(working) memory’, and ‘learning’ are ubiquitously used in everyday parlance, it is still a mystery how this is achieved by the brain. We have very limited insight into the mechanisms that enable these cognitive abilities and we have little knowledge which brain chemicals are involved. So far it has been demonstrated that attention increases our perception of the attended objects, and learning improves our ability to detect subtle changes in the environment as we obtain experience. Somehow brain cells (neurons) that respond for that object of interest make themselves better ‘heard’ during attention and memory, and improve their filter characteristics over time during learning. It is unresolved how this is achieved. The research aims to determine the chemical systems</td></tr>
</table>

	involved in these processes, and which areas of the brain are involved. It aims to delineate the 'language' used by neurons while communicating information between different brain areas, and the mechanisms that ensure the information is passed on. To obtain detailed insight into the chemical substances involved in attention, working memory, decision making and learning it is necessary to investigate what single neurons in the brain do while subjects perform specific, complex tasks. It is necessary to analyze (1) what neurons do locally, (2) what they do globally, i.e. their interaction between brain areas, and (3) understand which brain chemicals are involved in the process. The latter requires to selectivity influence the brain chemicals (the so called transmitter systems). These studies are inevitably invasive and can only be done in animals, not people. In order to study cognitive functions, these processes need to be investigated in awake, task performing animals. These functions will be studied using a variety of techniques that allow the investigation of neuronal processing at the microscopic level in the brain, at the medium scale level, and at the macroscopic level (whole brain activity). This integrated approach is essential, because it allows combining a very detailed mechanistic approach with the more holistic approach of whole brain activity monitoring. The minimally invasive technique of functional brain imaging (fMRI) will also be used on animals whenever possible and every attempt will be made to cross calibrate findings with non-invasive human psychophysical and fMRI studies. It is not possible to perform these studies in isolated cells, or even in brain slices in a test tube, as it is the interactions between individual cells and the rest of the brain that is of importance in these studies.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	Proper understanding of the mechanisms of attention, working memory, decision making and learning is crucial in understanding deficits associated with schizophrenia, attentional, memory and learning disorders (such as

project)?	Attentional Deficit Hyperactivity Dysfunction, Alzheimer disease and Lewy body dementias and stroke of the parietal cortex). Once the exact mechanisms are understood in the intact brain, we are likely to have a better understanding of what goes wrong during the development of attentional and memory disorders , and a targeted approach to treat these disorders is more likely. The research therefore is likely to have long term clinical implications. Moreover, ultimately it will yield a proper understanding how attention, learning and memory are implemented in the brain. • We will learn which brain chemicals (i.e. transmitters) and which receptor subtypes are involved in cognition and goal directed behaviour. • We will learn which brain areas are critical for cognition and goal directed behaviour, and how different cell types enable these. • We will learn how different brain chemical orchestrate the complex interactions between different brain areas, and we will learn which mechanisms are utilized therefore. • Crucially, we will obtain a thorough understanding how specific cognitive dysfunctions (such as Schizophrenia, attentional disorders, dementias) arise, and thereby our studies will inform psychopharmacology. It may in the long term aid in the development of better drug design for the treatment of a variety of cognitive dysfunctions.
What species and approximate numbers of animals do you expect to use over what period of time?	It is necessary to use old world monkeys, because these are the only animals whose brains are sufficiently similar to ours to enable us to relate our results to the situation in people. Old world monkeys are also the only species who are able to perform the complex tasks that are needed in these studies. The animals used will be between 2.5-~10 years old and a maximum of 40 macaque monkeys will be used over the 5 years of the project. A specific (but highly important) subset of questions can only be studied by a special (and difficult) technique, whereby the electrical activity is recorded intracellularly (so called intracellular

	recordings). This technique is very difficult to apply in primates, but it is comparatively easy to perform in rats and mice. Performing these particular experiments in mice or rats will still provide valuable answers and 40 mice and 40 rats aged 2-12 months will be used for this purpose. Optogenetics: is a technique whereby cells are coaxed into expressing specific receptors (which they normally would not express—i.e. the term 'genetics' in the word), and these receptors can be activated by light ('opto'). This technique allows unprecedented control over cell activity which is possible in primates and in rodents (and we aim to perform it in both). Optogenetic approaches, by clever genetic design, ensure that only specific cell types express (build) the receptor. However, this clever design is currently only possible for rodents, and here specifically in mice. We therefore aim to study certain aspects of cognitive function and cognitive circuitry (where we believe is adequate similarity between rodents and primates) in behaving and anaesthetized mice (280) and rats (40). Thus the 2 rat/mice studies combined will use a maximum of 400 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 macaque monkeys will be gradually trained to perform cognitively demanding tasks, in a laboratory setting. A cranial surgery will be performed to implant a head post and recording chambers, Some animals will have recording electrodes implanted which will allow long term recording of selected neuronal populations. The head post allows the animal's head to be stabilized while recording neuronal activity, without causing any discomfort. The chambers will be used to access the brain for short-term electrode recordings and investigation of the neurotransmitter systems involved in attention, learning and memory. Some animals will have MRI scans, and these animals will be introduced gradually to this apparatus, so that they become accustomed to it, and will perform their tasks while being imaged. All of these techniques are designed so that after the surgery the animals make a full recovery, and can then be

	studied in ways that cause minimal distress. In order for the animals to be motivated to perform the various tasks, the amount of fluid (or food) they are given each day will be restricted, and they will be given fluid (or food) as a reward when they complete a task successfully. The degree of restriction will be very carefully monitored, and kept to the minimum needed to motivate them to perform their tasks. Eventually the animals will be killed humanely by an overdose of anaesthetic. To maximize the use of the animals, some of them will be anaesthetized and other types of brain recordings made, then after keeping them anaesthetized for several days, they will be killed without having allowed them to recover consciousness. In some animals, small pieces of brain will be removed during this final stage under anaesthesia, and the retina (the cells at the back of the eye) may also be removed. These tissues can then be used for other types of studies, without the need to kill animals just to obtain this material. The following problems may be encountered: • Some monkeys (generally <2%) have problems adapting to the daily routine of getting used to the chair and training, and will therefore be stressed and not co-operate in the learning tasks. These animals will not proceed to further stages, as it would be too stressful, but will be used in non-recovery experiments under general anaesthesia. • Cranial implants may become infected, and this could cause distress, but these infections will immediately be treated with antibiotics and animals will receive pain medication if necessary. • Cranial surgery could cause pain postoperatively but animals will receive effective pain relief to prevent this. • Implants may become loose, and so need replacing. This will require additional surgeries under general anaesthesia. • In the rare case of an animal developing

	meningitis or encephalitis (<2%), they will be immediately treated with antibiotics and given pain relief, and their health assessed several times a day by the veterinary staff, in order to make a decision as to whether the treatment is proving successful, or whether the animal should be killed humanely by an overdose of anaesthetic. • Animals used in non-recovery experiments will not suffer from any adverse effects as they will be anaesthetized throughout and their level of anaesthesia will be constantly monitored. The well being of animals used in behavioral experiments will be checked at least once a day by the experimenter and the animal technicians responsible for their care. Their weight, water and food intake is continuously monitored, and their cranial implants are cleaned and checked for infections daily during the week (and if necessary on weekends). Any signs of infection or signs of distress will immediately be reported to the veterinary surgeon or his deputy and appropriate treatment given. Rats and mice The animals will be trained to perform specific tasks, in a laboratory setting. A cranial surgery will be performed to implant a head post and recording chambers, Some animals will have recording electrodes implanted which will allow long term recording of selected neuronal populations. Some animals will receive intracranial injections of viral vectors to allow for optogenetic manipulation of neuronal activity. The head post allows the animal's head to be stabilized while recording neuronal activity, without causing any discomfort. The chambers will be used to access the brain for short-term electrode recordings and investigation of the neurotransmitter systems involved in attention, learning and memory. All of these techniques are designed so that after the surgery the animals make a full recovery, and can then be studied in

	ways that cause minimal distress. In order for the animals to be motivated to perform the various tasks, the amount of fluid (or food) they are given each day will be restricted, and they will be given fluid (or food) as a reward when they complete a task successfully. This can be complemented (or even replaced) by electrical microstimulation of reward centers. The degree of restriction will be very carefully monitored, and kept to the minimum needed to motivate them to perform their tasks. They will receive at least 50% of their free access intake or 20ml/kg/day, whichever is higher. Eventually the animals will be killed humanely by an overdose of anaesthetic. To maximize the use of the animals, some of them will be anaesthetized and other types of brain recordings made, then after keeping them anaesthetized for several days, they will be killed without having allowed them to recover consciousness.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cognitive functions do not exist outside the animal kingdom and even there the abilities are only properly evolved in birds and mammals. While imaging studies in people can reveal the basic anatomical aspects of cognitive control, it does not allow insight into the underlying mechanisms. Computational modelling can make predictions regarding the implementation of certain functions and I have a well established tradition of close collaboration with computational scientists to use this approach. However, these modelling studies require experimental validation, which in this case requires invasive animal work. Whenever possible, use will be made of rodents, since some specific investigations can be done in these species. In order to minimise the numbers of animals used, especially the numbers of primates, a series of linked studies are carried out in each animal, and the maximum information is obtained by conducting a final study under anaesthesia, and

	during this final study taking tissue for other, in vitro, investigations.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our methods provide a high yield of data from each experiment. We often use multiple electrode systems, which gather data from multiple sites simultaneously. Chronic methods in awake monkeys allow recordings for many days from one animal. Our anaesthetic methods are highly refined, and allow us to record in terminally anaesthetised animals for 2-3 days, again greatly increasing the yield of data. As a consequence, it is often possible to produce publishable data from only two animals – the minimum accepted in the field to ensure that results are reproducible across individuals. In all studies, we ensure that data analysis is performed soon after each experiment. This enables us to see trends emerging, and to stop gathering data as soon as sufficient data have been obtained for statistical significance.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	As described above, some basic results on the mechanisms of cognitive control can be determined from *in vitro* studies or experiments in anaesthetised rats. However, the primate sensory system and the areas involved in cognitive control differ in some key aspects from rodents (e.g. the existence of a dorsolateral and ventrolateral prefrontal cortex and a frontal pole in primates, which is absent in rodents). These areas are probably the areas that are most involved in cognitive control in humans. For our results to be applicable to human patients, it is necessary that much of the work is carried out in old world primates, such as macaques. Behavioural training requires some food or fluid restriction to ensure that the animal performs sufficient trials of these demanding tasks for valid analysis. By using a staged approach to food and fluid restriction, where this is built up gradually, we ensure that the impact on the animal is minimised. By the time an animal is working under the most restrictive regime, task performance is high, and a

	large quantity of fluid or food rewards are earned in the training session. Our methods have been refined over many years. To the best of our knowledge, we were the first (worldwide) to tailor fluid restriction protocols to individual animals, in line with their normal fluid intake. For each animal, we make regular attempts to decrease the fluid restriction to the minimum degree necessary, while still being able to record high quality data to inform the science. We are currently running a study which explores to what extent different motivational strategies can be used to minimize restriction regimes (the study will also be part of this licence). We are also running a study which explores to what extent different protocols result in 'cumulative experience', i.e. to what extent many procedures performed on an individual animal add up cumulatively in terms of the subjective animal experience. Our anaesthetic methods are highly advanced, and at the forefront of current practice, led by our NVS. All experiments are conducted within the outstanding facilities and the support provided permits surgeries to be carried out to a very high level of asepsis.

<table>
<tr><td>Project 35</td><td colspan="3">Investigations into Disorders of Movement</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Parkinson’s, Dystonia, neurodegeneration, movement, dyskinesia</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>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 colspan="3">There are a number of movement disorders which are poorly treated and for which there is no 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.

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 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 including the substantia nigra resulting in progressive loss of control of movement as well as other symptoms including anxiety, depression,</td></tr>
</table>

	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 without side effects, and to find drugs that will slow or stop the progression of Parkinson's disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice (including genetically modified mice). We expect to use less than 1000 per 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?	During these studies the animals may experience mild to moderate discomfort as they will undergo procedures that induce the symptoms of diseases, such as uncoordinated movement. In addition they will experience some of the side effects of the treatments for the disorders. This may include increased or decreased movement, abnormal involuntary movements, weight loss or altered gut function resulting in constipation or diarrhoea. These will only be mildly or moderately uncomfortable. Anaesthesia, pain killers and unilateral lesions will be used where appropriate to reduce the pain associated with surgery and the

	severity of the incapacity. We have introduced stringent limits for the frequency of injections, blood sampling and behavioural assessment that any one animal can experience. The majority of our studies will be short term (less than one week of treatment), however, we are searching for new treatments for long-term disorders, and we will perform extended studies to investigate the long-term effects of drug treatment. Overall, the severity of this license is expected to be moderate. At the end of the experiments the animals will be humanely killed and tissues may be investigated biochemically.
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 cell culture models of PD derived from human and rodents to investigate the effects of drugs and toxins. Similarly, we will continue our analysis of drug activity using isolated tissues. However, brain function is extremely complex and neurodegenerative diseases such as Parkinson's disease and dystonia present an equally complex neuropathology with associated motor and non-motor symptoms. It is, therefore, vital to confirm the positive effects that may be apparent in an in vitro situation 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. We will continue to use rodent models of PD and dystonia as these are well validated and predictive of the efficacy of therapeutic treatment. 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 minimum numbers of animals	For these studies the appropriate group size will be determined by power analysis allowing robust and statistically significant results. Expert advice will be obtained from statisticians as necessary, and appropriate statistical analysis will be used. We have considerable experience in the design and performance of these types of experiments and

	have published extensively in peer-reviewed journals. Overall numbers of animals used has also been reduced through the use of cell culture techniques described in the previous paragraph.
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 neuropathology induced by the toxins we will use reflects that seen in PD and dystonia and therefore provides 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 MPTP-treated primate, however, it is unacceptable to use this model at early preclinical stages of therapy. Hence, rodent preclinical models are accepted as the most suitable for the investigation of symptomatic and neuroprotective treatments.

Project 36	**Characterisation and Modulation of Traumatic Brain Injury**	
Key Words (max. 5 words)	Trauma Brain Injury	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 injury to the brain is common and causes death or long term disability in tens of thousands of people throughout Europe every year. Many of these victims are young people. Injury to the brain frequently causes life threatening swelling of the brain itself. The process that causes this swelling is called inflammation. It is believed that inflammation increases the damage to the already injured brain. This starts a cycle of further swelling and damage. At present the care of patients with severe traumatic brain injury is limited to basic support of body systems. There is no specific treatment to prevent the cycle of brain swelling and progressive damage that follows traumatic injury. To date the inflammatory response is poorly understood and attempts to control it have not been effective in patients. A better understanding of the process of inflammation will lead to the development of treatments to limit this process effectively, prevent brain swelling and improving recovery. *Study of Brain Inflammation*	

	We have developed a suitable animal model of traumatic injury which allows the study of brain inflammation in rats and mice. New methods of looking at inflammation in live animals have been developed and are central to this project is the development of these techniques to visualise the inflammatory process in live animals following experimental brain trauma. Furthermore new techniques to control the inflammatory process have been developed locally that have not been tested in brain injury. Together the development of imaging and the use of more specific techniques to investigate and ultimately control the inflammatory process could lead to better treatments to control brain swelling. *Brain and Lung Injury* In patients with brain injury a serious infection in the lungs called pneumonia is common. This makes the patients much more difficult to care for. The reason why these patients developed pneumonia is not understood. Furthermore the development of injury to the lung can exaggerate the injury to the brain. Again the mechanisms causing this are not understood. This project will study the effect of brain trauma on lung inflammation and infection. This will require the development of a model of combined brain trauma and lung infection. A better understanding of the lung injury process after trauma to the brain may lead to the development of treatments to control it. This might also improve brain function in survivors.
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)?	An increased understanding of the inflammatory likely to derive from this response in the brain and lungs to brain trauma. More specific and potent treatments of inflammation, animals could benefit from the developed locally, and tested in our brain injury model will help to identify key regulators of the process in both the brain and lungs. This will help to define how and when to best treat brain and lung inflammation after traumatic injury. The development of new imaging methods to study inflammation will reduce the number of animals required to study the response of brain swelling to new treatments. This could also potentially apply to other studies of the inflammatory process in different

	diseases. Lung injury and infection following brain trauma prejudices patient survival and is associated with further injury to the brain. By increasing our understanding of this process and how to control it deaths from traumatic brain injury could be prevented and the quality of survival be improved.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and Mice. Approximately 800 rats and 1200 mice over the period of the licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Brain injury model The injury is produced whilst the animal is anaesthetised. Through a cut in the skin on the top of the animals head the skull bone is exposed and a small “window of bone” removed to reveal the surface of the brain. The surface of the brain can then be briefly compressed for a fraction of a second, in a controlled manner. This produces a bruise on its surface of the brain which extends to deeper structures and causes inflammation in the brain and damage to nerve cells. When formally tested this can cause measureable weakness on the affected side and detectable impairments in memory and learning. However, despite this, animals recovery promptly and are able to move freely without obvious impairment, exploring their environment and feeding appropriately. Animals receive post procedure pain killers. Unexpected distress from the injury is exceptionally rare. Severe weakness, inability to feed or convulsions are possible consequences of a brain injury. However these complications do not occur because the size of the injury is carefully limited. Administration of substances In order to either visualise the inflammatory process or modulate the severity of inflammation substances will be administered to animals following injury. By modulating inflammation and assessing the effect of this on the injury key elements of the inflammatory response can be identified. Potential novel treatments to limit inflammation in the brain and lungs following injury can be investigated. Harmful effects associated with the administration of substances will be avoided by using known safe doses. Lung Injury This will take one of two forms. To model lung

	infection complicating brain injury animals will be anaesthetised and bacteria introduced into the respiratory system through a fine cannula. This is not expected to cause more than transient increase in the rate of breathing. Following the procedure the injury resolves on its own. To model the effects of patients being on a ventilator (breathing! life support machine) following brain injury anaesthetised animals will have their breathing performed for them by a machine. This will stimulate inflammation in the lungs. These animals will not wake up from the anaesthetic before being humanely killed. Behavioural testing This will be conducted under direct supervision. These tests will measure the animals limb strength, coordination and ability to learn and remember new information. These tests involve the assessment of behaviours often natural to rodents, including swimming, climbing and balancing. t Animals will be kept warm and where necessary dried. For balancing tasks foam padding will be placed under the animal to prevent injury in case of falls. Moderate severity Any animal showing signs of distress which cannot be easily resolved will be humanely killed. At the end of each experiment animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To date it has not been feasible to produce an adequate *non animal* (cells grown in a dish) model of the immune animals and why you cannot system and the inflammatory response. Thus in this use non-animal alternatives regard there is no substitute to live animal studies. A central premise of this application is to develop imaging methods to improve our understanding of the inflammatory process and to aid the development of treatments more efficiently than current animal methods allow. This will only be possible in an animal model. Non animal based experimentation will always precede *animal* work to optimise techniques, thereby minimising the number of animal experiments

	required. Mice and rats represent the simplest models for performing whole body imaging. Their size permits detection of molecular signals within the brain and lung, not possible in larger animals, or man. Study of the brain's inflammatory process in human subjects is not possible without inducing further brain injury. Furthermore it is not practical to study sufficient numbers of patients with identical injuries to draw valid scientific conclusions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The use of repeated imaging of the inflammatory process and the response to treatment over days in the same animal drastically reduces the numbers of animals required for each experiment. We shall use preliminary experiments to establish optimal conditions for each experiment. The brain trauma model used in this work is already well defined in our hands. By studying the effects of injury on both the brain and lung in the same animal we will increase the information gained and reduce the animal numbers required. Further examples of reduction include the use of suitable stable non animal derived cell lines, where 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 use a well characterised model of traumatic brain injury in rats and mice. We have successfully introduced the model in both rats and mice. The brain injury severity used is the minimum required to produce evidence of injury, with features relevant to human injury and yet it is well tolerated. Animals recover quickly without distress. Analgesia is provided in each case. Mortality is very low and exceptional in experienced hands. The combined brain and lung injury model will be developed carefully in a stepwise manner in order to ensure that a relevant model is achieved that is well tolerated. We have developed experimental protocols with refinement in mind, and key examples are shown below.

	Animals will be monitored both by ourselves and by experienced staff in the animal facilities. For the brain injury only work, experience has shown us that animals can be reviewed at the end of the working day following the brain injury and daily following that. Animals should be moving around their cages, curious about people coming to see them and be able to feed. In the combined brain and lung injury work we plan to initially limit the time animals recover to the day of injury to allow continuous monitoring. As experience increases this period will increase. Planned post procedure checks will be performed at the end of the working day, between 21.00 and midnight first thing the following day, and daily thereafter. As experience increases the intensity of this monitoring may be reduced to that of the brain injury only work. Any animal showing signs of breathing difficulties will be humanely killed. In most of the experimental protocols described adverse events can be expected to be established within a few hours of injury. Therefore wherever possible we shall time experiments to begin as early as possible in the working day. This will ensure close monitoring is possible soon after the injury. When extended monitoring is required this will be performed as detailed above. Provisional studies will guide the dosing of injury inducing agents to ensure the minimum injury required is used.

<table>
<tr><td>Project 37</td><td>Primary visual pathways in the rat</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Rat, brain, vision</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>No</td></tr>
<tr><td>Translational and applied research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></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><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td><s>Yes</s></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><u>To ask how the cerebral cortex controls its own inputs from lower visual structures</u>. Information from our sense organs “flows” through lower brain structures “up” to the cerebral cortex, but we know that this part of the brain sends a significant amount of information back to the lower parts. While we do not currently know why this is, we do know that it is a common plan in all mammals, and throughout all cortical connections. It is thought to modulate the extent or power of the information flowing upstream. It may also compare incoming information with already “known” examples and act to enhance our attention to objects of interest. We propose to examine the visual properties of individual neurons which are receiving input from lower and higher centres to see how these two main inputs integrate with non-sensory, attention-like inputs from non-visual areas.</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>Our understanding of the functions of our cerebral cortex would be greatly enhanced if we knew why this part of the brain sends such a large and rich feedback to the lower regions from which it gets the original sensory input. This is common to all mammals, and to all our senses (except the sense</td></tr>
</table>

project)?	of smell).
What species and approximate numbers of animals do you expect to use over what period of time?	~30 adult rats per 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?	The animals will be painlessly anaesthetised, and remain anaesthetised throughout the experiment. They will never regain consciousness. This puts these experiments in the “unclassified” group, as the animals will feel nothing throughout. They will be euthanized painlessly in order to examine the brain tissue post-mortem. This is a necessary step to be able to localise the recorded cells.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The brain is a complex structure which cannot yet be understood by other means. The use of the laboratory rat as a model will reduce the need for and refine experiments on higher animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Each animals will be used in experiments that can last up to 24 hours (typically 15-18), to derive the maximum amount of information from each.
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 rodent is not normally considered the animal of choice in vision experiments. However, its “simpler” visual system allows basic questions to be addressed which can inform experimental work which may later be carried out on higher mammals, including humans. The animals will be group-housed in enriched environments.

<table>
<tr><td>Project 38</td><td>Investigating ADHD and common co-morbid conditions</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Attention Deficit Hyperactivity Disorder

Co-morbid conditions</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></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>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>There are three scientific objectives:

1. To determine the brain-basis of ADHD by examining brain structures relating to specific symptoms of ADHD using behavioural, physiological, anatomical and pharmacological studies
2. To elucidate the actions, both beneficial and detrimental, of drugs known to impact on ADHD, by examining the effects of the drugs on relevant behaviours and brain regions
3. To investigate the relationship between ADHD and its co-morbidities beginning with characterisation and modulation of co-morbidities in an existing rodent model of ADHD.</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>1. As well as furthering our understanding of the brain basis of the symptoms of ADHD, improved understanding of the brain basis is likely to have a significant impact on treatment. Currently available treatments remain ineffective in 20%</td></tr>
</table>

project)?	of individuals. By improving understanding it may be possible to establish why this group are unresponsive and what mechanisms a drug should possess to elicit a response. 2. Improving our understanding of the actions of treatment drugs will feed into our understanding of the neurobiology and development of new drug treatments. In addition, the work will address safety concerns of treatments for ADHD by examining their detrimental effects. 3. ADHD in isolation from other conditions is less common than ADHD with 'co-morbid' or co-occurring conditions and yet at present we have no model to investigate co-morbid conditions alongside ADHD. This project aims to characterise comorbid conditions in a commonly used rat model of ADHD. A careful characterisation of conditions that co-occur with ADHD will allow better understanding of how the condition exists in the clinic and has the potential to establish how different conditions can affect treatment outcomes. In addition, to the benefit to our understanding of ADHD, the careful characterisation of the existing model will allow refinements of future experiments using this animal.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use rats because previous work has done so and because the best validated model of ADHD is the Spontaneously Hypertensive Rat (SHR). We will use this strain and appropriate controls to investigate the brain basis of ADHD and the effects of treatment drugs. It will also use this strain as a basis for modelling co-morbidity. In total the project will use 1450 rats from weaning age to adulthood.
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 a significant proportion of the animals used there are no adverse effects because they will be humanely sacrificed for tissue analysis or all data collected under terminal anaesthesia (~505 animals). However, the remaining animals may undergo: a) Behavioural testing. This testing, where possible, will draw on natural behaviour or be carried out in ways known to avoid any pain, lasting harm or distress and therefore are not associated with adverse effects. In

	some cases testing will require motivation to be induced through food or water restriction. The level of restriction will be such that normal growth occurs and health will be monitored throughout to minimise any distress. b) Drug treatment with an ADHD treatment drug. Wherever possible this will be given orally using a method that causes no pain, distress or lasting harm. Where other methods of administration are used good practice will ensure no adverse effects beyond the transient pain of an injection and all animals will be monitored for signs of distress. If drug treatment is given it may also be necessary to take blood samples to check drug levels. Where this is necessary, sampling shall be done using appropriate techniques to minimise distress and only withdrawing safe volumes. Adverse effects of the drugs themselves are rare but take the form of increased locomotion, weight loss and diarrhoea. Should an animal display any of these signs advice will be sought from the animal care staff and, if necessary, the animal will be humanely killed. The overall level of severity is classed as moderate because animals may undergo repeated drug treatment and behavioural testing which cumulatively leads to moderate severity. This is also the necessary severity level because some of the animals used will be the spontaneously hypertensive rat which is known to have a compromised immune system and therefore vulnerable to poor health. We will safeguard health by using appropriate housing and husbandry.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	ADHD is a behavioural condition and therefore in order to examine ADHD we need to use a whole animal where these behaviours can be observed. In some cases, useful studies can use ex-vivo measures and therefore not using a living animal. Where this is possible we will do so. For our final objective we are attempting to model ADHD with co-morbid conditions and this requires use of an

	existing ADHD rat model – the SHR.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Previous work optimised drug treatment and surgical procedures. In addition, we have carefully considered the optimum amount of data that may be collected from an individual animal and employed a type of design that allows us to get the maximum data from the lowest number of animals, including data on how different factors (e.g. age and diet) interact with each other, something which would not be possible in other designs. We have also used our knowledge of the behavioural tests involved and the size of expected effects to calculate the number of animals used. We will ensure appropriate statistics are used to avoid unnecessary animal use. Furthermore, two of the strains of rat we will use (the SHR and control strain) are inbred strains which means that they display less genetic variation and this should reduce inter-animal variation and, therefore, the overall number required.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We require the use of rats, specifically the SHR, because they are the most validated ADHD model. Every effort will be made to refine experimental procedures to minimise suffering e.g. modifying behavioural tasks. Animals will be single housed where appropriate due to the increased aggression in SHRs. In addition, when administering treatment, only safe volumes and needle sizes will be used. A preference for oral and subcutaneous administration will be given. Careful monitoring by trained staff will begin *prior* to procedures because of the vulnerability of the SHR.

Project 39	**Development and plasticity of sensory pathways**	
Key Words (max. 5 words)	Development, plasticity, sensory, pathways	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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
Summarise your project (1-2) sentences)	The programme of work focuses upon the development of pain pathways in the mammalian central nervous system.	
Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.	The lack of knowledge of pain processing and of the actions of pain relieving drugs in infants and young children is an area of great concern in paediatric medicine. The aim, therefore, is to investigate pain development in newborn rats and mice and provide the basic scientific knowledge required to effectively treat childhood pain.	
Outline the general project plan	A range of biological techniques are used to investigate the mechanisms whereby somatosensory connections and pain neurotransmission develop in the newborn and juvenile spinal cord, brainstem and cortex in young animals. Furthermore, the effect of tissue damage upon the developing somatosensory system is examined with the aim of understanding the long term consequences of infant pain and trauma and its treatment on the developing central nervous	

	system. Our results are shared with scientists and clinicians dedicated to the understanding of children's pain and the alleviation of suffering.
• Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.	Animals will undergo surgical procedures of moderate severity which are all performed under anaesthesia. Some discomfort may result from these procedures but animals will be closely monitored upon recovery from anaesthesia and treated with analgesics as appropriate. If there are any adverse effects, such as poor wound healing, the animals will be humanely killed.
• Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.	Our work may be viewed in the context of the average human premature infant in intensive care, who will receive up to 200 painful procedures in the absence of any analgesia (Carbajal et al., 2008). It is imperative that we understand the nature of these infants pain and how to treat it. Our extensive clinical collaborations mean that we are able to apply our scientific knowledge directly to clinical situations, such as the treatment of pain in premature infants in intensive care or in children undergoing major surgery.
• Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.	Our research group uses 800-900 adult and neonatal rats or mice per year on a wide range of projects of mild or moderate severity. Many of these animals will be used for terminal electrophysiology under anaesthesia. Larger numbers are required for the removal of tissue after Schedule *1* killing for histological or molecular biological analysis of small traces of key proteins or RNA in the intact nervous system. We take statistical advice to check the efficiency of our experimental designs and have steadily reduced animal use by restricting the number of people working on animals in my lab and reducing the number of time points studied.
• Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will	The use of animals is necessary because pain is not a molecular or cellular event; it requires signals to travel through the entire, intact nervous system. Rats and mice form good models as their pain pathways are very similar to those of humans and the newborn rat is at an equivalent stage of development to a

use non- animal studies in parallel with the project.	premature infant. The number of animals is kept to a minimum through careful experimental design and the use of small pilot studies. Detailed examination of signals between neurons is carried out ‘in vitro’ on isolated spinal cord slices.
Explain why the protocols and the way they are carried out should involve the least suffering.	All surgical procedures are performed under anaesthesia and in cases where animals recover from surgery, care is taken to ensure that there is no discomfort through the appropriate use of analgesics.

<table>
<tr><td>Project 40</td><td colspan="3">Primary headaches and associated conditions</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Headache, Pain, Electrophysiology, Imaging, Translational Research</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">Migraine and related headache disorders are the most disabling brain disorders in the world. They present as severe attacks of throbbing pain which may be accompanied with symptoms, such as visual disturbances and nausea. There is a major unmet need to advance our understanding of this severe group of conditions from a scientific point.

Currently very few specific medications exist and the most effective acute treatments work only in approximately 30% of cases. The treatment of migraine is also confounded by the use of for example, anti-epileptic drugs which may have unwanted side effects.

When you consider that up to 16% of the population suffer from migraine alone, not to mention the more severe but rarer cluster headaches (females often describe the pain as worse than childbirth) the depth of the problem is clear (approx 10 million</td></tr>
</table>

	sufferers in the UK). A further major scientific and clinical need is to understand why the number of attacks an individual gets may increase (chronic migraine etc.). It is known that medication overuse is a major risk factor for chronification and the very drugs (including over the counter pain killers) used to treat the pain can worsen the clinical disorder. Separate to the suffering highlighted above to sufferers, the economic cost of headaches is unacceptably high. It is estimated to cost the EU over €20 billion per year and places an enormous burden on the NHS. Resulting in a large proportion of A&E admissions and 1 in 4 neurologist appointments. Thus a greater understanding and improved treatment of headache disorders would substantially benefit sufferers and help ease the burden on the NHS.
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 first potential benefit from this project is an increased understanding of what causes headache and why different individuals such as females are more prone to migraines for example. This will also include an improved understanding of associated symptoms such as the aversion to light and sound common in some people during attacks. A second potential benefit is an increased understanding of the role that headache plays in other disorders such as epilepsy. The information gained from this project will advance general scientific knowledge, but also strive to lighten the burden of sufferers and help reduce the financial burden of the disease. The project will benefit from close collaboration with clinical colleagues and industrial partners ensuring rapid sharing of data to advance the development of novel treatment options.
What species and approximate numbers of animals do you expect to use	The project has been designed to minimise the number of animals with human studies or other methods used when possible. Only when essential

over what period of time?	for specific aims will rats and mice be used. We expect to use 3250 rats and 3250 mice over the duration of the project; however every effort will be used to minimise this number.
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 housed and supervised by trained staff ensuring the best possible environment to minimise any stress. For the majority of the animals used in this project (over 60%) of interventions will be conducted under full general anaesthesia with the animal humanely killed while still under full anaesthesia to minimise any adverse effects and prevent any post operative pain. Where absolutely necessary for example during chronic drug administration and brain imaging (under short term general anaesthesia) animals may receive multiple interventions but these are expected to cause only mild discomfort with no long lasting adverse effects. This will provide an excellent method of comparing results with patients as the same imaging methods are commonly used, highlighting the safety of this approach. For studies which aim to identify basic connections within the nervous system (less than 5% of animals) it is necessary to allow animals to recover following surgery. With this in mind surgical interventions will be minimal and conducted as always by expertly trained individuals. We do not expect any long lasting adverse effect on the animals; however if the animal shows any unexpected signs of suffering it will be humanely culled. To further minimise the impact on the animal the maximum post surgical phase will be 1 week for this component of the project. At the conclusion of all experiments animals will be humanely killed and where possible to minimise further animal use tissue collected and stored for further analysis. The use of as much tissue as possible from each animal will help reduce the total number needed for the project.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As we are investigating a complex condition which only affects species with a nervous system we have no alternative but to conduct aspects of this work in animals. Where possible we will aim to work with clinical colleagues to use human models and we will only conduct work outlined in this project in rodents. We will utilise a number of approaches to minimise animal use including using stored samples from previous work or human post-mortem tissue.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All work which will be carried out under this project is and will be carefully planned by experts to ensure the minimum number of animals is needed for each study. This includes working with trained statisticians and our own expertise in this area which allows a pre-calculation of the number of animals needed to answer a specific question. No study will be conducted unless the scientific goals are deemed to be of significant impact and this will always be balanced against the impact and number of animals required. We use a number of novel highly effective models related to different primary headache disorders and where possible we use internal control data to minimise the need for placebo groups. To enable a detailed record of all work and as part of good laboratory practice, we will generate a protocol for each experiment outlining the objective(s); a description of the experiment, covering such matters as the experimental treatments, the size of the experiment and the methodologies to be used. Protocols will then be reviewed by appropriate staff prior to work commencing to ensure they are optimal. We will further use novel genetically modified mice which carry human mutations which predispose individuals to forms of migraine. While these conditions are rare we can use their animal models to gain detailed information about more general headache disorders. As these mutations are known

	and controlled the number of animals needed for this type of work is often lower than for unaltered rodent strains. The work will also be conducted with careful consideration of gender, using only male animals where appropriate to minimise variation as a result of female hormone cycles, which can result in the need to use more animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We intend to use a number of models of primary headache disorders, the majority of which have undergone rigorous scientific review for their benefits and ability to detect meaningful clinical predictions. Each of the models allows a different component of the headache pain system to be targeted. We and others have used these models for a number of years carefully characterising their benefits and refining the procedures in rodent models to minimise the use of higher species. The majority of the work (over 60%) is conducted under non-recovery general anaesthesia to minimise the impact; however where appropriate animals may undergo chronic drug administration which has been shown to be a promising model of medication overuse headache, which as mentioned earlier is a major current clinical concern. The repeated administration of drugs is expected to have minimal impact on the animal which will be very carefully monitored throughout and last for no more than 20 weeks. We will provide post operative analgesia where appropriate and when it does not interfere with the experimental aims, for example during certain surgical recovery experiments. For genetically altered animals the adverse signs are not always known but existing ones, show for example only mild experimental aversion to bright light (common in migraine). In such cases the housing of the animals will be carefully controlled to ensure they are not unnecessarily exposed to any bright lights.

	At all times during the project should any unexpected adverse events occur animals will be humanely culled and immediate help requested from the named veterinary surgeon to ensure we understand what went wrong and make sure it is unlikely to happen again.

<table>
<tr><td>Project 41</td><td>Studies on the neurobiology of sensation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Touch, pain, itch</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></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>Chronic pain is widespread, estimated to affect 1 in 5 of the population. Most of this pain is not well treated by existing medication because the drugs we have show only limited effectiveness and they all have side effects. By understanding how the brain generates sensory experiences, we hope to identify ways in which pain can be better treated.</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>New treatments for pain depend on understanding what causes it in the first place. By providing that understanding with this work, we hope to facilitate the development of novel analgesic drugs.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We will use approximately 2500 mice and 100 rats per year. Some of the mice bred in protocol 4 will subsequently be used in other protocols. We will undertake power calculations to estimate the minimum number of animals we need to study to obtain statistically robust results.</td></tr>
</table>

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 create animal models of clinical conditions such as arthritis, diabetes, cystitis, bone cancer. The primary intended adverse effects sensory abnormalities such as itch or pain. We will carefully monitor animals and use criteria established by the Home Office to ensure that animals experience only mild or moderate distress for limited periods of time.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are principally interested in how the nervous system generates sensations in health and disease. These sensations are multidimensional and emerge from the integrated action at multiple levels of the nervous system. Therefore while some information is available from the study of single cells in vitro (which we will exploit), a full understanding can only be obtained by studying intact organisms. A limited number of studies can be undertaken in human subjects (and we actively undertake such work), but for ethical reasons not all studies can be done in people. We will therefore study rodents, where a great deal of previous work has been undertaken.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers will be kept to a minimum by carefully planning all studies to ensure that the group sizes are kept to the smallest possible size at which a significant effect could still be obviously detected. Additionally as much data will be obtained from each individual as is possible; this will include data from a number of simple behavioural tasks to assess post-injury function, data from neurophysiological recordings and detailed anatomical data collected from the same 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.	Rats will be used for the majority of studies as they closely mimic the pathology of human nervous system. Mice will be used in some instances however, specifically when the use of a particular genetic modification can reveal valuable information (transgenic mice). The models we will use will either be discrete injuries of nerve fibre pathways in order to gain understanding of how specific sensory and motor

	projections respond to injury and various experimental therapeutics; or, we will use clinically relevant models which closely mimic the pathology, disease progression and functional readouts observed in human patients; in these cases we can test promising therapies in these valuable pre-clinical models as a first step towards translating a therapy to the clinic. In all the injury models selected for this project, the most substantial effects on animal welfare will be during the initial post-injury phase (up to 1 week post-injury) after which substantial recovery of general health will be observed in all animals along with significant functional improvements in the vast majority. All animals will receive intensive care, particularly in the acute post-injury phase, to ensure high standards of welfare are maintained. This will include cages remaining on heated mats, administration of analgesics and saline, provision of soft, easily digestible food.

Project 42	**Neurodegeneration and autophagy in zebrafish**	
Key Words (max. 5 words)	Zebrafish, neurodegeneration, autophagy	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	Many of the common neurodegenerative diseases affecting humans (such as Alzheimer's disease and Parkinson's disease) are caused by proteins that form clumps or aggregates inside nerve cells. These diseases are generally devastating to both the patients and their families and are currently incurable. The build up of these proteins can be compared to the accumulation of rubbish inside cells. We have previously shown that we can make cells "eat up" or dispose of these clumps of protein by increasing the speed of a naturally-occurring process called autophagy. The main aim of this project is to better understand how autophagy happens inside the cells of a living animal. We want to answer the following questions: 1. When does autophagy happen inside normal cells and what happens to this process when cells are old or diseased? 2. Is it harmful or beneficial to increase this waste disposal process in different cells (e.g. in the brain)? 3. Can we use medicines to increase the waste	

	disposal process and if so, does this slow down the progression of certain 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)?	At present, there are no known treatments that slow down or reduce the severity of diseases such as Parkinson's and Alzheimer's disease. This project will improve our understanding about how cells, particularly those in the brain, clear away harmful proteins found in these types of diseases. We will find out whether it is safe to increase autophagy, a waste disposal process that occurs naturally within cells. We will find out whether there are differences in cells of different ages and of different types (e.g. brain cells or muscle cells). We will then test new treatments to find out whether these treatments can reduce the amount of harmful proteins that cause Alzheimer's and Parkinson's disease, and related conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use zebrafish. We estimate that we will use —48,000 zebrafish over the 5 years of this project (approximately 9,600 per year).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the procedures carried out in this work are mild and are unlikely to cause any pain or suffering. In some procedures, the fish are anaesthetised and on rare occasions the fish do not recover from anaesthesia and so will be culled by a humane method, The other expected adverse effect results from the immobilisation of juvenile fish to allow us to perform microscopic observations. On rare occasions, bruising or damage occurs while getting the fish into the correct position. If this happens, the fish will be culled immediately by a humane method. There is one procedure in this licence which is moderate in severity. We use this procedure to find out the amount of drug that it is safe to give without causing harmful effects. At high concentrations of druqs, some fish will show signs of toxicity, such as increased heart rate, failure to swim in the correct position, sedation. Fish will be culled by a humane method as soon as any toxic effect is observed. All animals will be humanely killed at the end of procedures.
Application of the 3Rs	
1. Replacement	To understand biological processes in diseases that affect tissues like the brain or non-dividing cells like

State why you need to use animals and why you cannot use non-animal alternatives	nerves and muscle, we need to study these processes in living animals. We use zebrafish as they are vertebrates and share a high degree of genetic, tissue and pharmacological similarity with mammals. We need to be able to assess pathology in non-dividing nerve cells within a living brain, with the appropriate connections. When testing potential therapeutics, we need to examine possible side effects in all body tissues and to test whether these therapeutics can get to the tissue we are trying to treat (e.g. can compounds cross the blood-brain barrier). Cell culture models cannot easily represent long term behaviour of specific types of nerve cells through various stages of development.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are one of the main groups pioneering the use of zebrafish models to reduce and replace the use of mammalian (mouse) models on our research. We have considerable experience in developing zebrafish models of human disease and have developed assays in zebrafish which have short duration times (typically 5 -8 days long) and have performed power calculations to ensure we use the smallest group size (typically 5-10 animals per treatment or condition) to obtain meaningful and statistically 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.	Zebrafish are small tropical fish that have many advantages as an animal model for this work e.g. one pair of adults produces 100-200 fertilised eggs per breeding; young fish are transparent allowing internal organs such as the brain can be seen without surgery; there is a high level between the genes and tissues in man and other vertebrates. We have carefully developed genetically modified zebrafish which have aspects of human disease but in which we have limited the severity of the disease, e.g. by expressing the disease-causing gene in only one cell type in the eye. When these cells degenerate, only one type of cell dies (causing nightblindness) whereas if the transgene were expressed throughout the brain, the animal would have neuronal deficits, ill health and reduced lifespan. We also use a special technique (Ga14/UAS transgenic technology) so that parent lines do not express disease-causing proteins and do not have any signs of disease. The disease protein is only expressed the when two carrier fish are

	crossed together. In such crosses, only the offspring express the disease protein and show disease pathology. Using such lines, we only generate offspring for experimental purposes and the adult animals that are kept to maintain a breeding colony are viable and healthy, with no sign of disease.

<table>
<tr><td>Project 43</td><td colspan="3">Metabolic regulation in health and disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Metabolism; obesity; diabetes</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 section 5C(3)</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>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 colspan="3">Our laboratory is interested in how the body regulates appetite, blood glucose and adiposity; mostly from an academic view of how different organs function, but also with a view on what may go wrong during the development of obesity and diabetes. Although we sometimes take a reductionist approach to understand specific pathways (e.g. by in vitro electrophysiology), we prefer to take a more holistic, whole-animal view. There is no doubt that the obesity epidemic is the biggest medical problem facing industrialised societies. Therefore, any new therapeutic targets will have the potential of fulfilling an enormous medical need. We will play our part through the identification of new potential targets and by developing new transgenic disease models. Furthermore, by showing where drugs can have beneficial or adverse effects we will provide a useful service to other researchers in the field and to the pharmaceutical industry.</td></tr>
</table>

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 increase in the incidence of obesity and type 2 diabetes mellitus has reached epidemic proportions, due partly to the ageing population. Currently, it is estimated that there are 2 billion overweight people globally and 600 million of these are clinically obese. Without new and effective therapeutic interventions for the treatment of associated co-morbidities, the rising care costs could bankrupt the National Health Service and other health providers worldwide. Conservative commercial estimates of the annual market opportunity for anti-obesity and anti-diabetic drugs are over $100 bn. This project will guide future development of drugs, especially as co-therapies for lifestyle changes. The applicant has been involved previously in successful collaborative projects with preclinical, clinical and industrial partners, providing evidence for several novel targets for drug development that has underpinned programmes by a number of UK and European pharmaceutical companies. We will collaborate with pharma on projects to increase the quality of life of patients. For example, drugs used as anti-psychotics produce side effects that can greatly increase weight, leading patients to stop taking their medicine. We will validate a model of psychotic-like behaviour that is susceptible to drug-induced weight gain, and use this to search for co-treatments or alternatives which prevent or attenuate weight gain.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 4950 rodents (mice or rats), over a 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?	Our research requires manipulation of mice and rats which can lead to certain controllable adverse effects. Suffering is kept to a minimum through careful experimental design and through many years of refinement. Most types of experiment are non-invasive, in that the behaviour of the animal (e.g. eating, activity, metabolism) are monitored and then the animal is sacrificed so that changes in gene and protein expression can be measured in

	post mortem tissue. It is accepted that any manipulation, such as handling or putting an animal in a novel environment, is to some extent stressful. However, if animals are stressed, they will not behave normally and our studies will be less meaningful. Therefore, we strive to cause the least stress to the subjects by acclimating them to handling and to new environments. In order to modify activity in the brain or to measure hormonal responses, rodents will often first undergo surgery under general anaesthesia in order to insert tubes into the brain or into veins. The animals are given painkillers after surgery and they recover well within a few days without any lasting distress. With the tubes in place, we are then able to make injections of hormones or drugs which will affect appetite and metabolism, or remove small samples of blood in order to measure nutrients and hormones, in awake, normally-behaving animals. All of our experiments in normally-behaving animals are categorised as mild, but recovery surgery under general anaesthesia is classified as moderate severity. Wherever possible we will use anaesthetised animals or take tissue samples to allow experimentation either in non-sentient animals or *in vitro*. Following experiments, or to allow the collection of tissues, animals are killed by a Home Office-approved humane method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever it is possible to look at mechanisms in a targeted way, we use *in vitro* preparations rather than live animals. For example, if we wish to know how a drug or hormone acts on individual cells we can make, for example, electrophysiological recordings from brain slices or carry out hormone-release studies using isolated cells from the pancreas. However, live mice do need to be used to model the development of obesity and diabetes, as the diseases involve multiple, interacting tissues.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By using the latest transgenic mouse technologies that target specific cell types, we have been able to greatly refine our methods, so that we can use fewer mice, which also undergo fewer manipulations. When designing studies, we refer to the ARRIVE guidelines (http://www.nc3rs.org.uk/page.asp?id=1357). When

	necessary we will carry out pilot experiments to allow power calculations and ensure that, in the full experiments, we will use the minimum number of animals required to provide valid data. If there is any doubt on experimental design or statistical analysis, advice will be sought from the University's resident statistical advisor.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	By using latest techniques to breed transgenic animals, which express selected genes in only the cells we wish to investigate, it is possible to make many Refinements to our research. Since we can target single cell types selectively, we do not need to use the crude approaches previously required. We can generate much better data and much faster. For example, we have a number of mouse models in which specific nerve cells can be visualised outside of the living animal. These new approaches will lead to an exceptional Refinement and Reduction of animal experiments in the long term. Furthermore, our new transgenic models will be made available to other researchers, thus leading to a further Refinement of their experiments too.

Project 44	**Biophysical models of neural activity and neurovascular coupling**	
Key Words (max. 5 words)	Excitation, inhibition, models, LFP, neurovascular.	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project will address the fundamental question regarding the balance and interaction between neural excitation and inhibition in the intact brain. It will also investigate the dynamic relationship between changes in neural excitation and inhibition, and the ensuing changes in haemodynamic variables.	
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 proposed mathematical model of neural activity has a framework which is significantly simpler than the existing computational models in the current literature. This means that the model structure can be extended to include cortical depth and surface information, hence the extension to EEG modelling, with much ease compared with those using complex computational neural models. The ability of the model to separate neural recordings into components of excitation and inhibition means that the balance between these components can be monitored even if they cannot be measured directly. Furthermore as EEG is increasingly used for the medical diagnosis of diseases such as epilepsy, the interpretation of EEG	

	in terms of the underlying neural mechanisms will significantly enhance its ability to diagnosis disorders in which the balance between neural excitation and inhibition is interrupted.
What species and approximate numbers of animals do you expect to use over what period of time?	A rat model will be used, and the number of animals required under this project is estimated at 500 (maximum) 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 animal will be anaesthetised while all physiological variables will be monitored closely to ensure they are within appropriate ranges. Infection is uncommon during surgery and the subsequent experiment. Should complications arise due to infection (<5%), the animal will be euthanised using an appropriate Schedule 1 method. During the thinned cranial window preparation, small amounts of bleeding may occur from the bone. In most cases the bleeding is insignificant and swabs may be used to clean the surface of the skull. Should the bleeding becomes significant, swabs and bone wax will be used to stop the bleeding after which a layer of cyanoacrylate will be used to stabilise the thinned cranial window. Due to the duration of the experiment and the intervention needed, it will not be appropriate to re-use the animal. Thus the level of severity is non-recovery. At the end of the procedure the animals will be euthanised by a schedule I method, or by transcardial perfusion fixation with an appropriate fixative. If any procedural complications do arise veterinary advice will be sought immediately, or the animal will be euthanised by a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The proposed electrophysiological experiments and pharmacological intervention cannot be conducted safely in humans. Also in order to investigate haemodynamic responses to neural activity, blood flow and transmural pressure to the cerebral blood vessels must be maintained, hence the *ex vivo* preparation (e.g. brain slices) will not be suited for this research.
2. Reduction	To minimise animal usage, we will use concurrent

Explain how you will assure the use of minimum numbers of animals	recordings of neural and haemodynamic signals to establish models of neural activity and of neurovascular coupling, and a repeated measures design, if possible. We will also eliminate as much experimental noise as possible in order to maximise signal to noise ratio, further reducing the number of animals needed per experimental condition. At all stages of the project, we will consult a professional statistician, when required, to ensure an optimal statistical design and the number of animals required is minimised, yet sufficient precision and power are maintained.
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.	A rat model will be used as they have a very well understood cerebral anatomy. Also there exists a wealth of research and data which we can use to compare our results with in the literature. All animals will be under terminal anaesthesia which will be carefully monitored throughout the experiment to ensure that all physiological parameters (e.g., body temperature, heart rate, respiration rate, blood pressure, blood saturation) are within appropriate ranges and are stable to minimise animal suffering. To prevent overheating during drilling, the skull surface will be cooled with saline.

<table>
<tr><td>Project 45</td><td>Understanding and influencing neural responses in the rodent visual system</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Vision, cortex, rodent</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>
<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></td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td></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>The objective of this study is to better understand the nature of information processing in the brain, using the visual system as a model.</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>Greater understanding of how the nervous system processes information will contribute to development of therapies of neurological and psychiatric disorders including blindness, epilepsy, schizophrenia, and autism.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice; 240 per year</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>Animals will be trained to perform visual discrimination tasks to indicate their visual perception, while their brain activity is monitored. After the experiments, the animals will be humanely euthanized.</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
<tr><td>1. Replacement
State why you need to use animals and why you cannot use non-animal alternatives</td><td>Measurements of visual performance are currently only possible in live animals. Likewise, measurements of cortical responses to visual stimuli in mammals are currently only possible in live animals. Although technology to simulate neuronal circuits function is becoming increasingly powerful, the parameters needed to constrain such simulations are currently unknown. The data collected in this project will help constrain these</td></tr>
</table>

	parameters, bring closer the goal of accurate neuronal simulation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	New technology that allows recording from large numbers of neurons makes it possible to use much fewer mice than previously.
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.	This kind of research has historically been performed in cats, ferrets, and macaques. It is now becoming possible to perform at least some aspects of this research in rodents, and specifically in mice. Mice are a species whose needs are easier to meet in a laboratory environment, so moving to research in mice constitutes a refinement that is in agreement with the best principles of animal research. Indeed, mice are becoming the prevalent mammalian species in biomedical research, as only in mice is it possible to use extremely powerful techniques of genetic manipulation and targeting. Furthermore, by using mice we can employ techniques of simultaneous population recording, which dramatically reduce the number of subjects required. Indeed, this form of imaging is much easier to perform in mice than in other species, and allows one to record tens to hundreds times more neurons than an experiment using conventional methods. The behavioural paradigms we use have been designed to involve minimal discomfort, and mice will be constantly monitored should signs of discomfort appear.

<table>
<tr><td>Project 46</td><td>Neurorestoration following nervous system injury</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Spinal cord injury, peripheral nerve injury, sensory, motor</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></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>Spinal cord injury in particular leads to permanent sensory, motor and autonomic impairments and therefore has devastating effect on the life of the patient as well as the lives of their family and friends. There are currently no successful treatments for traumatic injury to the central nervous system, so there is a clear unmet clinical need to develop therapeutics that will lead to reduced tissue pathology and/or enable functional recovery following central nervous system injury.

Whilst functional recovery is often observed in patients with an injury to the peripheral nervous system, this recovery is often sub-optimal and can result in impaired function and/or neuropathic pain. There is therefore a clear clinical need to enhance the natural repair mechanisms which take place following injuries such as these.</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 results from this project will further ourunderstanding of the processes occurring following injury to the central or peripheral nervous system, which prevent or impair successful recovery of function. This understanding will aid in the development of potential therapeutics designed to enhance repair and improve functional recovery, many of which will be assessed as part of this project with the aim of developing a therapy which could be translated to the clinical situation to be used in human patients.</td></tr>
<tr><td>What species and</td><td>We will use approximately 300 mice and 700 rats</td></tr>
</table>

approximate numbers of animals do you expect to use over what period of time?	per year. Rats and mice will be the only animals used in this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The key procedures being applied in this project involve the induction of injury to either the central or peripheral nervous system. All surgical procedures will be carried out under general anaesthesia and animals will also receive post-operative medication to minimise any potential discomfort. This will often be followed by therapeutic intervention and various methods of assessing functional recovery, in particular the assessment of various functions whilst the animals perform behavioural tasks and the assessment of nervous tissue function by directly or indirectly recording the electrical activity of these tissues. Following injury, animals will show varying levels of impairment depending on the severity of the injury they have received. Impairment to motor function will be particularly obvious, all animals will display some form of paralysis at early post-injury stages. General well-being of the animals will recover quickly following injury (within the first week) and in the vast majority of cases this will be followed by dramatic functional improvements such that animals will be able to support body-weight on their affected limbs by 2 weeks post-injury. In some studies (transection injuries and in some severe contusion injuries) weight-support is unlikely to be restored, these animals will therefore receive intensive care to ensure their general welfare is maintained to the highest possible standard. At the end of the studies, animals will undergo euthanasia and their tissues will be used for further analysis of treatment effects; making maximal use of tissue will reduce the number of animals.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Many of our studies involve the assessment of novel therapeutics with the aim of finding a therapy that could improve functional outcomes in patients with spinal cord or peripheral nerve injuries. Such therapies will clearly be required to show robust efficacy in relevant pre-clinical models before clinical testing could be considered. There is no alternative system that can model the integrated actions and the complex pathology of the injured nervous system. Therefore, it is necessary to undertake much of our work in animals.
2. Reduction Explain how you will assure	Animal numbers will be kept to a minimum by carefully planning all studies to ensure that the

the use of minimum numbers of animals	group sizes are kept to the smallest possible size at which a significant effect could still be obviously detected. Additionally as much data will be obtained from each individual as is possible; this will include data from a number of simple behavioural tasks to assess post-injury function, data from neurophysiological recordings and detailed anatomical data collected from the same 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.	Rats will be used for the majority of studies as they closely mimic the pathology of human nervous system injury. Mice will be used in some instances however, specifically when the use of a particular genetic modification can reveal valuable information (transgenic mice). The models we will use will either be discrete injuries of nerve fibre pathways in order to gain understanding of how specific sensory and motor projections respond to injury and various experimental therapeutics; or, we will use clinically relevant models which closely mimic the pathology, disease progression and functional readouts observed in human patients; in these cases we can test promising therapies in these valuable pre-clinical models as a first step towards translating a therapy to the clinic. In all the injury models selected for this project, the most substantial effects on animal welfare will be during the initial post-injury phase (up to 1 week post-injury) after which substantial recovery of general health will be observed in all animals along with significant functional improvements in the vast majority. All animals will receive intensive care, particularly in the acute post-injury phase, to ensure high standards of welfare are maintained. This will include cages remaining on heated mats, administration of analgesics and saline, provision of soft, easily digestible food, bathing of animals whilst unable to groom and manual expression of the bladder and colon in animals in which these functions are disrupted.

<table>
<tr><td>Project 47</td><td colspan="3">Prion disease in ruminants</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Prion, scrapie, BSE, pathology</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></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>
</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">Scrapie (a prion disease) is a natural disease of sheep and goats. It is not known how it spreads between animals and between flocks. It is incurable and causes degeneration of the nervous system. The main aims of this project are to understand how these diseases spread, with particular interest in how infection travels from the site of infection to the brain.</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">The benefits of this project will come in improved welfare of on-farm sheep and goats and in the future control and elimination of an incurable brain disease. Our results will also be useful in the control of human prion diseases and development of diagnostic tests in all affected species.</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">Over the 5 years of the project:

Sheep, approximately 1,200
Mice, approximately 500</td></tr>
<tr><td>In the context of what you</td><td colspan="3">Many of the animals will develop TSE clinical signs</td></tr>
</table>

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?	and the level of severity is moderate. The animals will be killed by approved methods when disease is manifest but not severe. Experience allows accurate recognition of symptoms and euthanasia at a defined stage.
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 reliable methods for replacement of animals in this study. Work is underway to develop such methods but none is yet suitable for study of spread of prion disease through multiple cell types within an infected body. This project will however produce samples which will allow non-animal alternatives to be developed.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The project plans to use minimum numbers of animals while at the same time using sufficient to allow reliable interpretation of results. The numbers per experiment are continually reviewed statistical advice taken.
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 no invertebrates or fish models available to study prion diseases and non-animal methods will not allow us to study complex interactions between body cells and brain cells. This project will however provide tissue samples which will help in the development of non-animal methods.

<table>
<tr><td>Project 48</td><td colspan="3">MRI and IHC in a preclinical migraine model</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">MRI, immunohistochemistry, migraine, sumatriptan, CNS</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 section 5C(3)</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">The proposed multi-disciplinary project will utilise magnetic resonance imaging (MRI) and microscopy to investigate altered activity in the central nervous system in a novel preclinical model of migraine. The over-arching aims of the project are to further understand the generation of migraine and its symptoms, and to identify the specific brain regions involved. The results of our studies will enable us to correlate MRI changes with the development of migraine-like symptoms and the expression of specific indicators of altered excitability in the brain. This will advance our understanding of the mechanisms underlying the development of migraine. We also aim to establish how anti-migraine drugs affect MRI and behavioural responses in our model. This project has the potential to establish a translational MRI model that could be utilised to identify and determine the efficacy of novel therapeutic strategies.</td></tr>
</table>

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)?	Better understanding of the mechanisms underlying the development of migraine will help to provide better therapeutics to treat migraine pain in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	80 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?	Animals will be given an infusion of a drug that will induce facial allodynia (sensation of pain in response to light touch or pressure). Behavioural tests involving touch stimuli will cause no more than transient discomfort as the animal is free to withdraw from the stimulus as soon as it becomes painful. We do not expect animals to experience any other adverse effects. The model used is well established and known for not causing any signs of distress in rats. Animals will be humanely killed at the end of the procedures
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our goal is to mimic some of the signs of migraine experienced by patients, in order to study the mechanisms underlying its aetiology. This requires a functioning nervous system as similar to humans as possible. Rats are the lowest species that we could use which let us study changes in within the central nervous system (CNS) together with measurements of migraine-like pain behaviours As we are studying behavioural parameters which are only present in a mammalian nervous system, cell culture models and other in vitro/computer models will not suffice for assessment of complex behaviour. Therefore there is no feasible alternative that would entirely replace animals
2. Reduction Explain how you will assure the use of minimum numbers	The number of animals required was calculated using statistics to estimate numbers required to detect significant effects with confidence

of animals	
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use a well established preclinical model of migraine headache pain that involves the sustained infusion of sumatriptan. To our knowledge no other species have been used to investigate the effect of sustained exposure to sumatritpan to model migraine like pain. Using different species will require more animals in order to investigate the effects of sustained sumatriptan in this other species. The behavioural signs of migraine-induced pain do not cause the animals any difficulty in obtaining food or water. Data from unhealthy or stressed animals cannot be used, so every effort will be made to keep the animals as healthy and calm as possible during handling, housing and transportation. Pain and suffering will be kept as low as possible, surgeries will be performed under general anaesthetic and pain relief will be given if necessary. The survival will be kept as short as possible and animals will be handled using the best welfare standards so that no animals can suffer un-necessary.

<table>
<tr><td>Project 49</td><td>Cortical and sub-cortical motor control</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cortex, spinal cord, reticular formation</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></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>This project has the overall aim of improving our understanding of the different brain and spinal cord centres involved in the control of movement, and translating the knowledge into improvements in therapy for patients recovering from injury, such as after stroke or spinal cord injury.
Specifically, we aim to understand the relative contributions of different parts of the nervous system to movement control in healthy animals, and how information is processed within each neural centre. We will then map how the surviving centres change after damage. We also aim to understand the processes which can change neural connections within these circuits, and to use this knowledge to devise stimulus protocols which can modify connections.</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>Stroke is currently the leading cause of disability in the UK. There are around 150,000 new strokes annually, one quarter in individuals aged under 65. The UK has 1.2m stroke survivors, around half of whom live with a disability that affects their everyday life. Total care costs for stroke in the UK are estimated at £8.2 billion (all figures taken from</td></tr>
</table>

	The Stroke Association). Therapeutic options for improved rehabilitation are limited, especially for hand function – one reason for this is a poor understanding of the scientific basis for motor control, and the processes underlying its recovery after insult. The information gained by this project will allow us to devise principled new strategies for therapy to improve rehabilitation. If this leads to even small improvements in function, it will translate into major social and economic impact.
What species and approximate numbers of animals do you expect to use over what period of time?	40 macaque monkeys over 5 years 250 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?	Monkeys will be trained to accept some restraint (a neck collar), and to perform a behavioural task. They are motivated to perform the task by having restricted access to food, and occasionally fluid; food and fluid rewards are then given for correct task performance. After training is complete, they are surgically implanted with a headpiece to allow head stabilisation and electrodes to record muscle activity from the forelimb. Recordings will then be made from the central nervous system in the conscious state, whilst the animal performs the task. The most common adverse effects are associated with wound infections associated with the chronic implants. In a small proportion of animals, focal surgical lesions will be made on one side of the brain. In the days immediately following, these animals may need nursing help with feeding due to impaired movement ability. However, as in human stroke patients with small lesions they often show a rapid recovery. Rats may be prepared for recording by a surgery to inject neural tracers or novel genetic material, after which they are allowed to survive for a few weeks. Subsequent experiments involve terminal anaesthesia, and then making electrophysiological recordings or removing brain samples for analysis in vitro. Recovery from the initial surgery is unlikely

	to show adverse effects, and no adverse effects can occur in the final terminal procedure. The macaque experiments will have moderate severity, although the licence limit of 'severe' may be reached for short periods in some animals associated with the period immediately after a lesion. Rat experiments will be of moderate severity. At the end of experiments, all animals are humanely killed.
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 complex interplay of brain circuits in different regions, and as such must be carried out in intact organisms. The laboratory does run a substantial programme of experiments in healthy human volunteers and patients; however, these can only produce indirect data. Detailed understanding at the level of single neurons and their connections can only be achieved using the invasive approaches possible in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use sophisticated multi-electrode recording methods, which ensure that the maximum of data is gathered from each animal. Experiments in awake monkeys often yield sufficient data for publication from just two animals. Experiments under terminal anaesthesia use advanced anaesthetic methods to maintain the animals in good condition for extended periods (around 70 hours for macaques); this again enables us to gather extensive datasets from each animal.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	Some basic circuit properties can be investigated in rats. However, the neural centres and connections controlling movement differ in key aspects in primates compared with non-primate species. To ensure that our results are directly applicable to human patients, we must use old world primates such as macaques. Our techniques have been refined over many

(harms) to the animals.	years, and we continually seek to improve them. All recovery surgeries are carried out under full aseptic conditions, with advanced anaesthetic regimes which produce rapid and uneventful recovery. Full programmes of post-operative pain management are in place.

<table>
<tr><td>Project 50</td><td>Screening for aggression therapeutics in zebrafish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Aggression, zebrafish, drug screen, behaviour</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>
<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>The aim of this project is to better understand the genes and neural circuits that control aggression and to identify novel drug treatments for this behaviour.</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 work represents a first step towards developing improved therapeutic treatments for human aggression. Increased understanding of the neurotransmitter linked to aggression may also aid clinicians in developing biomarkers for this behaviour.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>I expect to use 770 adult and 20500 juvenile zebrafish (Danio rerio) during the 5-year period covered by this project licence.</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>No adverse effects are expected as a result of our behavioural testing or drug treatments. In case of unexpected adverse effects following drug application (such as reduced swimming due to</td></tr>
</table>

level of severity? What will happen to the animals at the end?	sedation) the experiment will be terminated and a vet contacted for advice. The amount of time spent recording behaviour will be kept to a minimum, in order to reduce any possibility of psychological stress to the animal.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The research described in this proposal uses behavioural analyses of zebrafish to examine the function of genes that control impulsive aggression in humans. Since it is difficult, if not impossible, to study behaviour in cell lines or organ cultures it is necessary to use animals in this research. I have looked at the FRAME website and other relevant sources for possible replacement protocols but have not found suitable alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The research to be carried out in the first part of this project will be based upon genes that have been identified in genome-wide analyses of human patients that exhibit heightened aggression. This will reduce the number of animals used since these genes have already been identified before starting this research. The numbers of animals to be used in these experiments have been chosen based upon data from published experiments (e.g. Norton et al., 2011) which in turn was based upon group size calculations for each protocol. For example, 13 adult zebrafish are required in each group of a behavioural experiment for an unpaired t-test to have an 80% chance of detecting a difference in aggression at the 5% significance level. These are the minimum number of animals required to ensure enough statistical power to detect significant differences. For immunohistochemistry, 6 adults are required in order to ensure a reproducible staining pattern (Norton et al., 2011). For the drug screen we will calculate the correct concentration of drug to be administered to animals based upon previous work, pilot experiments, online resources and manufacturer's recommendations including LD_{50} and EC_{50} information. When necessary, further pilot

	experiments will be conducted in order to reduce the number of animals needed in subsequent 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 zebrafish for this research because of the combination of genetic tractability, ease of visualisation of neurotransmitters in the intact brain and well-established behavioural protocols. Zebrafish are ideal for this research because drugs can be applied by immersion, obviating the need for potentially painful injections. Furthermore, their genetic similarity to other vertebrates will make it easy to translate information gathered in this research to other animals. Adult zebrafish (including both wild-type lines and GM fish such as transgenics and mutant lines) will be housed in the best possible conditions in our aquarium. This aquarium has constantly circulating water, the quality of which is monitored regularly. Fish are maintained at low stocking density in environment-enriched tanks. Fish will be minimally handled during the project and anaesthesia will be used during experimental manipulations when necessary.

<table>
<tr><td>Project 51</td><td colspan="3">Nervous system development and function in zebrafish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Vision, CNS, zebrafish, autism, epilepsy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3"></td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</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 Objectives are to discover:
1. To understand how neural circuits in the visual system develop.
2. To understand how the visual system works and how different types of neuron within the visual system contribute to visual system function and visually-driven behaviours
3. To study how neural circuit development and function are altered when genes implicated in human neurodevelopmental disorders are disrupted</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">1. Our research will provide insight into how the normal brain develops and how, once established, neural circuits work. Understanding these processes in the healthy brain is an essential prerequisite for understanding how they are perturbed in the diseased brain (see below).

2. Our research will also focus on how disruption of genes implicated in human neurodevelopmental disorders alters circuit development, function and</td></tr>
</table>

	behaviour. This will potentially provide valuable insight into how we may intervene to ameliorate the symptoms of human disorders such as Autism Spectrum Disorder and epilepsy.
What species and approximate numbers of animals do you expect to use over what period of time?	Over five years: Zebrafish: 10,500 Adults, 10,000 of which are used soley for the production of embryos which will be used in experiments. 25000 larvae, the majority of which will be used prior to the developmental stage at which they become protected by the act.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For the vast majority of zebrafish they will live a normal life within the animal facility with no adverse effects and be used for breeding until they are humanely killed around 18 months of age. The majority of experiments will be performed on zebrafish larvae prior to the developmental stage at which they become protected by the act.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are examining how the brain processes visual information. The complex neural architecture required for processing sensory stimuli and driving behavioural output cannot be generated in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To prevent the need for severe procedures, we employ advanced genetic approaches with single cell resolution to determine outcomes. A suffering animal is less informative than one with a specific minor defect that can nevertheless be analysed in detail. For this reason we use zebrafish, whose optical clarity permits use to track the behaviour of defective circuits in an otherwise healthy animal. Where more severe experiments are essential, statistical methods, such as the resource equation, will be used to ensure that cohorts are the minimum number needed to give reliable results. Pilot

	experiments will assess the likely appropriate size of these cohorts.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Most of our experiments will be performed on zebrafish, both because they have experimental advantages and because they are regarded as less sentient than mammals. All moderate procedures involve the use of anaesthetics and pain relief as advised by veterinary staff

<table>
<tr><td>Project 52</td><td>Develop and treat fish models of neurological disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fish, neurodegeneration, disease, ageing, animal model</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>
<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>Although neurodegeneration results in the loss of specific neurons, the exact mechanism or processes by which neurons die is unknown even where the exact genetic mutation that leads to the disease have been identified. Additionally, good models of disease that recapitulates many aspects of human disease and that are appropriate for identification of potential therapeutics is lacking. The goal of the project proposed is to develop genetic fish models of neurodegeneration with specific focus on genes such as SOD1, TARDBP and C9ORF72 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.</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>We hope that the fish model would complement rodent models and thus provide a more rapid and high 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.</td></tr>
</table>

What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use Danio rerio (zebrafish) and to test the disease models and plan to use approximately 12,200 zebrafish in our studies over a period of 5 years. Many of the animals generated for the study will be utilized just past protection (6-15 dpf) and a smaller fraction would be used for older age 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?	As we are primarily studying ageing related diseases, many of the phenotype we observe would mimic ageing and thus of marginal consequence to the health and welfare of the animals. As these animals are living sedentary lifes (as compared to living in turbulent river flows), the impact of their disability on their living conditions are minimised. Thus most of the animals in these studies will have no visible symptoms with a fraction of older animals showing moderate symptoms with minimal impact on their ability to obtain feed and move around. When studies are completed on the animals, they are humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Neurological diseases are complex diseases that require complex systems to study the disease process that occur during adult life. It is not possible to model the symptoms, pathology and behavioural changes observed in human disease in non-animal systems. Additionally it is becoming clear that diseases such as ALS also are system wide disorders with changes in other organ systems such as the immune system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our goal in using fish in addition to studying disease process is to uncover early disease specific changes that occur prior to the onset of symptoms and hence, we try to identify early disease biomarkers that can be analysed before they become protected (5.25dpf). This will allow great reduction in use of animals in research. Additionally, we power our studies to utilize the minimal number of animals to obtain robust statistics on the data collected and thus reduce the number of animals used and the number of times the studies need to be repeated.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	As mentioned earlier, our goal is to identify early disease biomarkers for disease. When such markers are identified, we can utilize these markers in our drug screening and therapeutic development program to refine the screening to utilize earlier readouts of disease to test efficacy of novel therapies. These allow refinement of our protocols to reduce the use of animal in research.

Project 53	**Gene function in neuronal circuit formation and maintenance**	
Key Words (max. 5 words)	Wnt, synapse, neurodegeneration	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 aim to elucidate the mechanisms that regulate the formation, function and maintenance of the contacts called synapses between nerve cells or neurons. Our objectives are: 1) To examine the role of endogenous secreted proteins in the behaviour of nerve cells during the formation of nerve connections. Here we will test the hypothesis that specific secreted factors stimulate the formation of synapses between nerve cells in the central and peripheral nervous system in vertebrates. 2) To analyse the contribution of secreted molecules in the maintenance of synapses in normal and diseased conditions. Here we will test the hypothesis that secreted factors such as Wnts protect neurons against neurotoxic effects of molecules such as beta-amyloid, implicated in Alzheimer's disease.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or	Our research will elucidate the function of secreted molecules on diverse neuronal functions and determine the molecular mechanisms by which they modulate neuronal connectivity. The identification of	

animals could benefit from the project)?	molecules that stimulate the formation and maintenance of neuronal connections is an emerging approach for the treatment/prevention of neurological diseases. We have previously found that the formation of contacts between motorneurons and muscle cells is controlled by a specific class of secreted factors, called Wnts. These findings may provide new insights into the cause, and possible treatment of neurodegenerative diseases like myasthenia gravis and Amyotrophic lateral sclerosis (ALS), a disease that leads to the degeneration of motorneurons. More recently, we found that blockade of Wnts induces the loss and dysfunction of synapses in the brain. Importantly, blockade of a Wnt antagonist that is elevated by Amyloid-B, protects synapses from the toxic effect of Amyloid beta, a molecule involved in the progression of Alzheimer's disease. Our proposed studies will shed new light into the mechanisms that control the formation and maintenance of synapses in the brain. This information is crucial for the development of therapies for the prevention and treatment of neurodegenerative diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	We are planning to use up to 5,000 mice and up to 1,000 rats over the 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?	Most of our protocols involve procedures of moderate and mild severity with one exception severe severity is applied. Expected adverse effects include post-operative stress or discomfort, drug-induced seizures, aggressive behaviour due to amphetamine administration and weight loss. In the unlikely event that a limb worsens with aging in one of our transgenic mouse lines, the affected mouse will be monitored on a weekly basis and wet food will be placed on the floor to ease its potential discomfort. If the mouse exhibits severe weight loss or difficulty in moving around the cage it will be culled. 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 culled according to schedule 1 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	We are studying the role of secreted factors in the development and degeneration of neuronal circuits and in particular their contribution to the formation and stability of neuronal contacts. Up to date, culture cell lines that form synapses are not available. Therefore, the use of animals and the isolation of primary neurons are crucial for our research program. The use of primary neurons can provide limited information on the function of genes and the molecular mechanisms involved. We therefore plan to use in vivo approaches that required the use of live animals to study their behaviour.
2. Reduction Explain how you will assure the use of minimum numbers of animals	First, when applicable, we are going to perform our experiments in vitro and the data generated will be used to form hypotheses that can then be tested in vivo. In addition, our vitro studies will allow us to better plan and execute in vivo experiments, reducing the numbers of animals required. For example, culture experiments may predict the best time points at which phenotypes may be observed in mutants. All mice will be examined for their phenotypic characteristics regardless of the severity of the main phenotypic appearance derived by the mutation or transgene. Animals will be shared with different member of the research team to maximize their use. Second, animal use will be minimised by importing lines of genetically modified animals generated elsewhere. Also, when transgenic animals are not needed, we will reduce the size of the colony to a minimum. If we believe that we will not use these animals we will consider freezing embryos to obtain a colony at a later time. Finally, all experiments will be analysed using the appropriate statistical tests that will allow us to determine the phenotype conferred by the transgene or mutation using the minimum number of animals.
3. Refinement Explain the choice of species and why the animal model(s)	To understand human development and disease we need to use a closely-related animal model. Rodents are the organism of choice. Mice, in particular, are the only mammalian model organism currently available

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.	where specific gene mutations can be easily generated. Genetically altered animals permit the analysis of precise gene alterations in an otherwise homogenous genetic background. This will allow us to careful dissection of the function of genes in vivo to obtain meaningful answers and reduce the numbers of animals required.

<table>
<tr><td>Project 54</td><td>Neural basis of motivated behaviour</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Obesity, food, dopamine, addiction, nutrition</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><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></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><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td><s>Yes</s></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>Obesity is a major problem caused, largely, by over-consumption of food. Junk food, in particular, is an important culprit. While we know how powerfully junk food can motivate us to eat, it is unclear whether it has this effect because of how it tastes (i.e. its sensory properties) or because it contains large amounts of calories (post-ingestive properties). Understanding how these two processes interact is essential for understanding and developing treatments and prevention strategies for obesity. We are especially interested in the role of the neurotransmitter dopamine as it may link how we perceive food (what it tastes like) to its nutritional value (how many calories are in it) and together drive motivation to eat it.</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>Understanding how eating food leads to the generation of signals in the brain and potentially changes in the brain and behaviour is an important goal for treating not just obesity and pathological eating disorders but normal feeding behaviour and</td></tr>
</table>

project)?	challenges to nutritional science. Potential advances include: development of novel foods, dietary regimes, or pharmaceutical compounds that effectively promote healthy eating by stimulating brain regions that encourage certain foods to be preferred. In addition, as the brain regions overlap with those involved in other addictions, for example, to drugs, gaining new understanding of how these circuits work will help combat these other addictions as well.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 1000 rats will be used over the five years of this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Rats will be surgically implanted with intragastric catheters. This procedure is well-tolerated and we expect few complications. In addition, rats for in vivo physiology experiments will have a craniotomy and be implanted with necessary apparatus to make recordings of neural activity (reference electrode, guide cannula, recording electrodes). This procedure is also well-tolerated. We will follow best advice in our pre- and post-surgical routines to ensure that rats experience the least amount of pain possible, for example, providing adequate analgesia that will be administered so that it is effective from before the animal starts to wake from surgery. In addition, we keep our rodents housed in groups and provide environmental enrichment as this is important for rats' psychological well-being. The expected level of severity will be either mild or moderate, depending on the experiment. At the end of experiment rats will be killed either by Schedule 1 or under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Intact communication between the gut and other visceral organs and the brain is fundamental to these studies. In addition, as we are ultimately concerned with the effect of these processes on behaviour, studying them in an intact animal is essential. Where possible, once mechanisms of

	interest have been identified, work will be carried out in vitro (e.g. testing characteristics of an ion channel).
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our experiments are all designed to use within-subjects statistics whenever possible – i.e. the control and experimental condition are performed in the same animal. Doing this increases the statistical power and drastically reduces the number of rats used. We are also involved in actively pursuing methods of studying these processes – or at least the cellular mechanisms – either in cell culture or less sentient rats. Even the in vitro studies requiring rats allow multiple treatments to be tested thereby reducing the total number of animals required
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats are chosen for this project as they are considered the least sentient of animals in which these procedures can be carried out. This project involves fairly complex behavioural tasks, e.g. learning of operant behaviour (lever pressing) and reward contingencies (this light predicts food availability) and rats are known to perform these tasks very accurately. In addition, while rats differ from humans, many aspects of their physiology are conserved and similar brain regions seem to be involved in producing the behaviours that we study. The proposed studies use positive reinforcement, rather than aversion, to motivate rats to behave. Rats will be closely monitored throughout procedures to ensure they remain healthy. In addition, rats will be group-housed and environmental enrichment will be provided.

<table>
<tr><td>Project 55</td><td>The behavioural neuroscience of adaptive behaviour</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Reward, habit, learning, addiction, risk-taking</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></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>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To determine the role of the brain’s dopamine system in changing behaviour to obtain reward; to understand how abnormal function of this system might contribute to addiction, risk-taking and schizophrenia.</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>Understanding the dopamine system is important for understanding how we make decisions, how habits are formed, and how the brain becomes addicted. The research might also allow us to develop a rat model of the subtle brain function impairments seen in people suffering from schizophrenia.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We expect to use at most 430 normal, outbred adult rats over the 5 years covered by the project.</td></tr>
<tr><td>In the context of what you propose to do to the animals,</td><td>Many of the animals will undergo mild thirst or hunger due to the dietary restriction we use to</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?	motivate them to work in behavioural tests. Some animals will experience mild, transient pain from injections of drugs used to alter brain function. Some rats will undergo surgical procedures under general anaesthesia to implant electrodes or cannulae, and the discomfort during postsurgical recovery is reduced by analgesia and likely to be moderate. Some of the rats might be used in experiments in which small regions of brain tissue are destroyed under general anaesthesia. However, the expected outcome is that these rats will have subtle changes in the way they behave, but will look normal. A small proportion of rats fail to recover well from the surgical procedures and in these cases the rats are humanely euthanised within 24 hours if their discomfort risks exceeding the moderate level. All the rats will be humanely euthanised at the end of the research.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals in order to understand the information processing performed by sets of brain cells during psychological tests. Tissue cultures and other *in vitro* preparations cannot provide this information because they allow one to focus only on a small number of brain cells rather than the entire nervous system. While this research can contribute to computer models of brain function, and indeed test whether these models are realistic, computer simulations cannot replace this research.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use research designs in which the animals provide their own baseline data before any experimental manipulation. By comparing measurements before and after the manipulation in each animal, we reduce the number of animals required to verify the results with 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	We chose rats for this research because their behavioural flexibility makes them well suited for studies of learning. Also we know more about the nervous system of the rat than we do any other animal, including humans. Like humans, rats have dopamine brain cells that influence decision-making

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	and the formation of habits. The behavioural tests the rats will perform are designed specifically with their behavioural capabilities in mind so that the rats do not become frustrated or require severe dietary restriction to motivate them to perform the given behavioural test. The ethical costs of the surgical procedures are minimised by the use of appropriate anaesthetic and post-surgical analgesia.

<table>
<tr><td>Project 56</td><td>Plasticity in spinal cord networks: development, disuse and activity</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Locomotion, spinal, injury, exercise</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><u>Yes</u></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td><u>No</u></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><u>No</u></td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td><u>Yes</u></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 circuits that control locomotion located in the spinal cord remains poorly understood which has several consequences to both health and disease conditions. Understanding how these networks of nerve cells work together to produce movement is the major objective of this project. We will determine how these circuits change when the spinal cord is isolated from the brain, such as in spinal cord injuries and also how these networks may change in different situations such as due to exercise, ageing or early development.</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>People with spinal cord injuries often suffer a severe loss of function below the level of the injury rendering them paralysed. This devastating condition has no current approved treatment, which results in tremendous emotional, physical, social and economic pressures on the individual their families and society in general. Any improvements in function will have an enormous benefit to their</td></tr>
</table>

	quality of life. Our experiments will pave the way to developing better interventions to treat conditions such as spinal cord injuries, but will also benefit other neurological conditions such as stroke, multiple sclerosis, motoneurone disease, etc. Additionally, the natural process of healthy ageing also results in changes to mobility, which are currently poorly understood. The results of the proposed studies will significant advance our understanding of how this process occurs generating new avenues for intervention. For example, how exercise may change the physiological function of these nerve cells.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats = 1750 Mice = 3250
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?	Several animals will go under anaesthesia and will not wake up from the anaesthetic. For the spinal injury experiments the expected severity level is severe, because of the loss in sensory and motor functions. However, pain is not an expected effect. The loss of communication between the brain and the rest of the body means that no pain sensation reaches the brain. Other than the loss of function in the affected limbs, animals behave normally in their cages, feeding, drinking and grooming. In addition, during such experiments each animal receives individual care at least twice a day, usually three times a day. In the unlikely event that an animal shows any signs of distress, the animal is humanely killed using one of the approved Schedule 1 procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Understanding movement and the neuronal circuits that control them require an in vivo animal model and such behaviours cannot be studied in any other way. In addition, the process of spinal cord injury is a complex and multifaceted one, which also requires an in vivo animal model. Cell cultures or computer models cannot at the moment replicate

	those processes, and are only helpful in this context once very specific questions develop.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All of our procedures have been maximized to reduce the number of animals by combining several procedures, behavioural tests and surgical interventions avoiding the need to use different animals for each one of procedures. We use sophisticated statistical designs to minimize the number of animals required, by maximizing the mathematical power of the 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 objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodents (rats and mice) are the preferred animal model for these projects for the following reasons. First, given the stage of our understanding a certain number of animals need to be studied, which precludes use of larger animals (pigs, cats, monkeys). Second, the spinal locomotor system of rodents have several similarities to humans, including external factors such as position of the foot, to internal factors such as the organization of sensory and motor pathways. Third, rodent models of spinal cord injury have been prevalent in the last years, and much is known about the disease process of the lesion in those species. Similarly, the events and changes that happen after a lesion in a rat are similar to those of the human. Any surgical procedure is done under anaesthesia. As mentioned before, each animal is individually taken care of at least twice daily. Also, pain and distress are unwanted outcomes because they are detrimental to functional recovery. Therefore, even the smallest changes, for example in skin condition, such as a small sore, are immediately treated.

Project 57	**Molecular neurobiology of circadian rhythms and sleep**
Key Words (max. 5 words)	Sleep, clock, circadian, neurodegeneration, brain
Expected duration of the project (yrs)	5 years

Purpose of the project (as in Article 5)

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		No
Maintenance of colonies of genetically altered animals	Yes	

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

There is a biological or circadian clock inside almost every cell of our body. These clocks control our daily rhythms of physiology, metabolism and behaviour (including the cycle of sleep and wakefulness). When this body clock goes wrong or gets out of synchrony with the day/night cycle (as in jet lag or shift work), people are more likely to suffer from serious conditions such as diabetes, cardiovascular disease and various forms of cancer. Similarly, mental health problems are more common when people do not get enough sleep, and circadian sleep disturbance is the principal cause of institutionalisation in neurodegenerative diseases.

We want to understand how the circadian clocks work in cells, in terms of genes, proteins and signalling molecules, how the main clock of the brain (SCN) controls all of the other clock cells in the body, and how clocks and sleep interact with serious conditions such as neurodegenerative and psychiatric 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)?	Circadian time-keeping and sleep are fundamental biological processes that, literally, set the tempo of our lives. This project seeks to advance basic science by improving our understanding of the complex interaction between our clock genes, brain circuits and sleep, with an added focus on their relationship to neurodegeneration and psychiatric disease. In addition, the work may provide basic scientific proof of principle that the clock contributes to the progression of diseases such as dementia. This knowledge may reveal potential targets for therapeutic intervention (by others) and management of sleep and other clock-related disorders, such as jet-leg and shift-work disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	The work will use mice, 56,000 over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	This project will use mice carrying genetic manipulations that are relevant to body clocks and to human disease. The former we know or have good reason to expect will be non-harmful. Disease-relevant mutations modelling neurodegeneration may produce moderate phenotypes, especially affecting movement and weight gain. These will be monitored closely and ameliorated by provision of suitable accessible food and water. In some studies animals will undergo surgical manipulation and thus encounter a moderate severity. Adverse effects arising from poor surgical technique or consequential to manipulations of the brain are expected to be infrequent (<5%) and will be dealt with by appropriate pain control and wound management. At the end of all procedures, or when the animals approach the moderate severity limit, animals will be killed humanely by an appropriate method.
Application of the 3Rs	
1. Replacement	There is no feasible alternative that would entirely replace the use of a living animal. This is because the

State why you need to use animals and why you cannot use non-animal alternatives	health-relevant mammalian biology we seek to understand (behavioural and physiological rhythms, sleep and wakefulness) are processes that only occur in the intact living organism. Moreover, for our tissue culture studies we require animals to provide brain and other tissues in a suitably differentiated state.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We significantly reduce our reliance on animal models by performing preliminary studies in cell lines, and as far as possible, *in vitro* techniques will always be employed to minimise the use of intact animals. Where animals are necessary, tight control over breeding programmes means that we produce very few animals surplus to needs (ca. 10- 12%), whilst robust experimental design enables us to generate statistically valid results from the minimum requirement of experimental stock.
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 least sentient animals that can be used for satisfactory tests of the roles of genes and signalling pathways in the circadian organisation of behaviour, physiology and sleep. Inbred strains will ensure consistency of results, and minimise the variations between individuals, thus allowing us to keep the experimental cohorts relatively small. Moreover, only in mice is the genomic knowledge and technology sufficiently well advanced to develop and apply conditional inactivation/activation of genes by inducing agents. The work will be carried out in dedicated, state-of-the-art facilities by highly trained technicians and scientists, all of whom are dedicated to the highest standards of animal welfare. The procedures to produce genetically new types of mouse, to breed them up into viable colonies and to experiment on them by recording cycles of behaviour and physiology have been refined over many years in our facility. The scientists and technicians will work closely with Named Veterinary Surgeons to ensure that animals are exposed to minimal adverse effects.

Project 58	**Assessment of the pathophysiology of brain dysfunction in diabetic mice**	
Key Words (max. 5 words)	Brain, Diabetes, Brain compounds, receptors	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 purpose of this project is to determine how high blood sugar levels (hyperglycemia) and diabetes mellitus (DM) affect brain function and what cellular mechanisms underlie brain diseases in DM. I will focus mainly on cell membrane proteins called receptors, which particularly express in both brain cells and in peripheral tissue and are activated by chemical substances released from neurons (neurotransmitter). These receptors are called GABA receptors. There are two types of GABA receptors, GABAA receptors and GABAB receptors in the brain. In this study I will mostly focus on GABAB receptors and examine which signalling mechanisms are affected by DM, and if these receptors can be a therapeutic targets for treating diabetes centrally and peripherally.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	Neuroinflammation has been reported in many neurological disorders including Alzheimer's disease, Huntington's disease and Amyotrophic lateral sclerosis (ALS). Therefore the techniques and findings gained from this programme can be used to	

project)?	study various brain disorders and will contribute to a better understanding of the causes of diseases and to the development of novel treatments. Moreover, diabetic retinopathy which is caused by complication of diabetes often lead to blindness. This has been thought as a result of Muller glial cell dysfunction (support cells expressed in retina). Since GABAB receptors are expressed in Muller cells, it is highly possible that the GABAB receptor signalling pathways in glial cells are novel target for the treatment of diabetic retinopathy. The expected benefits of this project are a better understanding of brain disorders such as neuroinflammation and neurodegeneration caused by imbalance of metabolic homeostasis (equilibrium requires an energy source of maintenance).
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 4800 mice, 600 mouse pups will be used over the 5 years of this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? Part 1	To study the molecular mechanisms of hyperglycemia and DM induced neurological dysfunction, I will prepare primary culture from mouse pups. Pups will be culled by schedule I methods. For cx vivo studies, I will prepare brain slices and whole tissue from mice. Tissue fixation will also be performed for histology to examine protein localisation and expression. Animals will be deeply anaesthetised and terminated by decapitation. Some animals will receive substances in vivo. Most of injections do not require anaesthesia however some substances may require surgical cannulation or stereotaxic injection in the brain. For cannulation, animals will be deeply anaesthetised throughout the procedure and post-op care will be given. Adverse effects such as haemorrhage, bruising, thrombosis and infection are not expected, but the animals will be carefully monitored and any problems promptly treated.
In the context of what you propose to do to the animals, what are the expected adverse	For in vivo studies, I will perform technique called two-photon imaging to visualise cell morphology and activity. This procedure will be done under terminal

effects and the likely/expected level of severity? What will happen to the animals at the end? Part 2	anaesthesia. MRI may be performed multiple times under anaesthesia to detect brain activity and structure. Several animal behavioural tests will be performed. Animals used for tail suspension test (TST) to study depressive-like phenotype will beterminated immediately after the test. For TST, medical adhesive tape will be applied in a % of thendistance from the base of the mouse tail. A cushioned surface will be used below the TST to help prevent injury to the animal from fall. No adverse effects are anticipated in the rest of behavioural tests (open field, home cage, rotarod, T-maze, and object recognition). Suitably trained and competent staff will handle animals. Blood sampling will be done to examine DM. It will not exceed 10% of total blood volume on a single occasion and will not exceed 15% total blood volume in any 28-day period. The use of skilled and experienced staff will minimise the chance of pain, suffering, distress or lasting harm to the animals. Glucose tolerance test (GTT) and Insulin tolerance test (ITT) will be performed. Blood sampling will not exceed 1% of total blood volume in 24 hours. Local anaesthesia (e.g. bupivacaine) will be dipped on the tail end to reduce pain. Animals will be terminated after the test.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The objective of this programme is to determine how hyperglycemia and glucose homeostasis affect brain function and what mechanisms underlie neuropathology in DM. Both mouse and rat model of DM are currently available to study from molecular to behavioural levels. These animals are well characterised and maintain symptoms reported in human patients. Therefore animals are required to study the cellular mechanisms in various tissues, symptoms such as high glucose levels, and the behaviour (e.g. cognitive impairment) caused by DM. Since several lines of transgenic mouse are available for studying GABA receptors and they can be crossed with diabetic mouse, I will use these mice and test how altered GABA receptor activity impact on diabetic phenotypes.

2. Reduction Explain how you will assure the use of minimum numbers of animals	This project is designed to use minimum number of animals possible within the subjects by using appropriate statistics. To estimate this number I attempted to perform normal power analyses with a significance level = 0.05 and a power = 0.8 while making reasonable assumptions about effect size. The source for the power analyses was via http://powerandsam plesize.corn!. Importantly, in vivo experiments will only be conducted when I have enough outcomes using in vitro experiments. This approach eliminates the need of unnecessary experiments.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodents are the best species for studying nervous system function in the DM and is directly applicable to human neurobiology. They will allow me to study the cellular mechanisms as well as the connection between central and peripheral tissues. I can also perform behavioural tests, which are important experiments to identify novel treatments for neurologic behaviours. Since there are mouse model of diabetes and transgenic mouse lines that are well characterised, if necessary, I will cross these mice and study the effect of neurotransmitter receptors in the pathophysiology of DM-induced brain disorders. Studying in the whole animals (mice) will provide direct translational implications for human nervous systems and health. The majority of experiments will be conducted ex vivo, and in vivo MRI and two-photon imaging will be performed under anaesthesia. MRI is less invasive method to look at brain activity and neurochemical changes. Multiple imaging sessions can be applied in one live animal and will help to reduce total number of animals used in the research. The two-photon imaging will be conducted only once under anaesthesia. Diabetic mice, which have mutation in leptin receptor genes, become obese by 4-5 weeks of age, hyperglycemia by 8-15 weeks depending on the line (ob/ob mice do not progress hyperglycemia).

	However the impact of mutation on animals up to 12 weeks of age is very mild according to Harlan Laboratories and there are no symptoms/discomfort linked to the mutation. All diabetic mice will be purchased before 11 weeks of age and will be used before any adverse effects are seen (typically by 15 weeks of age).

Project 59	**Inflammation and phagocytosis in CNS disease**	
Key Words (max. 5 words)	Inflammation Phagocytosis Neurodegeneration	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	Living or dead neurons may be eaten (phagocytosed) by glial cells, a process known as phagocytosis. The objectives of this project are: (1) to determine the contribution of inflammation and/or phagocytosis to loss of neurons in brain disease, and (2) to identify drugs and drug targets (proteins or processes) that prevent loss of neurons by blocking inflammation/phagocytosis in brain disease. The reasons for doing this are that inflammation and/or phagocytosis may contribute to the neuronal loss that occurs in brain diseases such as: Alzheimer's, Parkinson's, motor neuron diseases, vascular dementia, stroke, trauma, AIDS dementia, meningitis and normal brain aging.	
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 blocking the phagocytosis that accompanies brain inflammation we may be able to prevent the neuronal loss that occurs in these pathologies. We have found that this is true in: brain cell culture, an animal model of neuroinflammation and an animal model of stroke. In this project we aim to: **i)** test whether blocking phagocytosis is beneficial in other animal models of brain disease, and ii) test which genes, proteins and receptors involved in phagocytosis of neurons are	

	involved and therefore may be targets for therapy of brain diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Rat 480 Mice 1150 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 the experiments should cause only mild and temporary discomfort, largely as a result of the surgery. In most cases, the agent and dose of agent injected into the brain is designed to cause mild, local neuronal loss, without clinical signs. However, some experiments may cause moderate clinical signs with illness behaviour' such as humans have during a cold or flu, including clinical signs **such as interrupted sleep or reduced activity** and feeding. If the animals do show more than three moderate clinical signs, they will be killed. All animals will be 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	We have progressed as far as we can with non-regulated experiments in cell cultures. It is now essential to determine whether the processes we have identified in culture are also relevant and important in vivo. Similarly we have used disease models in culture, but these have limited application to human disease, where in vivo disease models are much more relevant to test potential protective agents. Only if we are successful in vivo in animals can we progress to preclinical development of these strategies for preventing neuronal loss in human disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The lowest numbers of animals will be used consistent with obtaining a reasonable estimate of experimental variability to test for significant differences between treatment groups. Previous results for brain injections indicate that about 8 animals per treatment group is the minimum required to achieve statistical significance. We will only test drugs in vivo, when they have been found to be beneficial in culture.

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 only be using rats and mice for this research (the lowest vertebrates for which there are established models of brain disease relevant to human disease). We will be using the minimum numbers of animals compatible with obtaining statistically significant results. And we will be using disease models with the minimum suffering compatible with relevance to human CNS disease — normally injection of pro- inflammatory agents and putative protective substances into very small regions of the brain, resulting in either no or mild signs.

<table>
<tr><td>Project 60</td><td>The function of neuronal networks underlying sensory processing</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Brain, neurological disorders, neurons, information processing</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>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>No</td><td>Yes</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>To understand the function and connectivity of neuronal circuits in the normal and genetically altered mouse brain. Understanding how neuronal circuits develop and wire up is fundamental to understanding brain miswiring, disease and pathologies.</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>In humans genetic disorders and neuron degeneration underpins cognitive disorder such as Downs Syndrome and Autism and dementia. Direct manipulation of the molecular and physiological functions of such circuits is the only means to establish the genetic, cellular and network mechanisms of such diseases. The mouse model system is the most widely used and accessible genetic tool to dissect the mechanisms of brain dysfunction. Results from this project will establish a framework of cortical connectivity and role various genes play in normal brain function and may be directly compared with human brain miswiring.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mouse (5yrs)
Breeding 15000
Experimental 11250

Rat (5yrs)
Breeding 300
Experimental 750</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>Mouse.

The overall expected level of severity is moderate. In less than 1% of cases animals experience</td></tr>
</table>

level of severity? What will happen to the animals at the end?	discomfort following recovery from surgery. In these cases animals are killed via Schedule 1 methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Use of animal models is the only means of determining the biological mechanisms involved in neuronal circuit development, function and disorders.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In many cases we also use computational modelling to assess the impact of neuronal dysfunction of on the physiological integrative processes underlying sensory integration. We have advanced imaging methods that enable us to collect physiology, anatomy and gene expression in the same mouse brain. This reduces the overall number of animals required. Using non-transgenic littermate animals also reduces the overall numbers of mice required for breeding.
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.	More is known about mouse genetics than any other mammal. Our choice of the mouse as a model system enables us to use existing published data sets thereby reducing the overall number of control experiments required. Furthermore by harnessing the power of mouse genetics we are able to refine and target experiments to specific populations of neurons and circuits. Again, this reduces the number of mice required.

<table>
<tr><td>Project 61</td><td>Leptin regulation of hippocampal synaptic function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Leptin, hippocampus, synaptic transmission, ageing, Alzheimer’s disease</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></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 key objectives are

(1) to examine how communication between brain cells is regulated by the hormone leptin during development and ageing.

(2) the effects of several bioactive leptin fragments in preventing hippocampal synaptic disruption, cognitive impairment and cell death in various models of AD.</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 study will increase our understanding of how brain cells communicate with one another, and also how this process is regulated by hormones like leptin. As communication between brain cells is a key event that underlies the ability of the brain to learn and remember information, this study will also give an insight into the impact of leptin and leptin dysfunction on memory processes.

This study will also examine the therapeutic</td></tr>
</table>

	potential of using parts of the leptin molecule in models of Alzheimer's disease; a devastating and incurable brain disease. This could ultimately lead to the identification of novel agents to treat Alzheimer's disease.
What species and approximate numbers of animals do you expect to use over what period of time?	This study will use mice and rats. We expect to use 1350 mice and 300 rats 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 expected adverse effects of the animal lines to be studied are well characterised and include obesity and/or polyuria. Some animals may exhibit weight loss. The expected level of severity is moderate. At the end of experiments 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	The use of mice and rats is appropriate as the cellular events underlying learning and memory are well characterised in these animals. There are currently no non-animal alternatives or model systems that mirror these cellular processes and/or the complexity of the brain.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To ensure the minimum number of animals is used, tissue from each animal will be used in several different 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.	The use of rats and mice are the most appropriate species as the functioning and complexity of the brain are not only well characterised but also very similar to the human brain. To ensure there is minimal welfare cost to animals, the health of all animals will be monitored routinely to ensure no deviation from normal health. In addition, animals will be killed in a humane manner in order to obtain brain tissue.

Project 62	**Understanding the pathophysiology of pain**	
Key Words (max. 5 words)	Pain, inflammation, diabetes, obesity	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 aim of this project is to advance the understanding of pain processing, in order to aid the development of new drugs for the treatment of long-lasting or chronic pain. Chronic pain in particular causes substantial distress in patients, and can have serious adverse effects on psychological, social and functional status, and quality of life. The Pain in Europe 2003 survey conducted by the Federation of European Pain societies (EFIC), which surveyed over 46,000 adults in 16 European countries, revealed that chronic pain affects 1 in 5 adults and persists on average for 7 years but often longer. Furthermore, almost two-thirds of chronic pain sufferers reported that their pain control was inadequate. Thus, clearly there is a substantial unmet clinical need. In some populations there is an increased incidence of chronic pain; for instance, a negative relationship exists between obesity and chronic pain conditions such as osteoarthritis rower back pain and other musculoskeletal disorders. Furthermore, obesity markedly increases the risk of developing type 2 diabetes mellitus: the risk increases continuously with body mass index and decreases with weight loss. Type 2 diabetes is associated with painful nerve pain,	

	which can presage loss of sensation and repetitive trauma, resulting in ulceration and possible amputation. The factors triggering painful diabetic neuropathies are not understood at present, despite the debilitating nature of this condition.
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 improve our understanding of pain mechanisms and the potential for new and effective pain management. These studies will help identify novel targets for drug therapy other methods of alleviating pain and improve quality of life. Furthermore, these studies will contribute towards a better understanding of the links between obesity, diabetes and chronic pain conditions, and to the development of new therapeutic strategies for the treatment of diabetic neuropathies and alleviation of suffering associated with these conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 1300 rats over a 5 year period, of these 600 will be part of the colony of genetically modified obese rats. Many of these rats will be used for the preparation of tissue samples for analysis, and therefore will not undergo any procedures. A number of rats (< 200 per year) will undergo minor operations under general anaesthesia, or be administered with a compound that induces a mild inflammatory response in order to allow us to evaluate the effectiveness of new analgesic drugs or treatments.
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 propose to use the following procedures: Breeding and maintenance of genetically altered rats constitutes the main animal usage in this project; this procedure is not associated with development of any adverse clinical signs. Similarly, induction of type 2 diabetes does not induce any adverse effects, although animals may display signs of increased water intake and urination. Induction of acute pain is a moderate procedure, and surgery will be carried out aseptically, and always under general anaesthesia. All procedures have been previously carried out in rats and adverse effects are rare. However, animals will be closely monitored for adverse effects such as weight loss I excess urination / abnormal behaviour and if this occurs advice will be sought from the named veterinary surgeon. At the end of the study animals will be killed by a humane method (as

	regulated by the Home Office).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal pain models are required to provide a basis for better understanding of clinical conditions and show how analgesic drugs work in the living animal. The proposed work is not possible without animals and cannot be performed in humans or using cultured cells. Animals are also required to study disease pathogenesis, prevention and treatment of obesity and diabetes. Consideration has been given to use of non-sentient alternatives by consulting the list of non-animal replacement methods listed at http://www.rdsonline.org.uk , however, there are no viable alternatives at present. The studies described adhere to the ethical guidelines for investigation of experimental pain in conscious animals laid out by the International Association for the Study of Pain (IASP, Zimmerman, 1983).
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals required for each experiment will be derived using a mathematical calculation to estimate the lowest appropriate group size which will enable us to achieve statistically significant results. Multiple tissues will be derived from animals upon completion of the terminal procedure, which will be distributed to other investigators within this Institution who are researching the same disease pathogenesis. This will serve to maximise the information derived from each animal and reduce the need for further animals to be 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. (Part 1)	Choice of species/animal model A good experimental model should reflect a similar developmental time course to that observed in clinical conditions and the models proposed here fit these criteria. Ten years of research has been conducted which has characterised rodent pain models and this work has shown that the proposed models produce robust and reproducible features of clinical pain. We have identified the induction phase, the time of maximum hypersensitivity and duration of altered sensitivity in each model, and therefore now have a sound basis for selecting appropriate time points post-surgery/tissue injury for targeting pain and tissue collection for use in gene expression studies.

	The proposed obese rat and type 2 diabetic rat models are well characterised, and animals display clinical features of pre-diabetes, or type 2 diabetes including insulin resistance, hyperglycemia, and blood lipid disorder. The Animal Models of Diabetic Complications Consortium (AMDCC) was set up by the NIH to develop appropriate and useful animal models of diabetes, and considers that a useful model of diabetic neuropathy should exhibit key pathologies present in human diabetes. These include: sensory loss, electrophysiological measures of nerve impairment, anatomical evidence of nerve fibre loss. Both models display all these features, and are therefore considered an appropriate model in which to study diabetes-linked pathologies.
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. (Part 2)	How animal suffering will be minimised: • Animals will be housed in comfortable conditions with environmental enrichment. • Where possible animals will be group housed. However, animals may be housed singly for short periods of time in order to record food intake / collect samples of faeces / monitor urination. • Diabetic animals will have their bedding changed frequently to compensate for any excess urination which may occur. • All procedures will be carried out by, or under the supervision of, an experienced competent person. • For all aspects of our work we will refer to the NC3Rs website for guidance (www.nc3rs.org).

<table>
<tr><td>Project 63</td><td>Stress, the brain and pregnancy outcomes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Preterm birth, stress, offspring, transgenerational</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>
<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 overall aim of this project is to understand the impact of stress exposure during pregnancy on the mother, pregnancy outcomes, the long-term health of the offspring (and subsequent generations) and the mechanisms involved. We will focus on understanding the changes in the brain that occur in stress-induced premature labour and whether these can be prevented. We will also assess the mechanisms in the brain and placenta that underpin altered stress responsivity and emotionality, and disrupted cognition, social behaviours, metabolic and cardiovascular function in the offspring of stressed mothers. Furthermore we will investigate whether any of these adverse effects can by reversed by post-natal interventions or prevented by blocking damaging placental signalling to the fetus.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>During pregnancy, stress and infection are risk factors which increase the likelihood of premature labour. Globally the rate of premature birth (before</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	37 weeks in women) ranges from 5-18%. In the UK 8%, of live births are premature, while in the USA this figure is 12%. Moreover, premature birth rates have been steadily increasing over the last 25 years. Understanding what triggers preterm labour and the cascade of events which ensue is important as surviving premature babies are at greater risk of serious health complications and long term disabilities which have huge economic and societal impacts. Current treatments used to try and prevent preterm labour target blocking uterine contractions, which occur late in the labour process. Enhanced understanding of the earlier events that occur in the birth cascade, particularly those organized by the brain, could lead to more effective therapeutic strategies. Maternal stress exposure during pregnancy can also result in the offspring being more susceptible to a range of adulthood diseases (e.g. heart disease, type 2 diabetes, anxiety and depression) and other adverse effects (e.g. impaired learning and memory, social behaviours) which have negative impacts on health and quality of life. By understanding the underlying mechanisms this project may also lead to new approaches to improve, prevent or reverse some of the adverse lifelong effects exposure to stress in pregnancy can exert on the offspring. Moreover, better understanding of the impact of the adverse experience of one generation on subsequent generations may lead to greater focused effort to prevent such transfer. We will also investigate whether it is possible to treat the placenta and not the fetus. Very few drugs are able to be used during pregnancy because of potential direct effects on the fetus if the drug crosses the placenta. Nanoparticles (attached to drugs that stop the signalling) will enter the placenta but will not pass through it, meaning the fetus is not exposed and creating a potentially new and safe way to prevent the damaging signalling from placenta to fetus that may cause the disease.

What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 2000 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?	Given the main objective is to investigate the effects of stress exposure on pregnancy outcomes, the animal's experience of stress is unavoidable, however the animals will generally be exposed to a mild stressor and exposure on any one day will be brief. Measures will be taken to exclude any extraneous stress caused by other procedures. To minimise animal suffering, all surgical procedures will be carried out by experienced investigators adhering to best practice to reduce pain and with the animal under deep anaesthesia. Animals will be regularly checked by trained technical staff, a veterinarian and the personal licensees, thus any adverse effects (which are expected to be rare) will be quickly discovered and be immediately treated. Any animal showing signs of distress, pain or suffering to an extent exceeding the severity limit of the proposed protocol will be humanely killed. All animals will be humanely killed at the end of the procedures and post-mortem tissue may be collected for further analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	A major element of this project involves studying the unique and complex physiological state of pregnancy (and the resultant offspring) and this cannot be done without living animals. Similarly, where assessing the effects of stress on behavioural outcomes, there is no reasonable alternative but to use living animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We minimise the number of animals used by making multiple measurements in individual animals (e.g. behavioural assessment combined with blood collection before and after a manipulation), thus maximising data collection and reducing future use of animals. However, the number of animals used for each study will be

	sufficient to make meaningful conclusions from analyses of data and for reliable statistical analyses.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Both the prenatal stress and preterm birth models were developed in rats. Rats display robust and reproducible behaviours and extensive physiological and behavioural data is available for this species. The size of the rat facilitates the investigative techniques needed to address the objectives of the project, some of which are extremely difficult in smaller species e.g. mice. Any refinement that minimises stress is beneficial to the animal and the science, as extraneous stress may influence the results. Thus, for behavioural studies, we familiarise rats with the test arena and the environment is kept constant and quiet, essential for reliable measures. Remote digital recording of behaviour minimises animal disturbance. We continually monitor animal welfare and review whether our next planned step in a study is justified by the preceding data. Surgical procedures are performed with appropriate anaesthetic and analgesic and in our experience are tolerated well by the rats.. Implanted cannula permit blood sample collection/drug administration and avoids disturbing the rats and the stress and discomfort associated with repeated restraint and venepuncture/venesection.

<table>
<tr><td>Project 64</td><td colspan="3">Role of AMPA receptors in synaptic plasticity</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">AMPA receptors, neurotransmission, plasticity, memory.</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>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 colspan="3">AMPA receptors (AMPARs) are channels in the brain that allow information transfer between nerve cells (or neurons). The contact points between neurons, termed synapses, are the places where the brain stores information. This information transfer and storage depends upon AMPAR activity. There are many different components to this receptor and it is not currently understood how these individually contribute to receptor function. Our objective therefore is to understand how these components including their many known variations, alter the way in which the receptor works and how they contribute to neurotransmission and memory. It is through full understanding of how this receptor works at the basic level that will allow us to tackle malfunction during disease states.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td colspan="3">AMPARs are central to basic brain function and are crucially required for higher cognitive brain activity including learning and memory. Malfunction of</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	these receptors results in various neurological disorders, including Alzheimer's type dementia, one of the most common age-related diseases in humans. Current treatments are purely symptomatic and do not modify the underlying disease process. Hence, understanding their operation at the molecular level is crucial for developing drugs targeting AMPARs and is a fundamental question in neuroscience. The benefit derived from this project therefore is the production of fundamental data on how these key receptors in the brain work. Due to their implication in many disease states, insights into how they function and what alters their activity will provide real avenues for clinical intervention.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years – Mouse: 12,000
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 mice will express altered versions of the AMPA receptor, which will be introduced into the brain via modified virus-like particles. This procedure involves anesthetising the mouse and injecting a small volume of virus-like particle directly into the brain. Following this procedure, the mouse is given pain medication and returned to its cage. Recovery is rapid after withdrawal of anaesthesia. Since the virus-like particle is contained in a very small area of the brain and due to the nature of the alteration, we do not expect to observe adverse effects in the animal from the injection. For the sleep study: this is a short-term protocol with no adverse effects expected. We will keep the mice awake for an extended period by providing new toys to explore on a regular basis and by touch if they try to sleep. At the end of any procedure the mice will be killed. Brain tissue will be subsequently analysed via electrophysiological recording or biochemical analysis.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To date, a substantial set of preliminary experiments has been carried out on cultured cells in the laboratory; however, we have currently reached the limit of our understanding from this type of preparation. Nerve cells are communicators and function in a network. In order to move forward and study how AMPA receptors contribute to a functioning network of cells within the intact fully functional brain we need to proceed to using animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will keep the numbers of mice needed for this work to a minimum. We will breed only the number of mice that we require for our experiments at any particular time and keep only the strains of mice relevant for this project. We will monitor breeding closely and adjust the number of breeding pairs and/or use timed mating if necessary to prevent overbreeding. For experiments, we will use the minimum number of animals required to answer the experimental question and plan accordingly to prevent excess breeding.
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.	These mice will have AMPAR manipulations (via the introduction of virus-like particles) in specific brain regions most relevant for the study thus producing novel and animal models. Current work is likely to be refined in the future as answers to questions about AMPAR function are revealed. Whilst not expected, any appearance of abnormal behaviour in the mice will be closely monitored, if necessary animals will be killed to minimise suffering.

Project 65	**Mechanisms of synapse function and disease**	
Key Words (max. 5 words)	Synapse function and disease	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 brain defines our personality, how we think, store memories, feel and behave. And yet we understand very little about how the brain actually works. It is our aim to expand the knowledge we have on how the brain functions, how it has evolved and then use this knowledge to understand disorders such as Alzheimer's, schizophrenia, autism and intellectual disability. In particular, we are focusing on a specific structure in the brain: The synapse. When we look at the brain we begin to appreciate its complexity. Brain cells, known as neurons, form networks of communication which relay messages between cells and brain regions. However the neurons don't physically touch, instead there are small gaps between the cells, called synapses, over which chemical messengers are sent between the cells to relay information. We are working to understand the proteins essential for correct detection and relay of a signal at the postsynaptic terminal once it has received a message. We know that these proteins are vital as research shows many postsynaptic genes encode proteins responsible for behaviour, including learned	

	and instinctive behaviours. A loss of these proteins has also been linked to brain disorders: disruption in over 300 genes encoding the postsynaptic proteins has been seen in over 130 brain diseases. We aim to understand how the proteins of the postsynaptic terminal interact and function, how this affects behaviour and learning, how the loss of a protein can lead to brain disorders and ultimately can we move towards better detection and therapy of these debilitating 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)?	Today we know that loss or mutation to particular proteins found in the synapse can lead to brain disorders of which there are no effective treatments. Before we can begin to develop treatments we must first identify where the fault lies. Our project will enhance our understanding of the molecules which allow the synapse to function properly. From this we will gain an understanding of neuronal connection and how this leads to the processes of learning, behaviour and disease development. A second benefit likely to be gained from this project is that our findings will pave the way to improving diagnostic screening and eventually treatments of neurological disorders. For example, by using mice genetically altered to allow us to see the location of the proteins in the synapse in the brain we can reveal precisely where the proteins are in the brain and how this changes with disease. By modifying the mouse protein with a tag this will allow us to purify the protein and any other that may interact with it in a functional complex revealing potential processes and candidates for development of new treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice have been proven to be excellent models for the human brain. We have the potential and the capacity to study many of the synaptic proteins known to be associated with brain disorders. Because of this we propose to use/create up to 6000 mice per 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?	We will use the mice for • Breeding to maintain our colonies for experiments. • Aging studies • Biochemical studies of synaptic proteins. • Image analysis of fluorescently-labelled synaptic proteins. • Behavioural studies • Treatment with commonly used compounds to see if

	these reverse the effects seen in mutants. • Studying the effects of drugs. Over 95% of the lines that we generate will not be exposed to any greater than mild severity limits and will not be subject to any procedures above mild severity. We have proven that mice that have been created to express proteins with tags show no difference in terms of behaviour and brain function to normal mice. Approximately 5% of our genetically modified mice are known to display symptoms of moderate severity (largely representing human brain disease). In these cases, the mice are monitored carefully and where possible experiments are ended before the animal endures adverse effects. Our experiments have been refined to reduce pain and distress to the mice with careful consideration to the cost-benefits of each model and procedure. Our behaviour and therapeutic protocols are expected to be below moderate severity. All of our colonies are monitored regularly for signs of behaviour and appearance that are not normal. Any mouse found to be in distress is handled humanely. When work under terminal anaesthesia is involved, the level of anaesthesia will be maintained at sufficient depth for the animal to feel no pain.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Mice have been proven to be excellent models for the human brain. Not only do we share with them a similar brain structure but importantly we also share the same key proteins that allow for correct synaptic function. Also a loss of these key proteins results in similar disease(s) in mice and humans, There is no other vertebrate model which we can use to study the human brain with such accuracy or efficiency, or one in which we can manipulate the genome with results that so precisely reflect our own. Also, in vitro systems do not allow for combinations of genetic mutations and genetic tags as efficiently or as easily as manipulating and interbreeding of our colonies.
2. Reduction Explain how you will assure the use of minimum numbers	We propose to create/use 6000 mice per year in order to support maintenance of up to single and compound genetic 90 colonies alterations; create new colonies of GA mice; produce cohorts of GA mice and controls in order to collect Our experiments have

of animals	been designed to include statistical methods which will ensure we use minimum numbers of mice to achieve statistical significance in the results. This is essential to provide robust and reliable results so that our findings can be used toward clinical trials in humans.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	In the absence of available human tissue we need valid models. Mice have been proven to be an excellent model for studies of normal and disease human brain processes of learning and behaviour. Genetic modification of these 'cognitive' genes permits studies that cannot presently be achieved by in vitro methods or human tissue. Welfare costs will be minimised by always seeking to implement protocols at the lowest possible severity rating. Where more severe protocols are unavoidable, all adverse effects will be considered as far as is practicable and severity endpoints will be clearly stated and observed.

<table>
<tr><td>Project 66</td><td colspan="3">The unfolded protein response in neurodegeneration</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Neurodegeneration, Alzheimer’s, prion, dementia, neuroprotection</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 section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></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><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td><s>No</s></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">Neurodegenerative diseases pose an ever-increasing medical, social, and economic burden on society and health care systems worldwide. Globally, there were 44.4 million people with dementia in 2013, a number projected to increase to 135.5 million by 2050, yet no disease modifying treatments exist. The problem is such that the G8 Summit on Dementia in December 2013 pledged to find a ‘cure’ by 2025. We recently discovered the cellular process that causes neurodegeneration in prion disease, which we manipulated pharmacologically, curing disease and preventing neurodegeneration in animal models. The same cellular process is dysregulated in human neurodegenerative diseases including Alzheimer’s disease. We now propose to understand to what degree this process is involved in dementias and other disorders, and to use our discovery to develop new treatments and biomarkers to cure</td></tr>
</table>

	dementia.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The impact of possible new treatment for neurodegeneration on the lives of patients and carers, on healthcare services and on the global economy in the context of an aging population, is potentially hugely significant. Manipulating the cellular processes underlying neurodegeneration benefit learning and memory as well as protecting brain cells from dying, so we can prevent disease progression and boost function of diseased brain cells.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, both wild type and genetically modified. To date we have used ~25,000 mice in 5 years. However, where possible we replace mice with worms and cells (see 3Rs section). We would nonetheless expect to use up to 20,000 mice over 5 years for developing new models of dementia and testing of potential treatments.
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 mice will be models of neurodegeneration. Some of the animals will never show any visible signs of distress however some will show progressive motor and behavioural signs. Those that do will be carefully monitored to limit both the length of the experiment and in the amount of suffering caused. Where needed, these animals will be given pain relief and easier access to food and water. In most cases mice will not exceed a moderate severity. In very rare cases prion infected mice can degenerate rapidly exceeding moderate severity, but we will check mice very frequently to reduce the chances of this happening and mice will be humanely killed as soon as relevant clinical signs are apparent. When novel compounds are used adverse effects may result, these will be rigorously monitored and appropriate action taken. At the end of each study, animals will be humanely culled and tissue taken for histological and biochemical analysis.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Neurodegenerative diseases are fatal disorders for which there is no cure. To understand the fundamental mechanisms and develop rational therapeutics, clinical, behavioural and pathological outcomes in experimental models are essential. Mice provide an excellent model for many aspects of human disease. They are ideal for studying prion diseases as they reproduce all key features of the disease: long incubation time, neurodegeneration and cell death. They also model brain cell loss and cognitive impairments of other neurodegenerative disorders such as Alzheimer's disease. Using mice with prion disease, we have discovered new pathways in neurodegeneration and new treatment targets for dementia, and described the first small molecule that prevented neurodegeneration in vivo and cured prion disease. We now need to understand the role of the pathway in neurodegeneration more broadly, and the effects of its modulation for new treatments for dementia. The complexity of the nervous system and the need to use cells or tissues which can accurately model neurodegenerative paradigms with clinical outcomes means that there is no substitute for animal experimentation. Insufficient information exists to generate accurate computer models that can predict the complex responses of brain tissues. While many studies can and will be done in cells in culture, intact brain with its full complement of brain cells is the only system in which mechanisms can be fully tested and therapies be accurately evaluated. Where possible we will use cell culture and nematode worms for evaluating modulators of neurodegenerative disease. However, the clinical validity of these and their relevance to human disease ultimately requires validation in mouse models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In all experiments, appropriate statistical power analysis will be used to minimize the numbers of animals required for a validated result. Multiple tissues will be taken from each mouse for multiple analyses.

	We will ensure high standards of animal care, welfare and utilise the most appropriate breeding methods. Colony sizes are monitored and adjusted within a formal forecasting system to meet the requirements of the research programme. Breeding colonies are always kept to their minimum size so as not to over-produce and avoid wastage.
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.	Invertebrates do not share sufficient commonalities in their CNS to make them appropriate models for human disease. Rats and mice share many similarities in brain structure with higher vertebrates. Biochemically and physiologically many of the mechanisms, processes and pathways are identical to those in humans. Furthermore the availability of transgenic animals with particular protein knockouts or overexpression make them useful tools for testing the importance of particular proteins in neurodegeneration model systems both in vitro and in vivo. Thus, these species are the most appropriate for testing basic hypotheses, which can be relevant to human health before moving into higher vertebrate species. When choosing any other particular transgenic strain, or in the generation of new models, we will use the least severe CNS phenotype with the least number of non-specific associated deficits. Should a particular strain have related complications (e.g. skeletal muscle atrophy) we will firstly minimize these by using the animals before the complications cause observable distress and/or modifying the animal habitat to compensate for the deficit (e.g. making food and water available without reaching). For genetically altered animals not infected with prions, the most invasive procedure is likely to be intracerebral injection with modified viruses or other substances. This is carried out under general anaesthesia and the animals are given post-operative pain relief for the craniotomy scar. The same applies to prion-infected animals receiving intracerebral injections. Most animals will receive one set of bilateral injections and not more than

	two, on separate occasions, not less than 24 hours apart. Administration of other substances will be given with due care and pain relief if appropriate as well as staged dosing, in general not more than daily dosing.

Project 67	**Molecular studies of calcium channel function and their role in disease**	
Key Words (max. 5 words)	calcium channel; ataxia; epilepsy; chronic pain	
Expected duration of the project	5 years	
Purpose of the project as in ASPA section 5C(3)	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 understand the role of calcium channels in various diseases including chronic pain, epilepsy and cerebellar ataxias.	
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 may gain a greater, understanding of potential therapies relating to these diseases	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 3000 rats, 50O, Xenopus 50 maximum 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 most of our experiments, mice, rats and Xenopus will be used as a source of tissue for in vitro experiments for our studies on mechanisms of ataxia and neuropathic pain. For these experiments, the level of severity will be unclassified (non-recovery), as the animals will be killed immediately by methods specified here. Most of the mutant mice to be used are expected	

	to have either no behavioural or other symptoms, or symptoms classified as mild severity. However, it is possible that symptons will be classified as moderate in some mice. In some experiments, nerve injury models of chronic pain will be used, in order to study how this pain develops and how it can be treated better (experiments are classified as moderate). Experiments will be of the minimum duration necessary for this type of study. All animals will be killed immediately at the end of the experiments, and their tissue will be used.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal models of disease (specifically neuropathic pain and cerebellar ataxia in our case) often require the use of mammalian models in which the symptoms can be monitored and relate to human disease. Mice and rats are the most widely used species for this and cannot be replaced without losing relevance to human disease. Our use of Xenopus (toad) oocytes is restricted to experiments which cannot be performed using immortalised cell lines, for example where specific gene expression is required. Therefore in recent years we have reduced Xenopus use, and wherever possible, we share the ovarian tissue between laboratories. We expect to reduce Xenopus use further in the future.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Most of our work involves in vitro experiments, in which either wild-type or genetically modified (mutant) mice are only used as a source of tissue of in vitro experiments. The minimum number of mice will be used to provide sufficient material for statistically significant results to be obtained. Experiments will be grouped so that the maximum amount of tissue for different experiments will be taken from each mouse
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take	The choice of species (mice because they are the smallest mammal that is routinely genetically modified) and rats because they are a standard animal used in many laboratories will be used. Xenopus will only be used as a source of oocytes when absolutely necessary. We will take all necessary general measures to minimise welfare

to minimise welfare costs (harms) to the animals.	costs (harms) to the animals, such as frequent observation, handling by trained staff, and use of anaesthesia to reduce pain.

<table>
<tr><td>Project 68</td><td>$GABA_AR$, neurosteroids and stress in brain function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>GABA, neurotransmission, stress, depression, addiction</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></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>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.</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>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</td></tr>
</table>

project)?	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 animals do you expect to use over what period of time?	The study will use mice and rats. We anticipate to work with a maximum of 10000 mice and 1400 rats 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.
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 however recovers to near normal values at adult stage (Gunn et al. 2013). However, we expect to be able to measure more subtle behavioural and cognitive effects in our experiments. 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 69	**Brain circuits controlling visual behaviours**	
Key Words (max. 5 words)	Zebrafish, brain, vision, neurons, circuit	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 main goal of the project is to understand how the brain processes sensory information, gathered from the environment, and then selects and executes a suitable behaviour. Except for the most simple reflex behaviours, there are few examples where we currently understand the complete brain circuit that controls a particular behaviour. Our aim is to produce a model of the complete brain circuit that controls a natural, visually-guided behaviour, which we study in larval zebrafish.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This is a basic biological research project that seeks to understand how vertebrate brain circuits process sensory information to guide complex behaviour. Therefore the primary benefit of the research will be to advance our scientific knowledge about how brain circuits are organised and how they function. Secondly, diseases of the nervous system, and in particular neurodegenerative diseases, are a growing human health concern. The development of effective treatments for neurological conditions is a massive challenge, due in large part to the great complexity of the nervous system: although basic neuroscience research has made considerable progress in	

	understanding many aspects of neurobiology, including the cell and molecular biology of individual neurons, the most poorly understood aspect of brain physiology is how the ~100 billion individual nerve cells that comprise the human brain function together, as a network, to perform the computations that control our actions, emotions and thoughts. This lack of basic scientific knowledge represents a major obstacle to understanding how genetic abnormalities, trauma, degenerative loss of specific cell types, and pharmacological agents affect the computational functions of neural networks. In this project we propose to uncover fundamental principles about how entire neural networks are functionally organized, and how they carry out the computations that control behaviour. This understanding of how neural circuits function in the healthy brain is necessary for understanding circuit dysfunction during diseases, and for developing improved diagnostic tools and treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	This project uses zebrafish, a small tropical freshwater fish species. Most experiments use larval fish between 5 and 10 days of age. Over five years we expect to use 50,000 larvae for experiments and for maintaining our fish colony.
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 experimental procedures have mild or undetectable adverse effects on the larval fish. They are tethered using gel and presented with visual stimuli whilst we monitor brain activity. In some experiments we will use laser-surgery to carefully remove small numbers of brain cells to help to work out what role they play in processing information. At the end of the experiments, larvae are euthanised using an overdose of anaesthetic. This is a humane procedure approved by the Home Office.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	How neural circuits are organised and the computations they perform are still very poorly understood. Therefore, to learn more about the patterns of brain activity that control specific behaviours we must perform experiments using animals where we are able to monitor brain activity in the context of a behavioural task.
2. Reduction	We use the minimum number of animals required to obtain high quality scientific data. This is achieved by

Explain how you will assure the use of minimum numbers of animals	*(1)* using best practices in animal husbandry such that our fish colonies can be maintained with the minimum numbers of breeding adults. (2) using cutting-edge microscopy techniques to image brain activity. This allows us to obtain large and comprehensive datasets from a single procedure in a single animal, which reduces the total number of animals required.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use larval zebrafish primarily because they are a simple vertebrate and have a tiny transparent brain. This allows us to use non-invasive optical imaging to observe patterns of brain activity whilst the animal views and responds to visual scenes. This approach to recording brain activity is harmless and causes less pain than traditional techniques using electrodes. Furthermore, it allows us to monitor many more cells at the same time, reducing the total number of experiments. To prepare animals for experiments we have to tethered them using gel. The larval fish are briefly anaesthetised during this procedure to minimise stress.

<table>
<tr><td>Project 70</td><td colspan="3">Spinal cord injury and potential therapies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Spinal cord injury, stem cells, regeneration, pain, spasticity</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 section 5C(3)</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">The overall aim of this project is to investigate and develop potential therapies for spinal cord injury. Spinal cord injury affects around 1000 people in the UK each year. The injury interrupts communication along the nerve fibre pathways which travel up and down the cord carrying signal between the brain and spinal cord. These signals are essential for producing movement, sensation, and control of bowel, bladder and other bodily functions. Following injury, loss of these functions can be devastating and the effects are often permanent because the adult mammalian spinal cord is not able to repair itself.

Traumatic (contusion) injuries are the most common clinical injury and are typified by damage to nerve fibres, death of nerve cells and the support cells around them and the development of fluid filled cavities which are surrounded by a scar.</td></tr>
</table>

	Approximately 50% of injuries are incomplete so that a varying proportion of nerve fibres remain undamaged (spared fibres) and support some residual function below the lesion. Although, some of this damage occurs at the time of injury, much of it develops over time as a result of secondary processes which it may be possible to prevent. At the moment however, there are no effective treatments for spinal cord injury. There are three main strategies for the development of treatments for spinal cord injury: 1) Neuroprotective: - minimizing the secondary damage that contributes to loss of function. 2) Compensatory: - inducing plasticity (strengthening or re-organisation) in undamaged pathways 3) Reparatory – stimulating the regeneration of nerve fibres and reconnection to other nerve cells in order to restore communication across the injury. Spinal cord injury may also lead to pain, to unwanted activity and stiffness in muscles (spasticity) and to poorly controlled blood pressure and cardiovascular complications. The purpose of this project is to test therapeutic approaches within each of the above strategies with the aim of limiting the consequences of spinal injury or restoring function and to investigate ways of preventing the undesirable pain, muscle and cardiovascular conditions that may accompany 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)?	The project should provide valuable information regarding the likely usefulness of different strategies aimed at repairing the injured spinal cord. It is unlikely that any “miracle” treatment will emerge from these studies. The nature of the problem is such that initial therapies for spinal cord injury are likely to be modest in their effectiveness. Nevertheless, the intention is that work of this type should eventually lead to the introduction of therapies that could preserve or restore a useful degree of function in spinal cord injured patients.

	The time scale for success is difficult to predict but such treatments may be in the clinic within the next 10 years. In addition the project will investigate ways of alleviating the pain and muscular problems that some spinal cord injured individuals experience and ways of preventing unwanted changes in blood pressure.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the five year course of the programme of work we estimate that we will need to use approximately 1600 rats and about 500 mice and in addition some animals will be bred to harvest tissues to obtain cells.
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 (other than those for breeding) will receive some type of injury to the spinal cord. These operations will be associated with mild pain and discomfort for a day or so. Most will also be associated with a degree of sensory loss and disturbance of gait. For many of the animals these will improve over a week or two so that the animal will return almost to normal. For a minority of the animals it will be necessary to perform more severe lesions from which the animal will not entirely recover. This is necessary in order to prove unequivocally that connections are formed across an injury by stem cells and to model cardiovascular and muscular problems associated with spinal cord injury. There are however, a range of procedures in place to ensure the animals are fully cared for after the operation and receive painkillers. There are also comprehensive husbandry procedures to ensure that the animals are maintained as comfortably as possible. At the end of the experiments the animals will be painlessly killed; for example by giving 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 use of animals is essential because the response to spinal cord injury is so complex that it cannot be fully modeled in any other way. In addition, it is impossible to carry out tests of functions like sensation and movement, which the therapies aim to restore, using anything other than

	an intact animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals we will use in the study will be kept to the minimum that give a clear answer to the questions we are asking. We continually think about the design of our studies to make sure that we obtain maximum information from each animal.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	It will be essential to produce injuries of the spinal cord in animals in order achieve the aim of testing potential treatments for spinal injury. However, the aim will be to use the least severe type of lesion possible and this is likely to vary depending on the question under investigation. We will therefore first carry out experiments that will determine the minimal lesions that are consistent with modeling the features we require in order to achieve the objectives of the work. We will select lesions with the least severe effects compatible with the aim of each part of the programme. Some of the injuries we will use will interrupt only one part of the spinal cord or produce diffuse damage but with large parts of the cord remaining undamaged (about 80% of the animals we will study). For some of the aims of the study however, it will be necessary to produce more widespread damage which completely interrupts the spinal cord. The lesions will be made using specialised equipment which enables the lesions or injuries to be produced with maximum precision. This means that the lesions will be carefully standardised and show little variability from animal to animal. This should minimise the variability of results and minimise the numbers of animals required to obtain clear results from the study. The aim is to understand spinal cord injury and develop potential treatments for eventual clinical use. The animal model must therefore accurately reproduce the important features of the injury in order that meaningful information is obtained. The choice of animals has been made taking into account this necessity and the principal of using

	the least sentient animal suitable for the study. Rodents are most appropriate as they are the least sentient animals that could be used; non-mammalian species like amphibia and reptiles show a capacity for regeneration which is not present in mammals and are not therefore suitable. Compared to mice, the response to injury in rats more closely resembles the pattern of pathology seen in humans as mice have a special wound healing response which means that they do not form the fluid filled cavities often seen in human injuries. The experiments are also quite technically difficult as they require microsurgical techniques so that the larger size of rats facilitates more precise lesioning and makes possible more complex investigations than is feasible in the mouse for example. For these reasons, the rat has become the standard animal for investigations in this field and much of the background knowledge on which the project is based, including our own work in the area, comes from experiments on this animal. As a result we have much experience with this animal and the chances of success are greater. Nevertheless, mice have other advantages. For example, the ability to produce genetically modified strains can offer a powerful tool. For this reason, we have chosen to carry out the study primarily on rats but using mice where they offer an advantage.

<table>
<tr><td>Project 71</td><td>Neuronal connectivity in development and disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Connectivity, neural development, developmental disorders</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></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 aim of this project is to map the development of connections between brain cells and identify how they are disrupted in neurodevelopmental disorders such as autism or schizophrenia.</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 will lead to a better understanding of how genetic abnormalities actually lead to changes in how the brain wires up during development. In the long-term, this will allow more rational design of new treatments for these disorders.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Rodents (mice and rats): approximately 6-7000 over the 5 year project</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse</td><td>The rodents will mainly be used to breed genetically altered animals (mild severity only at most). Additionally much smaller numbers will undergo</td></tr>
</table>

effects and the likely/expected level of severity? What will happen to the animals at the end?	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 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.

<table>
<tr><td>Project 72</td><td colspan="3">Production of fertilized Xenopus oocytes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Hormone assisted breeding of toads</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 section 5C(3)</td><td colspan="3"><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>Yes</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">The objectives are to gain a detailed understanding of neural circuits in the spinal cord and brain stem that are responsible for the generation of movements, especially locomotion. In particular we aim to understand how these circuits are assembled early in development and how they can be modulated to adapt behaviour. To achieve this aim we will use the tadpoles obtained as a result of the procedure of hormone induced breeding of adults.</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">Locomotor circuits in different vertebrates, including man, share the same basic organization and common design principles. However, the complexity of the mammalian central nervous system makes them difficult to understand in detail. Xenopus frog tadpoles develop rapidly and they are simple enough to understand at the level of single neurons. In turn, basic research can provide insights into movement disorders that occur after spinal cord injury or diseases such as motoneuron</td></tr>
</table>

	disease or Parkinsonian-like dystrophy.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use adult *Xenopus laevis* toads maintained in a laboratory colony. Each week two or three pairs of frogs will be given a small injection of hormone to induce natural breeding. The total number of injections each year will be approximately 240 to 300. With 105 toads in the adult colony, each individual will be injected a maximum of 4 times per 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?	The procedure involves the injection of a small volume of fluid subcutaneously into the dorsal lymph sac. This procedure is completed quickly and will likely only lead to mild, transient distress or discomfort to each toad. After mating the animals are returned to the colony and will only be re-used after a minimum of 3 months.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The aim is to understand how neural circuits assemble early in development and how they control behaviour; there is no alternative to studying these circuits intact and functioning normally.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reduction in numbers is achieved by hormonal synchronisation of breeding instead of killing animals to harvest eggs and sperm, selecting good breeders for re-use (through photographic ID of individual toads) and maximising the use of each batch of embryos by temperature controlled growth. Care is also taken not to repeat work by other groups by attending relevant conferences, maintaining good contacts with others in the field and regularly searching relevant abstracting and library databases.
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	Refinement in technique involves environmental enrichment of the housing for adult toads; regular handling of the same animals to reduce fear/stress and refinement of the injection procedure to prevent pain and distress. Toads are identified as individuals from photographs; this allows tracking of

measures you will take to minimise welfare costs (harms) to the animals.	fertility and maintenance of injection records.

<table>
<tr><td>Project 73</td><td colspan="3">Improving outcomes following nerve injury/repair</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Nerve injury, conduits, regeneration enhancing</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 section 5C(3)</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>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 colspan="3">The objectives of this project are to improve nerve regeneration through the development of bioactive nerve guides, the application of nerve regeneration enhancing therapeutic agents and the assessment of neuropathic pain levels following different treatments.</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">This project could lead to a new clinical treatment for the repair of nerve injuries, improving the quality of life of patients with severe motor/sensory defects or chronic neuropathic pain.</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">502 mice (462 for experimental purposes, 40 breeders) over 5 years.

160 rats.</td></tr>
<tr><td>In the context of what you propose to do to the animals,</td><td colspan="3">Adverse effects (for both mice and rats) include: sensory and/or motor deficiency, local discomfort,</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?	local infection at the nerve injury/repair site. Expected level of severity is moderate or below. Animals will be culled using a Schedule 1 method or via *perfusing the vascular system with histological fixatives via a major vessel or cardiac puncture, under deep anaesthesia*
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The physiological response following a peripheral nerve injury involves a complex series of events, with many interactions that are as yet not fully characterised. As our goal is to improve the overall outcome of peripheral nerve injuries - through the application of therapeutic agents or conduit guidance - we require a live animal model to assess the full range of effects and efficacy within the nervous system, which cannot be predicted using isolated tissues in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power calculations will be carried out prior to any individual studies in order to determine the minimum number of animals required to detect a clinically relevant difference.
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 no reason to expect any significant species differences in nerve regeneration between rodents and man, but there are differences in regenerative abilities in lower order species such as reptiles. We therefore believe that rodents are the appropriate choice for these studies, which is consistent with the majority of previous investigations. The majority of the work intended to be performed under this licence can be achieved using mice; however, due to size constraints mice are unsuitable for studies investigating the more clinically relevant trigeminal nerve branches or extended nerve defect lengths. Therefore, we believe that in experiments investigating the trigeminal nerve branches, or peripheral nerve regeneration over relatively long distances, the

	choice of the rat is justified. The nerve injury models intended to be used are well established, reproducible, and their associated adverse effects are well known.

<table>
<tr><td>Project 74</td><td colspan="3">Effects of exendin-4 in a novel model of early stage PD</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Early Stage Parkinsons’s Disease, Pre-motor symptoms, exendin-4, neuroprotection, hyposmia</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">Parkinson’s disease (PD) is characterised by tremors and movement loss. Prior to this, however, patients suffer a range of symptoms (e.g. hyposmia) which are often only recognised as being the precursor to diagnosable PD after a motor diagnosis has been made. There are very few models of early stage PD (ESPD). We consider that development of such a model will be of great value to understanding ESPD and provide a useful tool to test novel therapies. We also wish to investigate the effects of exendin-4 (EX-4) in an ESPD model as this drug has recently been shown to reduce the motor symptoms of PD in a small scale patient clinical trial.</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 colspan="3">A model of ESPD would be a very useful tool in order to better understand ESPD and study the utility of new therapies. We also believe that a model of ESPD may facilitate earlier diagnosis of</td></tr>
</table>

animals could benefit from the project)?	PD and allow more rapid clinical intervention prior to the development of severe movement disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using rats and mice and anticipate using 600 of each species 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?	Rats or mice will undergo relatively minor surgery. Both rats and mice will be given neurotoxins to simulate ESPD. The aim of this model is to use minimally effective doses of neurotoxins. We would therefore expect mild or moderate adverse effects. Any animals showing distress will be humanely killed immediately using a Schedule 1 method. Anti-parkinsonian drugs, such as EX-4, are not expected to produce any adverse effects. At the end of the experiment, rodents will either be killed by a Schedule 1 method followed by decapitation or transcardially perfused under terminal anaesthesia and decapitated.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The central nervous system in mammals is uniquely complex. We are studying the aetiology of a neurodegenerative disease which affects several distinct neurotransmitter systems which have a clear anatomical topography *in vivo.* It would, at present, be impossible to replicate this in an *in vitro* system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	It is important to strike a sensible balance between reduction and realistic numbers for a solid statistical analysis, particularly in the case of behavioural tests. Our choice of 10-12 animals per group is based upon previous experience with such tests. Moreover, it is intended that each animal is used to provide as much data as possible. This will include behavioural testing and *ex vivo* electrophysiology, neurochemistry, histology and immunohistochemistry.

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 have been chosen as we have considerable experience with this species. The rationale behind using mice is that this species in the most amenable to genetic modification which may, in the future, become highly relevant to this area of work. General measures to ensure maximum welfare will be taken during surgery and immediately after by use of topical analgesics/anti-inflammatories. Thereafter, rats or mice will be monitored to ensure that they are visibly in good health. Animals showing signs of distress will be humanely killed immediately using a Schedule 1 method.

<table>
<tr><td>Project 75</td><td>Zebrafish as a model of inherited renal disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cystic kidney, cilial, treatment, pronephros</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>
<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>We will assess the role and function of genes implicated in human cystic kidney disease and related conditions. The exact functional role of such genes and their encoded proteins remains poorly understood and there is a fundamental and clinical need to know more about these genes. In addition, this project will determine the response of cystic kidney disease to various proposed treatments.</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 work will generate novel insights into the disease processes underlying cystic kidney disease. A better understanding of cystic kidney disease may ultimately lead to novel therapies for human patients with these diseases.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We will use the tropical fish called Zebrafish. Adult fish will be housed in tanks and be mated to generate zebrafish eggs on a weekly basis. Adult fish of 3 months to 18 months of age will be used to generate eggs. Typically each pair of adult fish will produce 100 eggs per mating. Each zebrafish egg</td></tr>
</table>

	produces a single fish embryo, which grows to almost full maturity over the next 5 to 7 days. We plan to use around 10,000 eggs in this project which will be studied for up to 5 days post fertilization. Around 1000 embryos will be used between 5-7 days post fertilization.
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?	Adult fish will not be experimented on directly. Rather the eggs will be involved in experiments. Most of the fish eggs will be treated with compounds from birth to up to 7 days Some fish will undergo a small injection of dye at 3 days post fertilisation to allow measurement of kidney function. For any procedure which might be associated with any discomfort, all animals will undergo general anaesthesia appropriate for the species. We expect a mild level of severity for all these experiments and at the end of the procedure animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to understand the disease process in cystic kidney disease we need to study model systems, since ethical concerns limit the studies that can be done directly in people. The zebrafish provides a useful model which is an alternative to using mice. It offers considerable advantages over other animal models, for example it has transparent embryos allowing development to be studied easily, and it is also possible to modify gene expression by injection into the fertilized egg. Zebrafish are considered to be of lower sentience than mice and since our experiments use zebrafish at a very early stage in development, these experiments are much less likely to cause any suffering. For these reasons we aim to perform these studies in zebrafish in preference to mouse or other model systems. We have successfully used cell models to test drug treatments and mechanisms of disease. This has allowed a targeted approach to planning in vivo experiments. The present cell culture systems do

	not allow us to explore the mechanisms of kidney development and their response to mutations and treatments. For this the zebrafish is the ideal model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Healthy adult fish will be maintained at a minimal number in order to produce sufficient eggs for experiments. Fish will be grown up in the nursery to replace adult fish that are no longer productive, allowing maintenance of the smallest number of healthy adults in the aquarium.
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 zebrafish is a useful animal in which to explore kidney disease as it has a simple kidney with features remarkably similar to the human kidney. We aim to understand the events that lead up to a kidney cyst developing by mimicking cystic kidney disease in the zebrafish and examining the consequences in detail. This level of information could not be obtained from patients and would take huge numbers of mice to achieve a similar result. We wish to test drugs which change the activity of kidney cyst formation in zebrafish. We will also test the ability of drugs to alleviate developmental kidney failure and find out which drugs are most effective. The proposed treatments will cause minimal harm to the animals as they will not be prolonged beyond the initial developmental stages.

<table>
<tr><td>Project 76</td><td colspan="3">In vivo imaging in normal subjects</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Imaging, diagnosis, therapy</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">Imaging of disease processes in humans, either to detect disease, find out where it is within the body, or predict or measure the effectiveness of a specific therapy, is one of the pillars of the new “personalised” approach to medicine and therapy. The advancement of this field requires the development of novel tracer molecules which can be injected into patients, whereupon they find their way to the disease site and their presence can be detected by scanners. This can then tell us about the chemistry of biological processes within the diseased tissue. Each disease process needs a new tracer, which is developed through an interdisciplinary process involving chemists (to design and make them), biologists (to discover the biological target), medics (to set the clinical challenge and identify the needs of patients) and physicists (to optimise the imaging equipment and image reconstruction). The new tracers have to be evaluated in animals before they can be tested for</td></tr>
</table>

	the first time 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)?	Imaging is intrinsically a technique designed to extract biological information from animal studies with relatively low animal numbers. Thus, imaging offers better information quality, information yield per animal, and possibility to detect unexpected and unsought observations, all of which are highly advantageous in most if not all biomedical and clinical fields. The expected immediate benefit of research under this licence is the ability to make an informed decision whether to test the new contrast agent in humans, or to test further in an animal model of disease under another licence, or to abandon the agent, or return to the laboratory for further modification. Better contrast agents and chemistry will improve the quality of imaging. Making the radiochemistry of labelling tracers simpler and more robust, will eventually lead to wider availability to more patients. Whether directly by the development of new imaging technologies, or indirectly by use of imaging as a tool in basic biomedical research, better quality, availability and applications of imaging technologies will lead to better clinical decision making. Consequently this would lead to better quality of life for patients, reduced drug development costs, and reduced costs for health services. Once diseases have been identified in a patient, imaging also has the potential to non-invasively evaluate therapeutic efficacy, providing rapid feedback on therapeutic or interventional effectiveness. The beneficiaries will be patients, health services and pharmaceutical companies.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (7, 000) and Rats (5, 500) and Rabbits (500) over 5 years.
In the context of what you propose to do to the animals,	No adverse events are expected to be caused by the imaging methods used. Any adverse events

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	expected are related to induction and maintenance of anaesthesia (animals may die from respiratory depression <1 -2 % and/or hypothermia) injection of substances (but this is generally done under anaesthesia) and withholding of food prior to imaging (as done in the clinic) but efforts are being made to optimise anaesthesia, administration of substances and avoid unexpected adverse effects &/or deaths. Therefore the highest level of severity within this licence would be 'moderate' due to repeated anaesthesia but we anticipate that most animals under this licence will experience 'mild' severity. All 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	Animals have to be used because 1. Agencies which govern testing of novel drugs/probes/methods in man, require animal data which provide information and guidance to help design human clinical trials. 2. To validate mode of action, experiments are required that cannot be conducted in humans for ethical and scientific reasons. 3. Bio-distribution in whole organisms (i.e. tracking the injected agents route/ accumulation and excretion through the body), with intact biological barriers and excretion mechanisms, is key to clinical use. Non-animal alternatives cannot replace the complexities of the interactions of these probes in whole body systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Preliminary *in vitro* screening (i.e. cells or tissues) will eliminate unsuitable candidates which will not progress to *in vivo* studies, thus reducing the numbers of animals. The use of imaging to determine bio-distribution rather than conventional killing at sequential time points, dissections and organ analysis is a major contributor to reduction of numbers. Imaging allows repeated observations/measurements over a period of time (longitudinal study) on the same animal, with humane killing only at the last time-point. Thus, if a longitudinal study involves six time-points, the

	numbers of animals are reduced to one sixth by use of repeated imaging. Since each animal serves as its own control to compare different time-points, the data obtained are statistically more robust (reduction), requiring fewer animals. Moreover, distribution of contrast agent within organs, not just between organs, is obtained, and unexpected uptakes that may not be detected by conventional methods can be found by whole body scanning. All these attributes of imaging contribute to a greatly improved benefit:cost ratio (benefit = data quality and quantity, cost = animal numbers, procedures and their severity).
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Species: Mice and rats are the species of lowest neurophysiological sensitivity that provide the necessary size compatible with the scale of resolution or movement associated with the techniques being studied. Resolution of the whole body imaging techniques is of the order of 0.5 - 1 mm. Distribution within smaller animals will be beyond these limits. Some control experiments will be conducted in normal healthy rabbits prior to further work in appropriate disease models tested under other licences. Pilot studies are small experimental groups which help us to decide quickly how best to design a statistically and scientifically valid experiment. Thereby helping develop better larger study design and reduce possible suffering. Generally, inhalation anaesthesia will be used to minimise transient pain and distress and where possible, used for blood sampling, contrast injection, weighing and combined with imaging techniques where it is mainly used for restraint. In addition, there would be full and complete recovery between periods of anaesthesia and/or food withdrawal; rehydrating of animals during long imaging sessions; monitoring of respiration and/or cardiac function and maintaining body temperature during imaging. These steps will all be conducive to the animal's wellbeing.

Project 77	**Early-life and psychological impact on food choice**	
Key Words (max. 5 words)	Obesity, reward, mood, stress, early-life	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 controls appetite and food intake, and this is influenced by several broad factors including hunger, stress and the pleasure associated with eating. However, much less is known about how we make day-to-day decisions in what we choose to eat. This project will examine how certain influences (like stress, mood, habit and the palatability of food) can affect food choices in rodent models, with a particular focus on early-life experience.	
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)?	Unhealthy food choices can often lead to obesity and a host of associated diseases like diabetes and heart disease. In the UK, around 20% of the population is classified as obese. Current simple treatments for obesity (essentially diet and exercise) tend to be ineffective in the longer term. We need to better understand the brain mechanisms underlying how early-life and the environment influences food choice. With this understanding, we will be able to test means of promoting healthier choices in humans.	

What species and approximate numbers of animals do you expect to use over what period of time?	600 rats 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 level of severity? What will happen to the animals at the end?	The majority of the experiments involve voluntary food choices under different environmental conditions. As such, we do not expect the rats to suffer any adverse effects related to the procedures. Some procedures involve activation of the stress response or efforts to alter mood. 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 these experiments is to study complex interactions between the environment and behaviour and how they impact on voluntary choices made by the animals. As such, experiments must be done at the level of the whole animal. It is impossible to approach these questions using *in vitro* preparations or anaesthetised animals. Parallel studies will take place in human volunteers with human and animal work informing each other.
2. Reduction Explain how you will assure the use of minimum numbers of animals	At the experimental design stage, all studies in this project will be subject to statistical power analysis to determine the minimum number of animals necessary to show an effect of treatment. Being able to calculate the power depends in part on the variability of responses between animals. We have estimates of this variation from earlier studies. This study forms part of a broader project where we will use experimental data to develop computational models of food choice.
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	Rats have been extensively used as a model organism in this fields in our lab and many others. In terms of the brain regions and hormones involved in appetite control, the rat is well-understood and is comparable to the relevant systems in humans. Using rats lets us avoid repetition of earlier work, builds on current knowledge, and allows direct comparison of

measures you will take to minimise welfare costs (harms) to the animals.	our studies with others. None of our protocols exceed a moderate severity level and the majority will be mild. To minimise any unforeseen suffering all animals will be closely monitored and humanely killed if they exhibit signs of altered health status or when another specified end-point is reached. For all of our studies we will ensure best working practice, consult the NC3Rs guidelines and monitor improvements in refinement when published online. Furthermore, 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. The team also meets regularly, and matters such as experimental strategy and analysis of results are discussed.

Project 78	**Synaptic integration and plasticity in neural circuits**	
Key Words (max. 5 words)	In vivo, mouse, motor cortex	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 aim to understand how the brain initiates and controls muscle movement. Since the early pioneering work of Charles Sherrington, neurophysiologists have used the motor system to try and understand how cells in the brain – neurons – communicate with each other during motor behaviour, such as climbing the stairs or catching a ball. Over the past two decades we have made significant advances in understanding the 'code' that neurons use to communicate with one another but how these codes are generated in a single neuron and propagated throughout integrated networks of neurons still remains a mystery. To this end, we propose to use cutting-edge *in vivo* electrophysiological – measures the electrical activity of individual neurons in the intact brain – and deep imaging techniques to discover just how individual neurons generate 'motor commands' or action potentials that are sent down the spinal cord to	

	initiate muscle movements. The four main aims of the study are: **Aim 1:** To understand how individual neurons generate 'motor commands' or action potentials during behaviour. **Aim 2:** To investigate the importance of input from the thalamus to motor cortex – the thalamus being one of the main sources of motor-related information. **Aim 3:** To investigate the important of communication between the cerebellum and motor cortex – 2 areas involved in motor control – during the execution of a learned motor task. **Aim 4:** To investigate how noradrenaline and dopamine (important chemicals found in the brain) affect motor control both in health and disease. Our hope is that the results of this study will provide a platform of knowledge from which we and others can investigate the molecular, cellular and network underpinnings of diseases that affect motor control (e.g. Parkinson's disease, cerebellar ataxia, Rett's syndrome, 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)?	The motor system is of fundamental importance to our very existence. Almost everything we do, every thought we have ultimately results in a behavioural output that involves motor movement. Therefore, understanding how neurons in the brain generate motor commands and what patterns of activity underpin different coordinated motor movements is an important step towards understanding the mechanistic basis of diseases that affect motor control (e.g. Parkinson's disease, Rett's syndrome, stroke). The aim of this programme of work is to provide greater knowledge and understanding of how individual neurons and groups of interconnected neurons regulate muscle movement. We will build on previous rodent, primate and human data by providing a more in-depth understanding of how motor commands are generated, relayed and integrated in the motor system. It is our view that only by understanding how the motor system functions in

	health will we be able to make significant advances in generating designer drugs and therapeutic interventions focussed on alleviating motor dysfunction in disease. The first direct beneficiary of this work will be the local and international neuroscience communities. We will present our data at national and international neuroscience meetings with a view to forging lasting collaborations with others interested in motor control both in health and disease. Although our work is not directly focussed on generating translational outcomes, we will ensure that our work will be publicised to relevant Health Sector Agencies and pharmaceutical companies by utilising the resources of the Universities' Research and Innovation office. The mission of this department 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 mice and to achieve the outlined aims we will require approximately 10-15 animals per week, 50 weeks per year for 5 years = 2500 - 3750 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?	Some mice will be used for breeding purposes only. The experiments proposed in this study require surgery, for delivery of viruses to specific brain regions and for the placement of a recording chamber on the skull of the animal. For these experiments, pain will be controlled during surgery by general anaesthesia and post-surgery by analgesics. Stress related to the use of head restraint apparatus will be minimised by habituating animals to the experimental setup prior to each experiment. To facilitate learning food or water control paradigms may be used but the health of each animal will be monitored daily and the adverse effects associated are expected to be minimal. The application of drugs will be focal and restricted to particular brain regions so there should be minimal behavioural side effects. 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 e.g. use of heat pads. Risk of infection will be minimised by good surgical and 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.
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 coordinate muscle movement. To truly understand how the brain processes sensory and motor information it is imperative that we record neural activity in the intact brain of conscious mice. For this reason it is impossible to avoid the use of animals when addressing the aims of the outlined proposal. Alternative approaches: As part of the on-going work in the laboratory we have and will continue to use computer models of single neurons appropriate. 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 mice. However, these approaches will never 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	We will endeavour to reduce the number of animals used by the following means: 1) If animals fail to learn simple behavioural tasks we will use these animals to discover more about the anatomy of the brain using a variety of histological techniques on the same animals once they have

	been euthanased. 2) By employing analytical and computer models the overall number of animals required during the study will be reduced. The estimates for the numbers of animals required for the study are based on past experience and published literature. The use of highly controlled experimental setups and hypothesis driven science will maximise the chance of producing clearly interpretable results. From my past experience around ~10 animals are normally required for pilot experiments. The results of the pilot experiments will be used to estimate the number of animals required to address each aim of the study. For example, to identify subpopulations of cell that either increase or decrease their firing rates during movement (Aim 1), where a paired T-test will be used then assuming a standard deviation of 30% of the mean, and a desired power of 0.9, we expect to require ~14 mice in each experimental group.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The species that will be used throughout this programme of research is the mouse. The reasons for this are fourfold: 1) All preliminary data for this research proposal were generated from wild type mice 2) To understand how the brain processes sensory and motor information during behaviour it is necessary to use conscious animals. The majority of experiments will involve the use of head restraint techniques, which ensures stability for electrical recordings. The use of head restrained recording techniques provides a good estimate of the neural activity observed in freely moving mice but with the advantage that we can employ state-of-the-art recording techniques. 3) Advances in molecular genetic techniques to identify or manipulate single neuron activity or neuronal network function have been developed for the mouse. Thus, using mouse models provides us with the ability to manipulate brain function in a highly

	cell-selective manner – thus avoiding many of the adverse effects associated with brain lesions or drug manipulations. To minimise stress, mice will be handled extensively before and after habituation and behavioural training and we will ensure adequate recovery periods between surgeries. It has been well documented that stress reduces the ability of mice to learn simple and complex behavioural tasks so it is within the experimenters' interest to ensure a stress free environment in which the mice will train. Habituation and training will be the preferred option to encourage animals to perform tasks. Only if this this doesn't succeed will dietary or fluid restriction be used.

<table>
<tr><td>Project 79</td><td>Anatomy and physiology of midbrain dopamine system</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neurons, synapses, basal ganglia, plasticity</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></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>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Midbrain dopamine neurons are located deep in the brain and send projections to a variety of limbic and cortical areas. Dopamine neurons encode information about rewarding events. Dopamine released at projection sites modulates new learning and goal-directed behaviour. Dysfunction of this system has been implicated in drug addiction, Schizophrenia and Parkinson’s disease. The overall aim of this project is to provide a more detailed understanding of the anatomy and physiology of midbrain dopamine neurons and their associated neural networks. In addition, we will examine the effects of drugs of abuse on the brain, in an attempt to gain insight into the molecular basis of addiction. We will use a combination of high-resolution state-of-the-art electrophysiological, neuroanatomical and molecular techniques in rats and mice. This new knowledge may lead to advances in our understanding of a number of disorders.</td></tr>
</table>

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The main potential benefit of the work to be carried out will be the advancement of knowledge in this area through the publication of findings in peer-reviewed journals. I have a good track record of publishing my research in the highest quality journals (where it has been well-cited and influential) and at international conferences, ensuring it reaches the widest possible audience. In addition, where appropriate I have discussed my work with journalists. The midbrain dopamine system and the neural networks it targets are implicated in a variety of devastating disorders, including Schizophrenia, Parkinson's disease and drug addiction. Despite the enormous social, health and economic burden of these disorders few new treatments are available. One reason for this is that the underlying cellular and molecular mechanisms are poorly understood. We will provide new fundamental information regarding the dopamine system, which may provide insights into all of the aforementioned disorders. Secondly, we will provide specific information concerning the neural basis of drug addiction in particular, which may lead to the development of new and improved treatments which could greatly benefit society.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats (700/annum) Mice (4700/annum)
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 our protocols are not expected to cause adverse effects. A number of protocols involve surgical steps, which may cause postoperative pain. This will be monitored closely and treated with appropriate analgesia. All surgery will be done using aseptic techniques, appropriate analgesia and antibiotics given as required. In the cases of drug administration or dietary manipulation, animals will be closely monitored for unexpected weight loss or other adverse effects. In most cases the severity level will be mild, and in

	some cases moderate. Many of the experiments are ex vivo, in which case the animal is killed to obtain tissue. In the case of in vivo experiments, animals are killed at the end of the experiment, typically followed by further experimental analysis (e.g., anatomical or molecular).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Understanding the complex neural circuitry of the mesocorticolimbic dopamine system requires the use of animals, and rodents in particular for several reasons. This complex neural circuitry cannot be completely reproduced in cell cultures or in computational models. Although we are generating computational models of dopamine neurons, which we plan to use to generate experimental predictions they are somewhat limited in their scope and require further biological data. Moreover, we have conducted work using stem-cell derived dopamine neurons in cell culture with our collaborators, but it is still unclear how closely these neurons resemble ex vivo dopamine neurons, or indeed whether they can form neural circuits identical to those seen ex vivo. Importantly, it is not possible to relate activity in isolated neurons in cell culture to complex neural circuits and ultimately behaviour. In addition, to examine the in vivo effects of drugs of abuse or the effects of somatosensory stimulation, they must be administered to the intact neural system/animal. Although the dopamine system can be studied in humans (using fMRI or PET imaging), these approaches are extremely limited in spatial and temporal terms, and it is not possibly to examine synaptic, cellular level physiology and anatomy as is required in this project.
2. Reduction Explain how you will assure the use of minimum numbers	At every stage in the experiments consideration will be given to ways in which we can reduce the number of animals. Several of the protocols that we use are designed in such a way to obtain the

of animals	maximum possible data from a single animal. For example, we frequently conduct anatomical and molecular analyses following electrophysiological recordings, maximising the data collected from individual animals. For breeding genetically modified mice, where possible, we use strategies that maximise the use of offspring. When appropriate, we will cryopreserve mouse lines that are not required for extended periods, rather than maintaining stocks. We will consult with statistical experts and use power calculations to assist in planning for experimental group sizes.
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 in this project because they have a midbrain dopamine system similar to humans and therefore allow for extrapolation to human disorders. Although dopamine neurons are found in invertebrates, and some important insights have been gained from studying them, it is not clear whether the circuit organisations is similar to mammals, and some of their functions do not appear to be similar. Moreover, techniques for studying synaptic physiology are less well suited to, for example, flies or worms. In addition, the availability of transgenic mice provide powerful tools for examining these scientific questions. Where possible, we conduct in vitro experiments, rather than in vivo. In cases when in vivo work is necessary, where possible, it will be conducted under general anaesthesia. Experiments involving surgery will use appropriate anaesthesia, post surgery analgesia and antibiotics as required. Animals will be kept warm and monitored regularly during after anaesthesia. We will regularly consult with staff, vets and colleagues about best practice and potential further refinement of our procedures.

Project 80	**Zebrafish models of neurological disease**	
Key Words (max. 5 words)	Zebrafish, neurodegeneration, translational research, genetics	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our overall aim is to gain insight into human neurological diseases by generating and studying zebrafish models of these diseases, and testing the effects of therapeutic intervention in these models.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We expect to increase understanding of the mechanisms underlying human neurological disorders. We hope to identify the molecular pathways that contribute to these diseases, and to identify new diagnostic markers. Finally, we hope to identify novel therapies that can be developed further as potential treatments for the human diseases under investigation.	
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 33,000 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 spinal cord) so represent a simple yet appropriate model for studying human neurological diseases. We use close monitoring of adult zebrafish using score sheets 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.

<table>
<tr><td>Project 81</td><td>Breeding of genetically altered mice</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Transgenic, breeding, GA mice</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>~~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>The objective of this project is to breed and maintain mice with genetic alterations and to supply them for the use of their tissue in cell culture work. The aim of the in-vitro work is to improve our understanding of molecular mechanisms underlying natural ageing of the brain and in neurodegenerative diseases in man.</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 results from the experimental work using the brain tissue from these mice may reveal new targets to slow cognitive deficits observed in natural ageing and in neurodegenerative disorders.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>During the 5 year project we will use an estimated 7500 mice.</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>Mice will be bred using conventional methods which are not expected to result in pain, suffering or distress to the animals. Phenotypic effects are expected to cause mild or no adverse effects, however there may be a small number of mice which experience effects within a moderate severity limit.
Mice produced under this project licence will be killed for provision of tissue for cell culture work supporting research in to human neurodegenerative diseases.</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
<tr><td>1. Replacement</td><td>Animals will be used in this project where there are</td></tr>
</table>

State why you need to use animals and why you cannot use non-animal alternatives	presently no alternatives available to investigate in vitro or in silico the complexities of neuronal structure and function. This is particularly true for the aspects of this project in which we will analyse the functional role of signalling molecules in relation to the stimulation of neuronal cells, specifically at the dendritic spine compartment. Established cell culture systems are not available that reproduce the environment that exists in primary neuronal cell cultures forming active synaptic connections.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The breeding programme will be run centrally by experienced and competent animal care staff to ensure that the most efficient breeding strategies are used. Requirements and production will be regularly reviewed to ensure minimal wastage. Where appropriate (non-transgenic stock), surplus animals will be shared with other research groups who can make use of their tissues, or used in further breeding.
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 commonly used for work involving genetic alterations. The standard protocols, methods and reagents have been optimized for this species over the years and there are acknowledged benefits from their use. All mice breeding programme under this project will use standard, optimized husbandry techniques. Personnel working with the mice are experienced and competent animal care staff who will quickly gain familiarity with any new strains and their needs. Most strains of GA mice will show mild or no adverse effects, however, all mice will be closely monitored for indications of pain, suffering and distress. Any issues noted will be reported to the NACWO, PPL Holder and/or NVS and dealt with quickly.

Project 82	**Neural regulation of circadian rhythms**	
Key Words (max. 5 words)	Circadian, Eye, Neuroscience, behaviour, light	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 environmental light dark cycle presents itself as the predominant entraining stimulus of the circadian clock. Light information is relayed directly to the clock, located within the brain. Together with this photic information and our behavioural patterns, such as feeding, exercise and social interaction the biological clock is able to integrate and set rhythms for our daily lifestyles. The precise mechanism by which this complex interaction occurs is not clearly understood. Further, as individuals get older a clear deficit in the ability to entrain to environmental stimuli is apparent. Once again, this deficit may lie in a number of input pathways. Our experiments will aim to unravel the neural and cellular mechanisms that are responsible for mammalian circadian entrainment and further, establish where in the light input pathway deficits in visual acuity for circadian responses may originate.	
What are the potential benefits	Desynchronised circadian clocks can result in a	

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	number of adverse effects, such as the onset of depression and cancer. Shift workers and the aged are just two groups of people who are at high risk of such conditions. Our work aims to understand the mechanisms that underlie the process of mammalian circadian synchronisation and hence provide a framework for the development of treatments for circadian related disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using mice in our research. We expect to use approximately 2800 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 level of severity? What will happen to the animals at the end?	The adverse effects include mild to moderate discomfort from some of the procedures; however these will be regulated using appropriate pain relief. Following the protocol animals will be humanly killed and tissue samples taken for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The very nature of our work relies on understanding how a number of mammalian physiological systems interact and regulate the circadian clock. This is impossible to recreate in an in vitro setting.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise animal use by carefully constructing experiments and preforming the relevant statistical analysis that will ensure we minimise animal use and suffering.
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	The mouse is ideal for our experimentation as is displays a strong circadian behavioural phenotype that can be easily measured in the laboratory. This lends itself to our objectives in studying circadian entrainment. Most of the procedures we will use are non-invasive and rely on behavioural observations, thus are painless. Procedures that may induce discomfort will be performed with the appropriate level of pain relief. In order to reduce harm and

(harms) to the animals.	stress to animals we will provide excellent housing and environmental enrichment. In addition we will provide regular close monitoring of any animal that has undergone a procedure in order to ensure its wellbeing.

Project 83	**Neural circuit assembly**	
Key Words (max. 5 words)	Brain, synapse, cortex, development, GABA, neurodevelopmental disorders	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	Neuronal circuitries underlying the function of the mammalian cerebral cortex collectively constitute one of the most complex biological systems. As such, unravelling the mechanisms that control their development represents one of the most challenging questions in science. Understanding this process is also an imperative need in biomedicine, because abnormal wiring is thought to cause severe neuropsychiatric disorders. Thus, while there is growing awareness of the urgency for translation from basic findings to the clinic, it is also becoming clear that the translational bridge must be built on the solid footing of fundamental neuroscience. In other words, we need a better understanding of how the brain works in both health and disease. The function of neural networks in the cerebral cortex of vertebrates relies on the interaction between 2 classes of neurons, excitatory projection neurons and inhibitory interneurons. In these circuits, the output of excitatory neurons is fine-tuned and synchronized by	

	the function of inhibitory neurons, as the conductors of an orchestra do. Now, we know that the precise control of these inhibitory neurons on the network is key for cognitive function. The general aim of my research is to understand how these inhibitory circuits are formed and matured and what is the consequence of their disruption. The understanding of how the brain works in both health and disease, will represents a major opportunity to expand the search for novel targets to treat disorders in which cognitive deficits are at the core of the disease, such as autism and schizophrenia.
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)?	Neuropsychiatric disorders represent the leading source of disease burden in the developed world for people between ages 15 and 49. In contrast to heart disease or most forms of cancer, many neuropsychiatric disorders such as autism or schizophrenia begin early in life and contribute to lifelong incapacity or reduced longevity. Consequently, brain disorders will become an even greater public health challenge in the coming decades. Existing medications for most neuropsychiatric disorders are merely palliative, largely because our limited understanding of their causes. In this context, the development of new animal models with impaired cognition represents a major opportunity to expand the search for novel targets to treat disorders in which cognitive deficits are at the core of the disease, such as autism and schizophrenia. Mice are excellent animal models to investigate brain development. Given our ability to manipulate their genome and their susceptibility to some of the same genetic defects that cause disease in humans-we share 95% of the genes- mice are the gold standard for the type of experiments presented in this Project.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 15,000
In the context of what you	To reach our goals, we will breed and maintain

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 mice. We will use of cell-specific neuronal mutants, which will lead us to obtain more accurate results to implement refinement, although it will require a more extensive breeding to reach the appropriated mouse. We will also perform experiments both in vitro (neuronal cultures) and in vivo. This later set of experiments will include post-mortem analyses (e.g. immunohistochemistry, biochemistry), in utero and neonatal manipulations of mouse, behavioural analyses and electrophysiological recordings in adult animals under terminal anaesthesia. These experiments are either mild or moderate in severity. Animals will be killed at the end of our experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our understanding on the molecular codes underlying synapse formation and the consequence of disrupting this circuitry is still very limited. Therefore, it is difficult to built computer models based on what is still unknown. In addition, the architecture and function of cortical networks are very complex and can hardly be reproduced in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In addition, the project has been designed with the goal of reducing the number of animals used. For example, we have developed a database to register every piece of tissue obtained from these animals, which will be efficiency stored for future studies. I have more than 15 years of experience in using mice as a model system and in my laboratory experiments are always designed to use the minimum number of animals required to generate statistically significant data. All members of my lab will visit the web site of Dr. Michael F.W. Festing http://www.3rs-reduction.co.uk/ for any experimental design. They will use the http://www.biomath.info/ web site to estimate the sample size. Finally, to implement our standards, we will use factorial experimental design to maximize the data collected from each animal. We will also seek for statistic advice to improve the quality of our design and reduce 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.	Mice are excellent animal models to investigate brain development. Given our ability to manipulate their genome and their susceptibility to some of the same genetic defects that cause disease in humans, mice are the gold standard for the type of experiments presented in this Project. In this context, we plan to use cell-specific neuronal mutants by using the Cre-loxP strategy; this will lead us to obtain more accurate results to implement refinement. Additionally, I have established a mandatory induction for all new staff that will join my lab to improve the management of the colonies and the experimental design. This induction will be complemented by the use of databases like http://www.nc3rs.org.uk. To assure the welfare of the animals, anaesthesia, analgesia and general protection will be provided to the mice to avoid any suffering prior to manipulation or sacrifice for the experimental procedures, using approved methods. In particular, additional local anesthetize will be use for surgeries.

Project 84	**Brain development and function**	
Key Words (max. 5 words)	Mouse, development, cerebral cortex, interneuron, brain function	
Expected duration of the project (years)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 cerebral cortex is the nerve tissue that covers most of our brain. It consists of several billion neurons, and it is folded into many gyri and sulci to fit inside the skull. The cerebral cortex enables us to perceive the world around us, to think and make decisions. Unfortunately, we have a very vague idea of how it works. Our project aims to understand the general principles governing the formation of the cerebral cortex. In particular, over the next five years we will concentrate our efforts to elucidate the molecular mechanisms controlling the precise allocation and function of different classes of neurons into the cerebral cortex. We would like to understand how much of the information needed in this process is genetically encoded, and what is the influence of the environment in the formation of this complex brain structure.	
What are the potential benefits likely to derive from this	Understanding the development of the cerebral cortex will help us to decode its function. By studying	

project (how science could be advanced or humans or animals could benefit from the project)?	this process we also aim to understand human disease, because disruption of the normal development of the cerebral cortex causes disorders such as autism or epilepsy.
What species and approximate numbers of animals do you expect to use over what period of time?	The cerebral cortex is a brain structure that has changed very rapidly during evolution, but its general organization is basically the same in all mammals, from rodents to primates, including humans. For example, the cerebral cortex in all mammals consists of hierarchical networks of excitatory and inhibitory neurons. For this reason, we use mice as experimental model. Mouse genetics (i.e. the production of genetically modified strains of mice) allow us to manipulate the function of specific genes that control the development of the cerebral cortex, many of which have been linked to disease in humans. We plan to use approximately 20,000 mice over the next 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?	To reach our goals, we will breed and maintain genetically altered mice. We will also perform experiments both in vitro (neuronal cultures) and in vivo. This later set of experiments will include post-mortem analyses (e.g. immunohistochemistry, biochemistry), in utero and neonatal manipulations of mouse embryos, electrophysiological recordings in adult animals under terminal anaesthesia or following recovery from surgery, and behavioural analyses. Animals will be killed at the end of our experiments. Our procedures are either mild or moderate in severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is currently inconceivable that we could generate computer models to advance our understanding of brain development and the disorders that affect this process.
2. Reduction Explain how you will assure the use of minimum numbers	My research project is designed to minimise the number of animals used to obtain statistically significant results when testing our hypotheses, including the use of factorial experimental designs.

of animals	We use a highly developed database that aids us in obtaining the precise number of transgenic mice required for our experiments. In addition, we collect the brains of all the mice that we used in our experiments (including many from mice that are only used as breeders) and we store all the relevant information in a database. Because the mouse brain is relatively large and can be sectioned in hundreds of slices, in many circumstances we can use some of this tissue for pilot experiments in which we test novel reagents such as antibodies. This reduces sensibly the final number of animals that 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 genetically modified mice to study the function of genes in specific neural circuits during development. For this purpose the mouse is the best possible animal model because their genome is relatively easy to manipulate and their brain develops in a similar manner to humans. The use of conditional mouse mutants, in which only a specific population of neurons lacks the gene of interest, provides the most accurate results and hence refinement. To improve the welfare of the animals, anaesthesia, analgesia and general protection will be provided to the mice to avoid any suffering prior to manipulation or sacrifice for the experimental procedures, using approved methods.

<table>
<tr><td>Project 85</td><td>Tail docking in pigs</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Pig, pain, tail docking, tail biting</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>
<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>Tail biting is a behavioural abnormality which affects ~5% of farmed pigs worldwide and thus constitutes a welfare and economic issue. Tail docking is widely used as a measure deemed necessary to reduce the incidence of tail biting. This project addresses the adverse effects of tail docking, as a preventive measure, in comparison with the adverse effects of being tail bitten later in life, as well as developing strategies that will facilitate reduced risk of tail biting.

Tail docking is usually carried out soon after birth without any pain relief. It therefore causes pain during and after the procedure, though the extent of this is believed by many farmers and veterinarians to be negligible in relation to its value in reducing tail biting risk.

In humans, a considerable literature exists on traumatic injury or amputation and the so-called phantom limb pain. It is not known if tail docking in neonatal piglets produces long term changes in the</td></tr>
</table>

	nervous system which increase sensitivity to pain, and whether this is affected by the age of pig or the amount of tissue removed. Until strategies are developed to change pig production and make tail docking unnecessary, options to minimise the associated harms need further investigation. It has been suggested that behavioural changes can identify early signs of tail biting and facilitate intervention before an injurious outbreak occurs. In poultry, a method for forecasting outbreaks of feather pecking (which has many similarities to tail biting) has been developed based on automated processing of video images of group behaviour. Changes in activity have been reported to precede a tail biting outbreak, but no tool to exploit this knowledge currently exists.
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)?	Firstly, the provision of objective data which can be used by governments to determine policy on future legislation with regard to regulation of the admissibility or procedures for tail docking in pigs when traded against the harms of tail biting. Secondly, the provision of a tool which could assist farmers to detect impending outbreaks of tail biting at a stage when intervention to prevent widespread injury is still possible. This would, give farmers more confidence to reduce tail docking by allowing them to better manage the associated increase tail biting risk. At a more fundamental level, results will also provide benefits in human medicine by providing a model system for investigating the effects of nerve damage in early or later life on subsequent pain signal processing.
What species and approximate numbers of animals do you expect to use over what period of time?	Pigs. Over the 5 year project, up to 300 animals will be used in planned experiments. Up to a further 300 may be recruited if identified as victims of spontaneously occurring tail biting within a commercial pig unit.
In the context of what you propose to do to the animals,	Animals which are tail docked will experience short term pain and stress, which appears minimal in

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	neonates but will be more severe in older animals. They may also experience longer term pain associated with the consequences of nerve damage (analogous to phantom limb pain in amputees), although this is uncertain and the subject of this study. Animals which are victims of spontaneously occurring tail biting will experience the same adverse consequences. The incidence is less than 5% in current docked animals at this site, but the increase in risk with undocked animals is unknown. At the end of the experiment, animals which experienced tail damage will either be humanely killed to provide tissues for laboratory study or, if victims of minor tail biting which has fully healed, may be kept on the farm until they reach slaughter condition. At this point, they will be moved directly to a commercial slaughterhouse. Such animals will be appropriately marked, or batch traced, to ensure that re-use is prevented.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The pain system in pigs is complex and still poorly understood. The assessment of distress felt by the animals arising from tail docking or biting therefore requires behavioural and physiological measures which can only be carried out in living animals. We will combine this approach with use of tissue from humanely killed animals for laboratory assessments, which might validate such methods for use in future studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals necessary for controlled experimental work has been determined using power calculations based on data obtained in previous studies by the project team. The factorial design of the main experiment makes most efficient use of these animals to address the scientific questions under study.
3. Refinement Explain the choice of species and why the animal model(s)	Since the behavioural problem of tail biting is specific to the pig, this must be the species of choice. Suffering during tail docking will be

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.	minimised by provision of anaesthesia and analgesia during and post-surgery, except in the case of the neonates. This exception is because it is normal commercial practice to provide no pain relief during this procedure and a baseline to assess the painfulness is required to inform future policy. The animals in the experimental study of tail docking will be housed in environmental conditions known to minimise the risk of tail biting, with a thermally comfortable climate, readily accessible food and water and enriched pen conditions to satisfy exploratory needs. In the case of spontaneously occurring outbreaks of tail biting, immediate intervention will occur and perpetrators and/or victims will be removed to hospital accommodation and treated in accordance with veterinary advice.

Project 86	**The molecular pathology of axon death**	
Key Words (max. 5 words)	Axon, membrane traffic, microtubule	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	Our overall aim is to characterise the function of selected genes that are required to keep neurons healthy. We aim to analyse mice carrying genetic mutations that cause some of the longest neuronal connections (“axons”) to die. This process contributes to neurological dysfunction in patients with a number of neurological disorders, including common ones such as dementia and multiple sclerosis. We will also determine whether we can modify this axonal death by introducing other gene changes into 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)?	The benefits of this project will be two-fold. Firstly, the project will provide additional understanding of the basic cellular functions of the genes under study. We are focusing on genes that encode proteins involved in cellular pathways that control numerous critical functions, including synthesis and secretion of new proteins and regulation of plasma membrane receptor levels. Understanding these processes is of	

	fundamental scientific importance. Secondly, characterising how abnormality of the genes involved cause disease will allow us to understand the mechanisms by which axons die. This may help us to develop new therapeutic approaches for neurological disorders where axonal death is a feature. Our work may also help to understand the causes of the axonal loss that is a prominent feature of normal ageing.
What species and approximate numbers of animals do you expect to use over what period of time?	We work with mice and we expect to use up to 4000 over 5 years in the course 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?	The neurological abnormalities that we expect to observe are mild (e.g. decreased ability to run). We will carry out experiments to look for signs of neurological dysfunction. These tests are typically painless and include, for example, running on a treadmill. At the end of the experiments the animals will be killed by a humane method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use mice because they are an excellent model of human disease- i.e. they develop similar neurological features to human patients. This means that we can use tissues and cells from the mice, especially real neurons, to try to unravel the causes of, and develop treatments for, the human disorders. It would be impossible to obtain equivalent material from human patients. We also need to discover whether disease severity (e.g. running ability) is influenced by modifier genes. This is of course not possible with cell culture models and requires living animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use good breeding techniques to minimise the number of animals obtained. Where possible we carry out statistical analysis to estimate the minimum number of animals required to observe any significant behavioural or histological phenotype.

	We i) carry out pilot experiments in immortalised cell lines before we use animal tissue, ii) maximise the number of experiments carried out on a set of tissues derived from the mice, and iii) use the minimal number of experiments required to achieve statistically 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 work with mice as they are the lowest mammalian model organism. While lower animals can give useful insights, mice are required to model the mammalian nervous system, and so human disease, as accurately as possible. The specific mouse model that we are currently focusing on develops a very mild, late onset gait abnormality, and so distress to the animals is likely to be small. We will monitor the animals to check that more severe gait abnormalities have not developed. For analysing mouse gait, we use a digital treadmill device that generates multiple quantifiable gait data while the animals walk or run, maximising the amount of data obtained from each animal and so minimising the number of different individual tests required.

<table>
<tr><td>Project 87</td><td colspan="3">Vitamin A and retinoids in the central nervous system</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Retinoic acid, hormones, receptor, season, circadian</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>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 colspan="3">The essential nutrient vitamin A is converted to the compound retinoic acid and it is this molecule that controls the functions of cells. Relatively little though is known how retinoic acid regulates the function of the brain, and this project will explore this question. The project has three central objectives:

1. Examination of how vitamin A and retinoic acid, control the rhythms of the brain. These brain rhythms are essential to control the body and its ability to anticipate the rhythmic changes in the environment whether these be seasonal (6 month cycles) or change in day (24 hour cycles).

2. Investigate how vitamin A synthesized by glia controls the brain. We have discovered that the glia cells, the main cell type of the brain in addition to neurons, convert vitamin A to retinoic acid under some circumstances and we will explore under what conditions this may be important and whether it</td></tr>
</table>

	controls the function of both glia and nearby neurons. 3. Study of the mechanisms by which vitamin A and retinoic acid control the development of the embryonic brain. In contrast to the adult brain, the control by retinoic acid of the development of the fetal brain has been extensively studied. This Is not the case though for the processes that convert vitamin A to retinoic acid which is a key to comprehend how the actions of retinoic acid may be limited for normal regulation of the fetal brain but also to understand why excess levels of retinoic acid severely disrupt brain development.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The research performed in this project investigates fundamental changes in cell function and gene expression regulated by retinoic acid. Understanding this, in the long term, will be essential to comprehend several disorders. For instance: 1. The brain (specifically the hypothalamus) controls the desire to eat in the summer and conserve food during winter. We have strong evidence that retinoic acid in the hypothalamus controls this mechanism and may be used to control our desire to eat, a beneficial behavioural change given the current "epidemic" in obesity. 2. Our study of the actions of retinoic acid in the brain point to parallels with actions of a number of hormones and through such pathways retinoic acid may influence body metabolism and, in turn, play a role in diabetes. A number of studies point to such an interaction. 3. t Our study of retinoic acid synthesis by glial cells may point to a mechanism for their known role in Alzheimer's Disease. 4. Retinoic acid (isotretinoin) is a successful treatment of acne but is known to result in a high incidence of birth defects in pregnant women. Our studies will help point to the cause of this to help develop drugs which lessen this tragic side-effect.

What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats 13,500 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?	The adverse effects will predominantly be minor for instance ear notching, reduction of retinoic acid signalling and injection of cell labelling compounds. A small number of experiments will be more severe and will include studying the effects on the rodent of vitamin A deficiency which leads to eye irritation, surgery on the rodent brain to expose it directly to retinoic acid like drugs and exposing rodent embryos to retinoic acid which severely disrupts their development. In all cases suitable analgesia, anaesthesia (including terminal anaesthesia for some procedures), close monitoring including regular weighing, will be used to reduce all adverse effects. The effects will be mild for all but a small minority of animals for which severity will only be moderate. One of the main approaches of this project will be to give mice and rats substances which alter retinoic acid signalling. These may be given in the diet, by various routes or directly into the brain. In all cases, at the end of experiments, the animals will be humanely killed and tissues analysed. We anticipate that 5% of animals will be of unclassified severity, 93% of animals will be of mild severity and 2% of animals will be of moderate severity
Application of the 3Rs	
1. Replacement **State why you need to use animals and why you cannot use non-animal alternatives**	The principle aim of the proposed program of work is to understand how retinoic acid regulates the function and rhythms of the developing and adult brain. Simple in-vitro systems (e.g. cell lines) cannot substitute for this highly complex biological system that involves interaction between multiple organ systems and the use of animals in the proposed project is therefore unavoidable
2. Reduction **Explain how you will assure**	One of the underlying principles of the research will be to use a minimum number of animals to still obtain valid result. Careful planning of experiments together

the use of minimum numbers of animals	with statistical determination of number of animals required will allow this. The most sensitive methods for detection of molecules are used, with low background noise and variability, greatly reducing the amount of tissue required
3. Refinement **Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.**	Rodents are the species to be examined which provide a relatively “primitive” mammalian species on which there is a vast amount on information into which we can tap regarding brain function in order to understand what may happen in the human brain. Our research also includes studies on the human brain which allows us to determine in what way our research on rodents is applicable to the human brain. All adverse effects will be reduced to the minimum with suitable analgesia anaesthesia (including terminal anaesthesia for some procedures) , close monitoring including regular weighing. We constantly monitor the literature and other resources closely to refine our methods to enable the most effective possible and most likely to achieve the goals we have set. Such refinement includes the use of brain slices to study the effect of retinoic acid-like drugs directly on the brain which reduces the number of rodents required for such studies and removes any possibility of effect of the drugs on the living animal. A further example is our use of approaches to minimize any detrimental effect vitamin A deficiency may have on an animal by supplementing them with protective retinoic acid which maintains their health for most of the experiment.

<table>
<tr><td>Project 88</td><td>Zebrafish Models for Neuromuscular Diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neuromuscular Diseases
Genetics
Molecular Pathology</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>~~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>Inherited neuromuscular diseases such as muscular dystrophy are currently incurable and have a devastating impact on patients and their families as well as requiring substantial resources from the health service. Our overall aim is to examine the mechanisms underlying the development of disease in animal models and to provide basic understanding of disease mechanisms and to evaluate existing and novel treatments, thereby focussing resources on the most promising approaches. Muscle is a complex organ which consists of multiple cell types and this means that cell culture models, which generally consist of a single cell type, have limited applicability to patients. Therefore, many pre-clinical studies can realistically only be achieved in whole animals. In these very basic studies into the underlying basis and mechanisms of disease we have elected to use the simplest vertebrate model available, the zebrafish.</td></tr>
</table>

	Zebrafish have a number of significant advantages for these studies including a short generation time, transparent embryos (which can be viewed under a microscope) and a muscle structure closely related to mammalian muscle (unlike invertebrate models).
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The major benefit of this research is to the scientific community by progressing our understanding of how deficiencies in specific genes lead to a neuromuscular disease. By providing this basic understanding we aim to enable the development of novel and better treatments for patients with neuromuscular disease, although much of this more directed research lies outside the scope of this project. We also aim to test drugs for their efficacy and mechanism of action to inform ongoing drug development programs.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate that we will need to use up to 3000 zebrafish over the 5 year course of the project, the majority of which will be used for breeding purposes only. We minimise the number of zebrafish used by keeping breeding pairs to a minimum and using efficient genotyping strategies.
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 that we undertake involve the use of animals as models for human neuromuscular diseases and so the effects on the animals in part reflect those diseases (muscle weakness and wasting). However, we will limit the effects on the animals to the embryonic period wherever possible and so the expected level of severity is mild. Most animals will be humanely killed at the end of the experiments except those required for breeding who will be expected to be only mildly affected, if at all.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Muscle is a complex organ consisting of multiple cell types which interact both during development and as a mature tissue. Unfortunately, it is not possible to model this structure in cell culture (which generally consists of single cell type) with sufficient fidelity to make the conclusions drawn applicable to patients. Where possible, we use primary myoblast cultures from patients and other muscle cell culture

	techniques in parallel to investigate those aspects which can be addressed in this way (for example the sub-cellular localisation or molecular interactions of a specific protein), but these studies are of limited scope in terms of understanding whole muscle function.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Adult fish are required solely for the purposes of breeding and production of embryos (which form the basis of our experimental protocols). There is no aim to produce adults with a neuromuscular disease. We reduce animal numbers wherever possible by reviewing our experimental data rapidly following an experiment and planning follow up experiments to resolve outstanding experimental questions. In this way the information generated by our research is maximised while experimental animal use is minimised.
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 protocols on this license are designed to minimise any effects on zebrafish after 5 days of development, where the animals have more substantial capacity for suffering. We aim to restrict any harmful effects where at all possible to the embryonic stages where we can closely monitor the health of embryos and humanely euthanize severely affected individuals before they develop into hatchlings.

Project 89	**Mechanisms controlling axonal transport in neurons**	
Key Words (max. 5 words)	Axonal transport, neurodegeneration, neuron	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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
	X	Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our aim is to identify the mechanisms responsible for project (e.g. the scientific axonal transport *in vivo.* Axonal transport is an essential unknowns or scientific clinical process that allows long-range communication in needs being addressed) neurons, connecting peripheral structures such as nerve terminals with the cell body. Neurons are vulnerable to defects in axorial transport, resulting in progressive diseases, such as peripheral neuropathies and motor neuron disease. We plan to use a combination of imaging, pharmacological and genetic approaches to define the mechanisms of axonal transport and its relevance in neurodegeneration. We will determine the function of mutations found in factors associated with neurodegenerative diseases on axonal transport by using assays performed on primary neurons from wild type and mutant mice, and *in vivo* by high resolution microscopy. We will generate mutant mice in which axonal transport is compromised and we will identify the impact of these genetic alterations on mice survival.	

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 contribute to the elucidation of the causes determining neuronal death in neurodegenerative conditions. Understanding the mechanisms responsible for neuronal survival is paramount for a better diagnosis of these human diseases and for more specific and effective therapies. Information gained in our research will also have impact in other areas of human medicine, such as the delivery of gene therapy vectors to the central nervous system and its protection from infection by neurorotrophic pathogens.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use mice and a small number of rats in our research programme. Even though we use mouse embryonic stem (ES) cells for generating some types of neurons, mice remain the most reliable source for embryonic and adult neurons. Moreover, the results obtained with ES cells-derived neurons need verification in primary cells derived from mice. Many mouse models of human neurodegenerative conditions are presently available. These models allow us to test the role of alterations of axonal transport in neurodegeneration. Mice therefore remain the species of choice for our research programme. In view of our scientific targets and the size of our laboratory, we anticipate that we will breed and maintain less then 15,000 mice in 5 years, of which fewer than 5% will be used for intravital imaging. Additionally, we plan to use 100 rats in selected experiments and to validate the results obtained in mice.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Our research programme requires the generation of genetically-modified mice in which axonal transport is compromised. We will determine the phenotype of these mice at cellular, tissue and organismic level during development and postnatal life. Cell death in the nervous system and other tissues will also be determined. Furthermore, we will perform behavioural tests to assess the motor and sensory functions of these animals. Mice will be also used for intravital imaging under terminal anaesthesia. In selected cases, drugs that have been shown to modulate *in vitro* axonal transport will be used to revert axonal transport deficits in mutant mice to evaluate their therapeutic potential.

	The expected level of severity for the genetically-modified animals generated in our plan of work and the above procedures is expected to be moderate (or below). Examples of adverse effects are: abnormal posture of the rear legs, motility deficits and paralysis, progressive neurodegeneration, male sterility, lethality in utero or early life. Health conditions of the animals will be carefully monitored to adhere to moderate severity levels. Mice showing any unexpected harmful phenotype will be promptly sacrificed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Use of animals is required as a source of neurons and to test the effects of mutations of selected proteins on the adult nervous system. Furthermore, animals are essential to study the consequences of altering axonal transport during embryonic development. We have explored alternatives, such as the use of cell lines and mouse embryonic stem (ES)-derived neurons to replace mice and rats in our experiments. ES-derived neurons effectively replace primary neurons in some analyses, such as *in vitro* screens and preliminary test to assess the consequences of gene inactivation on axonal transport and neuronal survival. However, mice and rat are irreplaceable for testing the effects of these mutations in nervous system development, its maintenance in adulthood and to test the effects of small molecules improving axonal transport in vivo.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Established protocols for ES cell differentiation using wild type and mutant ES cells allow us to substantially reduce the number of animals required for *in vitro* screens and transport assays. A further reduction in the number of animals used in our work plan is achieved by using gene therapy vectors with high neuronal specificity. These vectors enable the expression of human pathological proteins in wild type animals, allowing us to induce the desired pathology only in the minimum number of animals required for the analysis. We also grow primary neurons in microfluidic devices,

	which better mimic the culture conditions found *in vivo* and facilitate imaging, thus increasing the rate of successful experiments and therefore decreasing the wastage of cells and animals. Experiments will be designed in consultation with statisticians to minimise the number of animals required and results will be assessed together using statistical software to assess significance and data dispersion.
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 remains the system of choice to dissect the mechanisms controlling axonal transport, since this pathway is similar in mice and humans, and several reliable neurodegenerative mouse models are presently available. Husbandry and many experimental protocols are well established in our laboratory or through collaborators, minimising the use of animals and their suffering during protocol optimisation. Furthermore, analgesia will be used whenever it is possible. We will use mouse models *in* which gene loss or expression of a pathological protein is induced by a specific drug, thus allowing a better control over the appearance of pathological phenotypes. Humane endpoints have been introduced to keep animal suffering within moderate limits, both for established disease models and newly established mouse strains. Local paralysis will be used as humane endpoint for the challenge with tetanus and botulinum neurotoxins. Furthermore, we will use a range of dosages inducing a slow appearance of the symptoms, making it easier to avoid unexpected adverse effect or symptoms exceeding moderate limits.