Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 14

Projects with a primary purpose of: Translational and applied research - Human Nervous and Mental Disorders

Project Titles and keywords

1. **White matter and glial function and pathology**
    - Glia, astrocyte, oligodendrocyte, white matter, nervous system

2. **Improving translation in Parkinson's disease**
    - Cell transplantation, side effects, disease models

3. **Brain mechanisms of cognition and behaviour**
    - Brain, cognition, behaviour, rats

4. **Brain injury and repair**
    - Brain, stroke, optic nerve, plasticity, neuronal growth

5. **Disruption of biological rhythms and stroke**
    - Stroke, hyperglycemia, hypertension, obesity, circadian rhythm

6. **Neuroprotective effects of novel incretin analogues in models of Parkinson's disease**
    - Parkinson's disease, rigor, tremor, drug development, dopamine

7. **Studies of transgenic rodents that express human amyloid protein**
    - Transgenic, rodents, human, amyloid, protein

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

9. **DNA Strand Breaks, DNA damage responses, and Disease**
    - DNA damage, Neurodegeneration, Cancer

10. **Understanding rare intellectual disability syndromes**
    - Cerebellar atrophy, ataxia, bone mineralisation, hyperostosis, intellectual disability

11. **Drug development in Dravet Syndrome**
    - Epilepsy, Dravet Syndrome, seizures, drug development

12. **The development of vaccine against specific domains of NMDAR to allow pre-treatment of patients at high risk of stroke**
    - Stroke, vaccine

13. **Therapies in Parkinson's and Huntington's disease**
    - Neurodegenerative disease model, treatment, behaviour

14. **Role of the environment in drug abuse development**
    - Drug abuse, rat, environment, context

15. **Preclinical screening in drug discovery**
    - Imaging, drug discovery, disease models

16. **Physiology and pathophysiology of motor circuitry**
    - Zebrafish, electrophysiology, motor network

17. **Sheep as a large animal models in health and disease**
    - Huntington disease, sheep, behaviour, electrophysiology

18. **Understanding locomotor control mechanisms**
    - Locomotor circuit; development; neuroregeneration; electrophysiology

19. **Mechanisms underlying Rett syndrome and other autism spectrum disorders**
    - Sensory processing, Rett syndrome, EEG, neurons, autism

20. **Regulation of body glucose and energy levels by the brain**
    - Diabetes, obesity, glucose, energy, brain

21. **Developing in vivo Raman spectroscopy in mice**
    - Raman spectroscopy, neuromuscular disease

22. **Mutations in matrix proteins and disease**
    - Stroke, vascular disease, collagen

23. **Molecular mechanisms underlying neurogenesis and neurodegeneration**
    - Nervous system, neurodegeneration, mouse models

24. **New medicines for amyloid and C-reactive protein**
    - Amyloidosis; serum amyloid P component; monoclonal antibody; immunotherapy; drug development

25. **Brain Protection for Birth Asphyxia**
    - Birth asphyxia, brain protection, newborn, therapeutic hypothermia

26. **Protein function in genetically altered and stroke mice**
    - Brain, Stroke, Brain compounds, Receptors

27. Therapeutic strategies in developmental disorders

- Neurology, autism, intellectual disability, Rett syndrome

28. Sensory feedback in artificial limbs

- Sensory, artificial limbs, stimulation, peripheral nerve

29. Repairing the damaged spinal cord

- Spinal cord injury, axon regeneration, neuroprotection, scarring, cavitation

<table>
<tr><td>Project 1</td><td>White matter and glial function and pathology</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Glia, astrocyte, oligodendrocyte, white matter, nervous system</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td>-</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g.the scientific unknowns or scientific/clinical needs being addressed)</td><td>About 50% of our brain is made up of white matter, which is damaged in important neurological conditions ranging from stroke to cerebral palsy. White matter is made up of axons and glial cells, which are also found at lower density in the grey matter of the brain. The glial cell type called the astrocyte, for example, makes up more than half of all the cells in the brain. Studying injury to white matter and glia requires the capacity to identify these cell types reliably.

This project will use transgenic animals where fluorescent proteins are expressed in selected glial cell types to examine injury in glial cells and white matter. The objective is to determine the injury mechanism operating in glial cell populations and within white matter generally, with a view to identifying likely pathways for pharmacological intervention to protect the brain.</td></tr>
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What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We aim to understand better the injury mechanisms operating in glial cell in the central nervous system, in particular white matter glia. Our work will move toward the development of effective interventions against ischemic injury of the brain. Our specific aims are: 1. To characterise regional differences in glial cell injury at various points in development. 2. To investigate the mechanisms of cellular injury in axon and glial cell populations, in particular within white matter structures. White matter injury is critical in numerous neuropathologies, including stroke, spinal cord injury and cerebral palsy. Stroke in the third most significant disorder in Western society while cerebral palsy is the most common neuropathology of immaturity. The project will move us toward clinical interventions for these prominent diseases, which currently have few and limited treatment options.
What species and approximate numbers of animals do you expect to use over what period of time?	For mice, we would plan to use approximately 600 per year in the generation and maintenance of transgenic colonies and for rats 240 neonates (birth to post natal day5) per year will be killed via decapitation. In addition, 10 rats/mice per year will be killed via anaesthesia/perfusion. Several strains of transgenic mice will be bred to maintain colonies and generate animals for experiments. About 50% of these will have no abnormal phenotype and will be used as controls and the rest will have a mild phenotype.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals are bred and killed via schedule one, with tissue used ex-vivo. The transgenic strains are already well characterized and no significant health concerns have been identified. A small number of animals will anaesthetize and trans-cardially perfused prior to killing. Breeding animals will be monitored daily by staff with extensive training and expertise in animal care. In the unlikely event of the animal showing significant discomfort for any reason, the named veterinary surgeon will be notified and the animal(s) will be humanely killed.
Application of the 3Rs	
1. Replacement	A significant amount of the work we do relies upon using cell culture systems, but cell injury in the

State why you need to use animals and why you cannot use non-animal alternatives	nervous system involves a complex interaction between many different cells types and therefore cannot, unfortunately, be accurately modelled in vitro. We can get useful information from the cell culture experiments but the validation of these results needs to be performed using animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of experiments in our work is kept to a minimum. Initial pilot experiments (2 or 3 animals of each genotype) are performed and, if positive, numbers expanded to the number required to determine statistical significance (determined by power calculation based on historical norms). For assessing cell death levels under a particular paradigm, this is typically an “n” of 8 sections from a minimum of 3 different animals. Precise “n” numbers used for each experiment will depend upon the scatter of the data and the appropriate statistical test(s) used to analyse the data set; advice will be taken from medical statisticians within the University where appropriate. Strict training and supervision of those post-doctoral workers and PhD students performing the work will limit the number of experiments (and therefore animals) rejected from analysis for technical reasons (failed experiments). It is important to recognize that using transgenic mice to identify cell type in far more efficient than methods, such as post-mortem cell staining, that are applied to wild-type animals. The breeding and utilization of these models will therefore represent a significant reduction in the total number of animals required to reach meaningful conclusion.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use mice and rats for these studies as there are a large number of tools (transgenic, antibodies, growth factors and molecular biology methods) available for these two species; more than for other species. Mice and rats also have a nervous system that is similar to that of a human compared to other model organisms such as fruit flies, fish or worms, which lack white matter.

Project 2	**Improving translation in Parkinson's disease**	
Key Words (max. 5 words)	Cell transplantation, side effects, disease models.	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		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 disease, Parkinson's disease, is a degenerative disorder affecting over 1 million people world wide. Current treatments are limited and cause severe side effects. New treatments are on the horizon and involve repairing the brain using cells or viruses. However, in order that we can test these new therapies appropriately, we need to be able to accurately represent them in animal models. This has been made even more important by the finding that when cell therapies were trialled in patients they induced unexpected side effects. Therefore I want to improve the models of disease and apply the therapies so that we can better gauge their potential effects	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	The potential benefits of this project are improvements in therapies for people with Parkinson's disease. Importantly we are aiming to reduce the side effects profile and achieve reliable cell transplantation or viral vector therapies in a well	

project)?	defined phenotype of Parkinson's patient.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use around 1100 rats and 600 mice over the next 5 years. Each experiment with its control groups needs to be relatively large as we are looking for subtle effects which have significant clinical ramifications.
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 moderate severity to be experienced by the majority of our animals, most animals will experience at least one short surgical intervention, always accompanied by pain relief. Likely side effects are rare and from surgery include – pain (analgesia is provided and additional may be given if necessary under consultation with the NVS), weight loss (as a result of the lesion), infection (animals are monitored daily post surgery), seizures (sedatives are administered during surgery). Drug administration may cause mild chaffing from repeated behavioural movements, no other side effects are anticipated. All the animals are killed at the end of the experiment because their brains are also a highly valuable part of the experiment and they are analysed in the laboratory in concert with behavioural data.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The two main outputs of these experiments are behavioural and anatomical: ie the behaviour of the animals and the ability to repair their brains. Neither of these can be replicated without a full neurological circuitry meaning that only whole animal experiments are able to provide the answers we need to move clinical therapies forward.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Many of these experiments will be based on those this group have carried out previously, allowing us to factor in power calculations to identify the optimal group numbers needed to produce significant results. Group sizes may be larger where variables are unknown but too will be based on extensive experience of these models. The use of imaging will provide the opportunity to reduce numbers of animals

	used and simultaneously gain greater insight into the longitudinal effects of medical interventions which are more applicable to patients.
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 most commonly rats are the best choice in this instance because they are large and sentient enough to display behaviours reminiscent of Parkinson's disease that then improve with interventions, their brains being similar and large enough to humans to identify grafts and accurately locate regions of the brain to lesion and repair. The protocols will be adapted to be in line with the latest published information on animal welfare, for example in the use of immunosuppressants and other anti-parkinson's medications.

Project 3	**Brain mechanisms of cognition and behaviour**	
Key Words (max. 5 words)	Brain, cognition, behaviour, rats	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The project will study brain mechanisms that mediate and integrate important cognitive and behavioural functions (memory, attention, cognitive control, sensorimotor, motivational functions). We will also study how dysfunction of these mechanisms gives rise to cognitive and neuropsychiatric problems and examine mechanism-based treatment strategies. We will focus on a brain circuit consisting of hippocampus, prefrontal cortex and connected subcortical sites. This circuit has been implicated in important cognitive and behavioural functions and its dysfunction has been associated with important neuropsychiatric and cognitive disorders. We will particularly focus on how this circuit links everyday memory functions (such as remembering where we parked our car) with appropriate behavioural responses (getting back to the car) (Aim 1) and how imbalanced neural activity within this circuit (too little or too much) affects cognition and behaviour (Aim 2). Moreover, abnormalities of this circuit have been associated with pain conditions, and we aim to clarify	

	the impact of pain on the cognitive functions mediated by this circuit (Aim 3).
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)?	•Improved understanding of the brain mechanisms of normal cognition and behaviour, as well as of the pathophysiological mechanisms relevant to neuropsychiatric diseases. More specifically, our findings will: i) clarify the functional significance of hippocampo-prefrontal-subcortical interactions; ii) clarify how imbalanced neural activity contributes to cognitive deficits relevant to neuropsychiatric disorders and cognitive ageing and inform novel treatment strategies; iii) clarify the impact of pain on brain and cognition. • Findings will pave the way for the development of novel treatment strategies for cognitive and behavioural deficits.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use no more than 1,540 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?	The welfare demands of rats can be well satisfied in captivity. The welfare of our animals is important for the success of our studies, which would be confounded by undue discomfort and stress of the animals. Our experiments require surgical procedures to manipulate selected brain sites and monitor their function. These procedures are performed under full anaesthesia, complemented by appropriate analgesia, and typically do not cause lasting discomfort. In fact, following a few days of recovery, rats are ready to take part in our behavioural tests that require attention and inquisitiveness. Some of the behavioural procedures we use to assess cognitive functions require mild to moderate aversive motivation by requiring escape from water by swimming to an easily accessible platform. Others rely on the motivation to 'work' for food and require that the animals are maintained with restricted access to food. The discomfort caused by these procedures is mild to moderate, and probably less severe than that experienced by wild rodents due to predators

	and other natural stressors. By examining how manipulations of neurotransmission in specific brain sites change the animals' performance measures on our behavioural tests, we can find out how these brain sites contribute to the behaviours under study. To examine the impact of pain on brain and cognition, we will use a well-established model of moderate chronic pain caused by knee osteoarthritis.. This imodel involves the injection of a toxin into the knee to induce knee damage and accompanying chronic pain similar to that resulting from knee osteoarthritis in people. The pain in this model will be moderate. At the end of the studies, all animals will be humanely killed. Brain and knee joints (in pain studies) will be collected for ex vivo analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Detailed mechanistic understanding of brain-behaviour relations requires combination of behavioural testing with invasive methods to manipulate and analyse function of brain sites with high specificity. For ethical reasons, this is impossible in human participants, and animal experiments are necessary. Although, no alternatives are available to replace the proposed in vivo animal experiments, our work is informed by complementary approaches. Non-invasive brain imaging studies in humans reveal important brain-behaviour correlations, but they cannot reveal the causal roles of specific brain processes in a behavioural phenomenon. We collaborate closely with colleagues studying related topics in human participants, using non-invasive brain imaging, and that we have begun reverse-translating some of our cognitive tests into human participants. Even though these studies cannot replace the proposed animal experiments, a close integration of studies in humans and in animal models will help to maximize the benefit of animal experiments. In vitro studies in brain slices can reveal important basic neurophysiological processes, but in vitro

	results are difficult to link directly to behavioural studies. Moreover, neuronal interactions within extended structures, such as the hippocampus, and between brain structures are difficult, if not impossible, to study using brain slices in vitro, as the brain “slicing” destroys the relevant neuronal connections. Using in silico computational methods to model the neurochemical and neural circuit mechanisms of cognition is possible to some extent, but these models depend on the type of data generated by experiments as proposed in this project. We have begun collaborative work with neurocomputational scientists to integrate or data into neurocomputational models. Even though such models cannot replace the proposed animal experiments, such models help to maximize the conceptual benefit of our experiments and they will enable us to devise more accurate and specific hypothesis.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We plan each experiment carefully and use state-of-the-art methods to minimise variability and to obtain robust, sensitive and reliable measures with the minimal number of animals. Experimental procedures will be standardised as far as possible to avoid unnecessary variance. The number of animals required for an experiment will be estimated based on previous studies that examined the influence of comparable experimental variables. If appropriate, effect sizes of treatments will be estimated based on pilot studies on a small number of animals, so that the required sample sizes to obtain acceptable statistical power (>0.8 at type 1-error rate of $p<0.05$) can be estimated. Where possible, within-subjects designs will be used, so that each animal serves as its own control in order to reduce variance and the number of animals. However, some experiments require between-subjects factors, e.g. when the measure under study changes due to repeated testing d. To increase the probability of revealing real treatment effects, the influence of confounding extraneous factors will be

	minimized by random allocation, counterbalancing and/or matching.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Our experiments are conducted in rats, because their brains, including the hippocampal-prefrontal-subcortical system, are similarly organised as in humans and a wide range of behavioural tests is available to study the cognitive functions of interests. In addition, the welfare demands of rats can be well satisfied in captivity. The brains of rats are large enough to tomanipulate and analyse specific brain regions with high selectivity and behavioural tests are well-established in this species, because it has been used for research for a long time. Animal welfare is vital for the success of our studies: behavioural tests require the animals' inquisitiveness and free exploration; insertion of infusion cannulae into pre-implanted guides (as necessary for our studies involving pharmacological manipulation of specific brain sites in behaving rats) requires tame and comfortable animals; undue stress would interfere with most of our measures. Surgical procedures are conducted under aseptic conditions and under appropriate general anaesthesia, complemented by appropriate peri-operative analgesia and care. Appropriate recovery from surgery will be allowed before any behavioural testing. We minimise undue stress of the experimental animals by carefully habituating them to the required handling before any procedure. If we require purely physiological, without behavioural measures, experiments will be conducted under terminal anaesthesia, thereby minimising suffering. Within our animal facilities, high welfare standards are maintained, and, if appropriate, we seek advice from qualified staff or the vet to minimise any suffering.

Project 4	**Brain injury and repair**	
Key Words (max. 5 words)	Brain; stroke; optic nerve; plasticity; neuronal growth	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Injury to the brain either acutely via direct injury (stroke or traumatic brain injury) or chronically via neurodegeneration in Alzheimers disease represent crippling conditions where the body is either paralyzed without sensation or left with dementia, respectively. Both conditions damage nerve fibres that connect the brain with the spinal cord and the body. In order to repair the damage, the damaged nerve fibres must be made to regrow across the injury to make connections below it. There are usually some undamaged nerve fibres, and these can potentially regain some function through stimulating intact fibres to sprout, bypassing the lesion. The project is designed to develop treatments that will repair damage to the brain through promoting nerve fibre regrowth (regeneration) and/or fibre sprouting from existing or uninjured axons (plasticity).	
What are the potential benefits likely to derive from this project (how science could be	This project is expected to provide novel information about growth-promoting molecules to aid neuronal plasticity and regeneration after neurological damage	

advanced or humans or animals could benefit from the project)?	to the brain. It will advance our knowledge regarding molecular and physiological processes that occur after injury to the brain. These processes can then be targeted or manipulated to aid neuronal growth and functional recovery after injury to the brain. In the longer term data obtained from work undertaken on this project are likely to play a critical role in developing new treatments for patients with acute and chronic brain injury, including the recovery of sensory and motor or learning and memory, as well as examining combinations of treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	This project aims to only use rats and mice for anatomical and functional assessment. As the majority of our lab work involves tissue culture models, we aim to use animals only when necessary; such as for testing potential therapeutic drugs or neuronal tracers in a complex living system. This will occur where successful data has been produced from non-animal based data and justifies the use in animals. Thus, we anticipate using approximately 5500 mice and 7500 rats over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	This project aims to elucidate potential growth-promoting candidates that are developmentally regulated in wild type and transgenic animals. Neuronal connectivity can also be assessed via anatomical tracing and electrophysiological assessment and also with the use of transgenic animals that have candidate genes overexpressed or eliminated. To accomplish this different fluorescent tracers or viruses can be injected into the brain or areas that innervate the brain to label different populations of neurons. Transgenic animals with the addition of viruses can also be used to either stimulate or inhibit neuronal signalling. Secondly, we aim to induce injury to the brain either acutely with lesions models such as stroke (by physically or chemically occluding arteries) or traumatic brain injury (by physically impacting the brain using a controlled device). Other models aim to

	chronically injury the brain via chemical neurotoxins to induce neurodegeneration and/or neuronal ablation and consequently mimic diseases such as Alzhemier's. We aim to also induce partial injury to the optic nerve or retina by inducing physical (cut or crush injuries) or chemical lesions and consequently mimic direct physical damage or focal lesions to neurons or supporting non-neuronal cells in the eye. Neurons present in the eye are slightly more permissive than neurons present in the brain and spinal cord and are present as a bundle of easily accessible neuron fibres, consequently this allows us to study and evaluate candidates in a simpler and more permissive model before the movement into the brain. Clinical signs from these brain injuries include sensory and motor impairments, which are subtle deficits that affect the ability to sense and move the affected limbs. General walking, grooming and feeding behaviour are not affected as lesions are only partial. Chronic brain injuries that mimic Alzheimers Disease will include impairments of learning and memory, which are subtle deficits that affect the ability to recognise common objects. Clinical signs from optic nerve injury include minimal visual impairments in one eye, as it includes the use of one eye vision impairments are not complete and the animal is able to still visualize its environment. In all animals general walking, grooming and feeding behaviour are not affected as lesions are only partial. Therefore the majority of animals are likely to show signs of mild sensory, motor and learning impairments which equate to the moderate severity category and only a small number (<10%) will develop signs that reach the moderate severity limit and will need to be killed.
	The progression and development of damage will be investigated and studied to assess differential changes before and after neuronal damage. Finally, different treatment strategies will be administered either directly into the brain or into areas that innervate the brain such as the spinal cord or

	peripheral nerves to assess their effects both on anatomical reorganisation of neuronal connections and functional recovery. Treatment strategies include enzymes, viruses, antibodies, graft implants, etc. The majority of drug treatments are not expected to show adverse effects. Although in the case of graft implants, there may rejection from the host and immunosuppression may have to be used. Again, changes in neuronal connectivity and function can be assessed through anatomical tracers, behavioural function and electrophysiological assessments. At the end of the study, animals are humanely killed. Tissue is collected for anatomical studies or used for molecular/protein assessment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The basic concepts and treatments for brain injury repair are performed using tissue culture models, and the majority of papers from our laboratory involve little or no animal work. Concepts developed in tissue culture have to be tested and refined in a real brain where the complex environment of the adult nervous system is present, and where functional recovery can be measured. No treatment for brain injury could be tested in human patients without extensive prior validation in animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	No animal experiments are performed until a well developed treatment concept has been developed using tissue cultures. For animal experiments, we aim to achieve minimal variation between animals thus enabling the use of smaller experimental groups. Animal group size is determined from previous experience, pilot studies and statistical power calculations to ensure the number of animals used is sufficient to achiever statistical significance.
3. Refinement Explain the choice of species	These projects are performed in rats and mice as their biology of brain and eye damage, repair and

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.	neuronal growth is similar to humans. Rats are also capable of complex visual and motor behaviour and skilled paw use, making it possible to achieve good behavioural outcomes. Mice are used for some experiments because they can be genetically manipulated, allowing molecular hypotheses to be tested. Their behaviour is almost as good as that of rats. We minimise suffering by developing and/or using behavioural outcome tests of high resolution that pick up deficits in fine movement control. Therefore, it is not necessary to make large and disabling brain injuries. We use well established lesion models that have been extensively used in our lab throughout several previous studies, thus we have achieved a high rate of reproducibility with our brain and optic nerve injury models (consistent size and outcome).

<table>
<tr><td>Project 5</td><td colspan="3">Disruption of biological rhythms and stroke</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Stroke, hyperglycemia, hypertension, obesity, circadian rhythm</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td><s>Yes</s></td><td>No</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 colspan="3">Global urbanisation associates strongly with obesity, high blood pressure and diabetes and factors related to the urban lifestyle may induce increased susceptibility to cardiovascular and metabolic disease. In this project we propose that disruption of daily biological rhythms caused by exposure to erratic light dark cycles generated by artificial light is one factor that contributes to the diseases of urbanisation. Specifically our focus is on stroke which is the leading cause of neurological disability in adults as well as the third major cause of death in the UK. Obesity, high blood pressure and diabetes increase the risk of having a stroke but also make the effects of stroke worse for the patient who suffers one. In this project we will expose rats to erratic light dark cycles, which mimic patterns encountered by shift workers, to disrupt their biological rhythms. We will then experimentally</td></tr>
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	induce a stroke and measure the amount of brain damage using MRI in anaesthetised animals and by postmortem analyses. Our hypothesis is that rats with disrupted rhythms will experience a greater amount of brain damage.
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 findings will give us insight into how urbanisation affects health and can inform how society addresses issues associated with shift work.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats: up to 600 over 5 years Mice: up to 200 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Approximately 30% of the experiments will be conducted with animals under general anaesthesia and they will not recover from the experiment. Stroke is a serious medical condition and therefore animal models are classed as substantial severity. Stroke-induced brain damage causes functional impairment in animals that recover from the surgical procedure used to induce it. However, rodents have a good capacity for functional recovery and regain movement, feeding, drinking and social behaviours within 2-3 days after the surgery. At the end of the experiment animals will be killed so that the amount of brain damage can be measured or biochemical analyses can be performed on the brain tissue.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Stroke is a complex condition because it originates within the blood vessels supplying the brain (a clot is formed and blood flow is reduced) but the major effects are in the brain tissue which is damaged by reduced delivery of oxygen and glucose. Moreover factors in the body such as blood pressure, blood glucose and temperature can affect the amount of brain damage after stroke. Therefore it is essential to model the intimate relationship between the two

	tissue compartments: vascular and neural and also between the brain and the rest of the body. It is possible to model the effects of stroke in brain slices or cultured cells by oxygen and glucose deprivation and these are widely used. But they do not replicate one of the most important elements which we wish to study in this project, namely the region of brain tissue that is functionally impaired due to reduced oxygen and glucose delivery, but is not irreversibly damaged because it receives just enough blood flow to allow it to survive for a few hours. This area of brain tissue can be saved if blood flow is restored. In this project we hypothesise that this "salvageable" area of tissue is compromised by the metabolic changes that accompany disturbances of biological rhythms caused by exposure to erratic light/dark cycles. Therefore it is necessary to use animals so that the interplay between factors such as blood glucose, blood pressure, obesity and the evolution of brain damage can be examined.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our laboratory has built up experience over the last 25 years of experimental stroke research using animals. We therefore have a wealth of information and data which can be used to determine the minimum number of animals required to test the scientific question being asked in each individual experiment. We also have expertise in statistical design that informs us on the minimum number of animals required in any given 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.	Mice are the lowest species that can be used for experimental stroke studies of the intact brain and this species is used in studies of how genetic modification influences stroke outcome. However, two important factors mean that rats are the preferred species for this project: rats have a larger brain volume than mice and for MR imaging studies they give greater image quality and resolution; there is considerable variability in the anatomical organisation of the blood supply to the brain in different strains of mice and these abnormalities compromise the quality of the experiments and

	mean that greater numbers must be used. The induction of stroke and any MRI scanning will always be carried out under anaesthesia and where possible, animals will be used in experiments where they do not recover from anaesthesia following the stroke (approx 20% of experiments). The mildest form of stroke which produces a reproducible degree of damage will be used to minimise animal suffering. Post-operative care (regular monitoring, subcutaneous fluids, soft bedding, softened diet, etc) will be provided. Animals under procedure will be checked regularly for any deterioration in their condition. Should this occur, prompt veterinary advice will be sought. If the severity limits of the licence are exceeded, the animal will be promptly and humanely euthanised.

Project 6	**Neuroprotective effects of novel incretin analogues in models of Parkinson's disease**	
Key Words (max. 5 words)	Parkinson's disease, rigor, tremor, drug development, dopamine	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Parkinson's disease is the second largest neurodegenerative disorder. More and more people are affected each year, as the life expectancy of people in the industrialised countries increases. Currently, there is no effective treatment to stop disease progression. The drugs to be tested have shown positive effects in animal models of Alzheimer's disease and other disease models. It is likely that these novel drugs will have protective effects in Parkinson's disease. In a pilot clinical trial, an older version of the drugs tested in this proposal has shown improvement in patients with Parkinson's disease. It is important to test the newer, more effective drugs in animal models first before they can enter clinical trials in patients.	
What are the potential benefits likely to derive from this project (how science could be	It will be tested how effective the drugs are in protecting the animals from Parkinson's disease type motor impairments. Also, different drug doses will be	

advanced or humans or animals could benefit from the project)?	tested. The drugs that show the best effects will be tested further in clinical trials in Parkinson's disease patients. One drug from this class of drugs already has been tested in a pilot study and showed good effects. As some of these drugs have been tested in toxicology tests already, they can be immediately advanced to be tested in patients.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice will be used, as they are the standard experimental animals in these types of tests. Several animal models of Parkinson's disease have been developed and are well established. In addition, a transgenic mouse model of Parkinson's disease, which expresses a human mutated gene, will be used. The numbers used are the lowest possible for the experimental designs. A lower number would lead to ambiguity of results and might not produce a significant result, despite the drug's actions. This situation would invalidate the experiments, and the animals would not have been used in the most sensible and effective manner. We will use up to 2000 animals in the five year period. That is a maximum number, and the actual numbers will be very likely lower.
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?	Expected adverse effects for each protocol will be different and thus, described with protocols. At the end, animals will be perfused for brain tissue collection or will be killed by 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 processes observed in PD are slow in development and require studies in the brains of living animals and humans. Furthermore, the testing of motor activity and control requires research on living animals. In addition, invasive work can only be done on animals, not on humans. No cell culture studies or computer models can replace these essential toxicology and physiology studies. Using

	rodents will be the lowest form of mammal that can be used for this work while ensuring results that can be translated into human application.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In the behavioural tasks, each group consists of 10-12 animals. This ensures that enough data points can be gathered to apply statistical analysis such as a Two-way ANOVA (analysing the difference between test and control group, and the effect of time) or of T-test. In the event that data are not normally distributed, U-tests and Kruskal-Wallis tests will be applied (see publications in the reference list for examples). All experiments have been discussed with statisticians to obtain the best data analysis with the lowest numbers of animals required for the research. The numbers used are the lowest possible for the experimental designs. A lower number would lead to ambiguity of results due to variation and might not produce a significant result, in spite of drug actions. This situation would invalidate the experiments, and the animals would not have been used in the most sensible and effective manner.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	For the research of Parkinson's disease, chemical lesion of dopaminergic neurons and transgenic mice are the only available animal model in biomedical research. No other animal models can be currently produced to research the cytotoxic basis of PD. Recently, transgenic rats and higher species have been developed, but for the tests proposed in this application it is entirely sufficient to work with wild type rats and transgenic mice. The protocols used are standard protocols in this type of research that have been streamlined to be the most effective testing method without causing any distress to the animals while using the lowest numbers of animals required. All work will be conducted by trained and experienced staff, and animals will be monitored by staff and the named veterinarian of the animal unit.

<table>
<tr><td>Project 7</td><td>Studies of transgenic rodents that express human amyloid protein</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Transgenic, rodents, human, amyloid, protein</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 yrs</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</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 proposed project will analyse the effects that proteins (amyloids) found in the brains of people with Alzheimer’s Disease (AD) have on behaviour and on neuronal activity in mice and rats. Several novel drugs will be tested to see whether these new compounds can improve on the symptoms observed in transgenic mice and rats that express these human proteins in their cells. Currently we have no effective drugs to prevent the accumulation of amyloids in the brain, and to prevent the degenerative processes in the brain that are activated by amyloids. It will be tested whether novel drugs that activate specific receptors on neurons can prevent the neurodegenerative processes seen in the transgenic animal models of AD. For example, it has been observed that people with type II diabetes run a higher risk of developing</td></tr>
</table>

	AD. This is linked to the fact that the insulin signalling cascade does not work properly in conditions of type II diabetes. The insulin receptor is also an important growth factor in neurons, and the lack of signalling activity in the brain appears to increase the risk of chronic neuronal damage development. Novel drugs that have been developed to treat type II diabetes will be tested in the experiments to see whether they can reduce or prevent the neurodegenerative processes seen in the transgenic animal 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 proposed research could lead to the development of novel drugs that can help prevent the development of AD. Currently, no drugs are available that can achieve this. The NHS does not consider any of the available drugs to be effective in treating AD. It will be evaluated how the novel drugs affect the degeneration and behavioural changes. A reduction of the degeneration could help millions of AD sufferers that are currently without treatment. One drug tested by me in the described experiments is now in a clinical trial in patients with Alzheimer's disease.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use up to 2500 animals in the five year period, approximately 1000 rats and 2000 mice. These are maximal numbers and the actual numbers will be very likely lower.
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 treatments listed in this application are mostly behavioural tasks that will not have side effects to the animals. They will be handled to avoid any stress by the treatments. The surgery is the treatment that will induce some level of discomfort. All efforts will be made to minimise this. All animals will be observed for negative side effects by trained staff. The protocols used have been tested and improved over years and cause the least suffering possible for these types of experiments. Group sizes are optimised so that the minimum of animals will be used for these procedures.

	Animals will be bred and some of them will be implanted with cannulae for drug delivery. Animals will be injected with drugs and tested in several behavioural memory tasks. The animals will age and will develop plaques over a course of 5-8 months. The effect of drugs on preventing or slowing the neurodegenerative processes of amyloid accumulation will be tested. Neuronal activity and synaptic plasticity (brain function) will be tested to analyse in more detail how the amyloid accumulation affects neuronal activity over time.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The processes observed in AD are slow in development and require studies in the brains of living animals and humans. Furthermore, the testing of cognitive processes requires research on living animals. In addition, invasive work can only be done on animals, not on humans. No cell culture studies or computer models can replace these essential toxicology and physiology studies. Using rodents will be the lowest form of mammal that can be used for this work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In the behavioural tasks, each group consists of 10-12 animals. This ensures that enough data points can be gathered to apply statistical analysis such as a Two-way ANOVA (analysing the difference between test and control group, and the effect of time) or of T-test. In the event that data are not normally distributed, U-tests and Kruskal-Wallis tests will be applied (see publications in the reference list for examples). In the electrophysiology measurements, groups of 6-8 will be used. Here, the variation is not as high, and statistically significant results are expected with such group sizes. Data will be analysed using T-tests, Two-way ANOVA, and *post-hoc* parametric tests. All experiments have been discussed with statisticians to obtain the best data analysis with the lowest numbers of animals required for the

	research. The numbers used are the lowest possible for the experimental designs. A lower number would lead to ambiguity of results due to variation and might not produce a significant result, in spite of drug actions. This situation would invalidate the experiments, and the animals would not have been used in the most sensible and effective manner.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	For the research of Alzheimer's disease, transgenic mice are the only available animal model in biomedical research. No other transgenic animals can be currently produced to research the cytotoxic basis of AD. Recently, transgenic rats and higher species have been developed, but for the tests proposed in this application it is entirely sufficient to work with mice. The protocols used are standard protocols in this type of research that have been streamlined to be the most effective testing method without causing any distress to the animals. All work will be conducted by trained and experienced staff, and animals will be monitored by staff and the named veterinarian.

<table>
<tr><td>Project 8</td><td>Bioelectronic Medicines</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Electrophysiology, implantable devices,</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The purpose of this project is to gain a better understanding of how the autonomic nervous system controls functions of visceral organs and to ascertain whether modulation of the nervous system (neuromodulation) can be accomplished electrically. If this is successful, it gives us reason to believe that neuromodulation could be used to treat diseases associated with those organs, such as asthma (lungs), hypertension (heart and vasculature), and bladder and sexual dysfunction.</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 the ways in which the autonomic nervous system affects organ function will provide justification for evaluation of whether this has the potential to treat diseases associated with those organs.

The early stage data that will be collected will allow dose predictions to be made on the efficacious levels of neuromodulation, as well as to support</td></tr>
</table>

	candidate selection of devices that could be developed for the clinical market.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse (300 over 5 years) and Rat (750 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?	Initial work will use tissues from euthanased rats and mice, treated with tracers, to identify nerve pathways. The next stage will be to implant stimulating devices in terminally anaesthetised animals and observe biological and functional effects of electrical stimulation. Blood pressure, heart rate and biological markers that measure changes in organ function will be monitored and used to define endpoints. If a nerve or tissue is inadvertently damaged by the injections or surgery (less than 5% of animals) it may cause neuropathic pain. If signs such as guarding a specific area of the body, hyper sensitivity to touch, vocalisation or spontaneous and repetitive movements are observed then the animal will be killed by a Schedule 1method without delay. All animals will be euthanised at the end of studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	A limited amount of testing has been done without using animals to give confidence nerve stimulation may treat disease. The science cannot be advanced further without using animals. Only a whole body system biology approach will give conclusive evidence and understanding that manipulation of the nervous system can be an effective treatment of disease. A computer model does not yet exist to test nerve stimulation as a treatment of disease.
2. Reduction Explain how you will assure	Pilot studies in small numbers of animals (e.g. 1 to 3) will be used to develop optimal methods, assess feasibility and outcome measures, and to estimate

the use of minimum numbers of animals	required group sizes for larger 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 will be used for all experiments because they have a nervous system that is anatomically and functionally sufficiently similar to that of humans. Where surgery is used aseptic surgical techniques, anaesthetics and pain preventing medicines will be employed to minimise potential of post operative infection and pain. Surgically implanted recording electrodes will be externalised and secured in a way as to prevent the need for repeated surgery when testing on multiple days and it will also negate the need for continual anaesthesia. The implantation itself will cause no lasting adverse effects. On the rare occasions where animals need to be singly housed to prevent fighting or damage to implants they will be given regular social interaction with other animals.

<table>
<tr><td>Project 9</td><td colspan="3">DNA Strand Breaks, DNA damage responses, and Disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">DNA damage, Neurodegeneration, Cancer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><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 colspan="3">Understanding how cells, tissues, and organs respond to DNA damage is crucial to our understanding of how DNA damage causes disease. DNA damage arises both during normal growth and from exposure to chemical toxins in our environment. The threat posed by DNA damage is illustrated by the existence of human genetic diseases in which our ability to properly repair damaged DNA is abnormal or absent. However, our understanding of how DNA damage causes disease is limited. Only by using mouse models can we address this deficit and thereby contribute to better diagnosis, management, and treatment of human diseases associated with defects in the response to DNA damage.</td></tr>
<tr><td>What are the potential benefits likely to derive from this</td><td colspan="3">This project will enhance our understanding of how mammals respond to DNA strand breaks/DNA</td></tr>
</table>

project (how science could be advanced or humans or animals could benefit from the project)?	damage and how these DNA lesions impact on tissue development and maintenance. In particular this work will address the mechanistic cause of diseases that arise because of genetic defects in DNA repair and DNA damage responses. This work will also enhance diagnosis of DNA damage-related pathologies and, in the longer term, provide new approaches towards clinical intervention.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate using a maximum of 38,000 mice over the 5-yr period 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?	Adverse effects arising from new genetically altered strains are difficult to predict. However, we anticipate that most mice will exhibit little or no phenotype, with only a small fraction (<10%) exhibiting mild or moderate phenotypes associated with developmental dysfunction and/or tissue degeneration. The mice will be assessed daily or twice daily as appropriate throughout their lives for adverse effects, e.g. signs of pain or distress, weight loss, reduced motility, neurologic symptoms. Animals exhibiting any severe phenotypes will be killed, or in the case of individual animals of particular scientific interest, advice sought from the local Home Office Inspector and the Animal Care Staff and Veterinarian. If severe phenotypes are observed i.e. causing substantial discomfort such as major reductions in body weight (20%), severe reductions in motility (e.g. difficulty to feed/drink or remain upright), mice 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	Where possible, we use microorganisms or immortalised human cell cultures for our studies, since these do not require animal models. However, mice are required for some experiments where the impact of unrepaired DNA damage on developmental and degenerative pathologies is under investigation. These questions are critical to our understanding of a plethora of human DNA repair-defective human diseases that are

	associated with neurodevelopmental dysfunction, neurodegeneration, and/or cancer predisposition. Finally, where mouse cell lines can replace whole animal experiments, we employ these to further reduce the number of animals we require.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We always ensure our breeding colonies are no larger than minimally required for stock maintenance, and we use cryopreservation to avoid housing animals in the laboratory in the long term. Where appropriate, group sizes will be determined by standard power analysis (SISA), and we ensure these are as small as possible by appropriate experimental design (e.g. by addressing multiple related questions simultaneously, with single test and control groups).
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the best available mammalian model for understanding how DNA damage casues diseases such as cancer and nervous system dysfunction in humans. Only in those cases where critical questions cannot be addressed by other methodology do we utilise mice. Under all circumstances we minimise animal suffering by daily/twice daily health and behaviour monitoring, including the use of health score sheets, and by humane killing of animals if the appropriate humane end points (see specific protocols) are exceeded. Where in doubt, advice will be sought from the Animal Care Staff, Veterinarian, and the Home Office Inspector.

<table>
<tr><td>Project 10</td><td>Understanding rare intellectual disability syndromes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cerebellar atrophy, ataxia, bone mineralisation, hyperostosis, intellectual disability</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
</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 sets out to develop and investigate models for rare developmental or childhood onset syndromes affecting the central nervous system, neuromuscular and skeletal systems. Mouse models will help us to better understand the onset and pathway of disease such as cerebellar degeneration with associated ataxia (loss of muscle co-ordination) or hyperostosis (increase bone production). Although we are specifically modeling rare diseases in the first instance, we anticipate that our findings will have implications for a wider set of related defects which may include the possibility of converse conditions such as osteoporosis (brittle bones) and osteoarthritis (swollen and inflamed joints), which could be linked in to the same pathways.</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 aim of our project is to gain a better understanding of the factors and their mechanisms of action, underlying novel, complex syndromes affecting intellectual disability, brain development and/or regulating bone physiology. Overall, our research aims to improve the quality of life and mobility of people with associated pathologies. Ultimately, we hope to help develop treatment options for patients with these and related pathologies.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use wildtype and mutant strains of mice to provide models of the human genetic diseases under study. We would not expect to use more than an estimated 3000 over the course of the 5 year study.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All procedures to be undertaken are performed in mice. Several of the animal models are already available and have no recognized adverse affects. It is expected that mice born with *Ptdss1* gain-of-function mutation will generate increased bone mass with age. Postnatal *Snx14* knockout mice may develop cerebellar hypoplasia, which may seen as progressively worsening ataxia and mental and behavioral abilities. These novel models will be carefully monitored by appropriately trained experimenters but are not expected to exceed “moderate” in severity for the proposed experiments. Animals at the end of experiment will be killed by approved methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The syndromes we seek to model are complex and involve multiple organs and tissues. We can and will use alternatives such as cell culture where ever possible, however, we can only fully reproduce certain physical characteristics in an animal model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We always aim to reduce the numbers of animals we use and include power analyses in order to identify the minimum number of animals that we need, in order to answer the specific questions

	being posed.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Cell culture models cannot easily represent the long-term behaviour of specific types of neuron and surrounding cells in the brain, or dynamic growth of head and face bones. It is critical to be able to measure the effects of genetic changes not only at the cellular level but also on the complex structures in the whole animal. The possibility of genome manipulation makes the mouse an invaluable model to study the genetics of complex syndromes in a live mammalian system. During this project we also take advantage of other disease models including standard cell lines and patient blood derived cells and fibroblasts (collagen-producing cells) where available.

Project 11	**Drug development in Dravet Syndrome**	
Key Words (max. 5 words)	Epilepsy, Dravet Syndrome, seizures, drug development	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Dravet Syndrome (DS) is a catastrophic childhood epilepsy that kills ~25% of those presenting before adulthood and profoundly limits the lives of those who survive. It is untreatable. We have discovered two drugs that are showing promise in clinical trials for the treatment of Dravet Syndrome. However, we still do not know the mechanism(s) by which our drugs exert their beneficial effects. Proving that they work in this mutant mouse model of DS which so closely resembles human DS will provide us with a way to determine underlying drug mechanisms of action. Understanding the mechanism of action will help us understand what makes some epilepsies resistant to drug treatment and also help us develop new treatments for drug-resistant epilepsies. We will establish and maintain a colony of mutant DS mice. We will use behavioural and electrical (like EEG) measurement methods to find out how effective our drugs are in these mice. If the drugs work, we will use tissue from these and additional mice for ‘test	

	tube' experiments to determine the mechanism of action of the study drugs. **Objective 1:** Establish, expand and maintain a colony of SCN1A mutant mice **Objective 2:** Determine the anti-epileptic efficacy of a given study drug using behavioural and electrical monitoring methods. **Objective 3:** Determine the mechanism of drug action by sampling body fluids and using 'test tube' methods.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Knowing how these drugs work will help us better understand why the drug-resistant epilepsies that affect 15-20 million people worldwide occur. It will also allow us to develop better medicines to treat these resistant epilepsies. Knowing whether our drugs work in this mouse model of DS will help regulators, industry, clinicians and patients benefit by informing and, if results are positive, speeding up the process of clinical licensing of these drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	SCN1A mutant mice; ~3500 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?	75% of these mice exhibit no symptoms. The remaining ~25% experience lethal seizures and, without intervention, do not survive past ~16 days old. Therefore this work is considered to be of severe severity. We will also treat animals with our study drugs. Whilst these have been well tolerated in our long experience we cannot rule out rare reactions in a tiny proportion of animals. We will also implant some animals with electrodes to monitor seizure activity or a small tube to sample brain fluids. This can be painful and cause suffering but we will use painkillers whenever possible.

	We will need to take tissue samples from some animals in order to determine which have the disease and which do not. This can cause some pain but will be minimised by pre-treatment with a local anaesthetic. All animals will be killed at the end of the study unless asymptomatic animals are transferred to other authorised users to establish colonies for their own research.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Epilepsy is a disorder of the whole body (brain and periphery) and, as such, is too complex for current 'test tube' approaches to properly model. Moreover, regulatory (EMA) requirements demand testing in whole animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiment sizes will be determined by statistical calculations which tell us the minimum number of animals required for a meaningful drug effect to be accurately detected in our experiments. A professional statistician will be consulted when required to ensure experimental designs are used that minimise the number of animals required yet maintain adequate precision. We will not breed more animals than we will need for our experiments.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use mice which are the least sentient species possible that can be used. The mouse genome has a sufficiently high homology with that of the human and the physiology and pharmacology of mouse nervous system is sufficiently similar to humans to accurately reflect DS. Wherever possible, we will not use of the C57BL6 SCN1A +/- strain in order to minimise the number of animals that experience recurrent seizures over several weeks. SCN1A -/- animals which exhibit seizures by P9 and, without intervention, die by P16 will be preferred as any suffering will be limited to the shortest period of time. We will only breed sufficient animals to meet our

	planned experimental needs and we will use animals as early as is scientifically possible for our experiments to minimise the time during which seizure symptoms manifest. We will use our strong links with both the creator of these mice and the EU Dravet Syndrome Foundation which funds DS research and supports patients to gain further experience with the model to improve and maintain welfare. We have already conducted several visits to these sites and will continue during the project.

Project 12	**The development of vaccine against specific domains of NMDAR to allow pre-treatment of patients at high risk of stroke**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	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 overarching objective of our research is to develop a new therapy for stroke. We have proposed three novel and compelling approaches that could lead to the development of new treatments for stroke. At the end of this body of work (~5 years), if the approaches we have proposed show efficacy and safety, we would hope to start clinical testing. These approaches are not interdependent and could independently lead to success. Stroke is brain injury caused, in most cases, by the blockage of a blood vessel that supplies blood to the brain. There is currently only one approved stroke therapy, tissue plasminogen activator (TPA),that must be administered to stroke victims within 4.5 hr of symptoms onset. TPA is a clot busting agent which breaks down the clot which is blocking the vessel. However, only 3% of patients will receive this medication primarily because most do not arrive in hospital early enough to be eligible; or because they are taking medications that make them ineligible for the clot busting therapy. Thus, there is a desperate need for new therapies.	

	We are proposing three novel approaches to stroke therapy development: 1. The development of drugs which have multiple mechanisms of action. These types of drugs have not been previously developed and tested in stroke. 2. The development of a vaccine for stroke that would allow the treatment of large numbers of patients who are at risk for stroke. 3. To better understand the body's own protection mechanisms against stroke and to understand how these messages maybe transmitted to subsequent offspring. This knowledge could be used to develop new therapies.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	If we are successful in developing a new therapy for stroke, this could have huge benefits for humankind. Stroke is the third biggest cause of mortality and the biggest cause of adult disability worldwide. A new therapy for stroke that could benefit large numbers of patients is desperately needed.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use only rats and mice for this body of work. Approach 1: species –rat. Approximately 500 animals over three years. Approach 2. Approximately 100 over three years. Approach 3: mice. Approximately 160 over three 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 model we will use is the middle cerebral artery occlusion (MCAO) model which models human stroke. Some of the animals are expected to reach severe level. However, these animals will be closely monitored and suffering will be minimised. Any animals exhibiting significant levels of suffering will be humanely terminated. All animals will be humanely terminated at the end of the procedure.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our previous work has used in vitro approaches to select candidate drugs and therapies which underpin the three approaches that we have proposed in this body of work. Our previous work has allowed us to select candidates with the highest likelihood of success. However, going forward, in vitro work cannot replace animal experiments which are needed to confirm efficacy and safety. The outcome measures that need to be tested include functional and behavioural testing which cannot be tested in cells. In addition, stroke injury involves a complex interplay of many different cells in the brain and many organs systems. This cannot be tested using in vitro approaches.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals will be kept to minimum by ensuring that the experimental designs to be used are rigorous and that all personal licensees (and dedicated animal care staff) working on this project are appropriately trained and suitably competent. This will enable a high success rate to be achieved with minimum number of animals being used. Statistical approaches such as Anova will be used so that the number of animals in each study is minimized by using multiple comparisons within a set of data, thus eliminating the need to carryout multiple group 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.	An ongoing aim is to constantly refine the use of experimental animal models and reduce the impact on the animal. We are working on refining our surgical methods that are used to induce MCAO which can affect the results and also reduce the impact/severity on animals.

Project 13	**Therapies in Parkinson's and Huntington's disease**	
Key Words (max. 5 words)	Neurodegenerative disease model, treatment, behaviour	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aims of this project are: To identify and evaluate existing and new substances and cell-based therapies to treat the neurodegeneration seen in Parkinson's disease (PD) and Huntington's disease (HD); To develop new animal models of PD and HD, especially modelling the dementia seen in these diseases, with which to test newly developed treatments; To better understand how drugs currently used to treat these diseases (especially dopamine agents) may interfere with both disease and the brain's own repair processes.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or	Parkinson's and Huntington's disease are both neurodegenerative diseases characterised as movement disorders because parts of the brain responsible for controlling movements have been	

animals could benefit from the project)?	affected by the death of cells. Parkinson's patients suffer a progressive loss of movement, stiffness and weakness in the muscles, as well as involuntary movements due to the uncontrolled release of dopamine from their drug treatment. Huntington's disease is characterised by uncontrolled movements as well as cognitive and psychiatric disturbances. Treatments are therefore aimed both at controlling the abnormal movement symptoms of the diseases, and the often distressing psychiatric symptoms, usually by replacement of the neurotransmitter dopamine or similar drug-based manipulations, also by surgical replacement of the cells which are lost to the disease. The benefits which we therefore hope to derive from this project are to identify drug treatments that can slow down or reverse the progressive loss of cells which lead the disease process. We are working towards developing new stem cell-derived nerve cells which can be grafted into the brain of patients to repair the relevant diseased brain regions in PD and HD. We work to better understand the brain's own repair systems and how we can help it to improve thinking, memory and other deficits in HD and PD using simple drugs that are already being used in clinics for these patient groups. Finally this project will help us to gain a greater understanding of how the proteins which are faulty in these diseases interact to bring about the cell dysfunction and death, and how associated inflammation responses help or hinder the disease progression.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use no more than 13,525 mice and no more than 4,500 rats during the 5 years of this project. We will use the minimum numbers of animals possible to achieve biologically and statistically meaningful data.
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	For the majority of our animals, we anticipate no more than transient discomfort and no lasting harm. Some animals (20% of mice) carrying a genetic mutation will gradually progress to a disease state modelling the human disorder, showing gradual

happen to the animals at the end?	weight loss, reduced movements, a persistent tremor, and abnormal gait, eventually reaching the moderate severity level, where the defined end points will determine that they are humanely killed. Some animals (20% of mice, 50% of rats) will undergo surgery, with a moderate severity limit in the immediate post-operative period, during which time analgesia will be present. Post-surgery, they may show reduced or altered movements, where they have received a lesion to mimic an aspect of the human disease. These animals may later receive a treatment to repair the lesion, such as cell transplantation, which will assess the effectiveness of the cells to ameliorate the effect of the disease model. Animals are expected to have mild to no lasting adverse effects after they have recovered from the surgery itself. Some animals will undergo mild food or water restriction to motivate their performance in behavioural tasks which are reward-driven, and they will be carefully monitored to ensure that no sign of dehydration occurs, and any weight loss is kept within a defined limit. We expect no adverse effects due to behavioural testing, other than a possibility of transient distress. All animals will be humanely euthanized, and in most cases tissues will be taken to inform the relevant study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This research is only possible because we need to study whole brain diseases as we do in patients. Human studies in patient groups are used as much as possible, with functional imaging and post-mortem studies but new therapies to arrest the disease can only be studied using animal models because of the experimental nature of the agents being tried.
2. Reduction Explain how you will assure the use of minimum numbers	We carefully design our experiments such that we maximise the behavioural and histological data collected from each animal. We use the minimum number of animals required to yield statistically and

of animals	biologically meaningful data, and we will work towards designing more refined behavioural tests such that the number of animals needed to generate this data can be reduced.
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 models we use are the least sentient beings still able to mimic the human neurodegenerative disease we are studying, with a brain which is anatomically similar to the human brain. Rats are used where we test cell based therapies as they have a slightly larger brain to repair and a greater behavioural repertoire to probe and test. Transgenic mice are used as they contain the genes responsible for unique human disorders such as some forms of Parkinson's and Huntington's disease. They allow us to more faithfully study the pathology and behavioural deficits of the human disorder. We pilot new treatments on a small number of animals, and any adverse effects are discussed with the named veterinary surgeon. We take animal welfare very seriously, and if adverse effects cannot be quickly ameliorated animals will be euthanised to prevent suffering. We are currently testing a new method of delivering substances to the brain through a simple injection to a peripheral blood vessel, which, where applicable, will replace surgical delivery to the brain.

Project 14	**Role of the environment in drug abuse development**	
Key Words (max. 5 words)	Drug abuse, rat, environment, context	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Y	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Environmental factors have been shown to play a major role in modulating the behavioural and subjective effects of addictive drugs and the vulnerability to develop drug addiction. However, the neurobiological bases of the interaction between drugs and environment are still poorly understood. The aim of the present project is to help filling this gap by investigating the neurobehavioural response to addictive drugs as a function of context in rodents. We will study the effects of drugs such as heroin, amphetamines, cocaine, and alcohol in relation to the setting in which they are administered (or self-administered), as well as the underpinning neural adaptations. We will also perform experimental manipulations aimed to change behavioural reactions, thus the addictive process. This means that we have to manipulate the animals housing environment, always in the least stressful way for the animals themselves.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Drug addiction is a complex phenomenon with deep social and economic impact. At the current state there are few treatments with little efficacy. Research performed under this Licence will provide a better understanding of the behavioural and neural consequences of drug abuse, and will contribute to our comprehension of addiction basis. Our findings may provide insights into the biological substrates of• individual vulnerability to addiction and may identify features of the human environment that may promote the development of addictive behaviour. In turn, this may lead not only to the development of new therapeutic approaches, but also to the identification of innovative prevention strategies,
What species and approximate numbers of animals do you expect to use over what period of time?	7000 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?	The great majority of the behavioural tests are either observational in character (e.g. activity behaviour) or designed to produce responses aimed at obtaining rewards (e.g. self-administration behaviour), and will cause minimal discomfort and no adverse effects. We will use the lowest drug dosages that are behaviourally effective without producing acute or chronic adverse effects on animal's physiology or neurobiology. We will also use central nervous system (CNS) manipulations such as selective lesions, infusion of pharmacological agents or recombinant (AAV) viral vectors. Such procedures are carried out under anaesthesia with recovery. We will implant intravenous (jugular) catheters under anaesthesia to allow animals to self-administer drugs, to study voluntary drug taking. In both cases, surgical success is high, and typically we don't experience problems; occasional infection or irritation can be appropriately treated with appropriate topical medication(s). All surgical procedures will be carried out under aseptic conditions, to reduce the risk of infection and aid rapid recovery. Pen-operative analgesia will be used to minimize pain. Some procedures may require mild food or water

	deprivation in order to motivate the animals to perform the behavioural tasks; we carefully monitor body weights and health in order to avoid adverse events and suffering. On some occasions we might use mildly aversive stimuli such as electric footshock of moderate intensity to reinforce learning; in such cases shock levels are kept to the minimum threshold of efficacy so that no chronic adverse effects are produced. At the end of all experiments animals will be killed and where applicable tissues collected and analysed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Addiction is a behavioural phenomenon that reflects disorders of motivational and executive control systems, as well aberrant learning. For these reasons, the use of conscious animals that possess these abilities is essential. It is not possible to investigate such behavioural disorders in *in vitro* systems. Although rats do not display the full behavioural repertoire of humans, the brain systems thought to underlie the behaviours of interest are largely homologous. The project is part of a wider effort in which we make use, on the one hand, of patients and human volunteers, and on the other of *in vitro* cell culture. Within the narrower limits of the project as described, we are beginning to use *ex vivo* materials for electrophysiological work that will in the future guide our choice of drugs for testing therapeutic potential.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of rats to be tested will be the minimum number required to obtain statistically reliable results, based on previous experience **in** the laboratory, and the literature. Where appropriate, power calculations will be used to estimate the appropriate numbers of animals based on expected variability, and anticipated effect sizes. Whenever possible, controls groups will be shared across studies to further reduce the number of animals used. Where possible we will use within-subject comparisons to increase the statistical power of the

	experiments, and to limit the numbers of animals used. Whenever possible, behavioural testing will be coupled to immunohistochemistry and/or electrophysiology procedures, so to provide tissue for *ex vivo* studies and avoid additional exreriments.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Our experiments use rats, as the lowest species that have sufficient resemblance in brain function and in specific behaviours (such as conditioning) to humans, to allow the knowledge acquired in experiments to be applicable to understanding human addiction. The use of rats allows a considerable knowledge of behaviour and genetics from the extensive literature available, reducing the need to repeat experiments carried out elsewhere. The knowledge that we have gained from our previous research will be used to refine the protocols and minimize the discomfort of the animals. For example, within the limits of experimental protocols, the rats will be handled as pets and care will be placed in reducing noise to a minimum to avoid startling. Careful attention will be paid to the wellbeing of the animal, and where necessary animals that are severely affected will be killed humanely. Some procedures may require mild food or water deprivation in order to motivate the animals to perform the behavioural tasks; we carefully monitor body weights and health in order to avoid adverse events and suffering. On some occasions we might use mildly aversive stimuli such as electric footshock of moderate intensity to reinforce learning; in such cases shock levels will be kept to the minimum threshold of efficacy so that no chronic adverse effects are produced.

Project 15	**Preclinical screening in drug discovery**	
Key Words (max. 5 words)	Imaging, drug discovery, disease models	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to accelerate the discovery of new drugs to treat people suffering with distressing and disabling psychiatric and age-related brain disorders, such as Parkinson's disease, stroke or schizophrenia. This will be achieved using our panel of screening techniques in living animals, which include high-resolution magnetic resonance imaging (MRI), electroencephalography, autoradiography, behavioural and histological measurements. This allows us to comprehensively measure the effectiveness of drugs in the brain. These screening techniques are incorporated within our imaging research facility and will be offered either as a service to pharmaceutical industry, or utilized through academic collaborations. The project has two main objectives: 1) To measure structural and functional brain changes in rodent models representative of age-related and psychiatric disorders to develop biomarkers of pathology that can be measured in	

	both humans and animals 2) To provide a service for assessment of new drug treatments for age-related and psychiatric disorders using our preclinical screening techniques
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 value of this approach is three-fold. Firstly, rodent models allow one to assess the precise effects of genetic, environmental or drug-induced manipulations on brain structure and function in the absence of confounding factors usually present in human studies. Secondly, the use of MRI (clinically comparable technology) allows the collection of parallel assessments in living rodents and humans, maximising the possibility for translation of experimental findings to the clinic. Thirdly, and most importantly, in rodents one can measure brain structure and function in ways that are technically and ethically impossible in living human subjects, bridging the gap between imaging and neuropathology to identify the underlying mechanisms. This will accelerate the discovery and translation of new drugs for the treatment of disabling and distressing psychiatric and age-related brain disorders such as e.g. schizophrenia, stroke or Parkinson's disease. Currently there are only limited treatment options for these disorders and these are only partially effective at relieving patient symptoms. Furthermore, no treatments exist to slow the progression of these disorders and thus cure patients of their illness. Together this represents a serious unmet medical need our research will attempt to address.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 1700 rats and 1000 of mice over the length of project that will be 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	In all cases we endeavour to minimise any suffering of experimental animals. More than half of all protocols will be performed under anaesthesia and therefore animals will not be conscious or feel any pain. However, for purposes of translating the results of our experiments into clinical

end?	findings and to understand how the living systems respond to pharmaceuticals, we will sometimes perform recordings in conscious animals. This may involve taking blood samples, injecting substances into the body and taking metabolic, behavioural and/or electrophysiological measurements from the brains of these animals through injection of radioactively labelled tracers or previously implanted devices. These will always be conducted in a manner that causes minimum amount of suffering. Any animal that shows unexpected adverse effects will be immediately humanely killed. All surgical and brain imaging procedures will always be conducted under anaesthesia, animals will receive pain killers, and local analgesics will be applied to wound sites. To aid recovery, animals will receive additional hydration, electrolyte replacement and wet-mash food for few days following surgical interventions. During such time they will be inspected daily and euthanised in case of any unexpected adverse effects. Most animals will be killed humanely within 12 weeks of starting any procedure; in a few cases we will keep the animals for up to a year. In such cases they will be housed in social groups, under standard housing conditions.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The animals have to be used because it is not yet possible to model complex brain diseases adequately in cells or by using a computer model. This is because our knowledge about the structure and function of the nervous system, and the pathological events in these diseases is not yet sufficiently advanced. Therefore, one of the main aims of this work is to help advance our knowledge of complex psychiatric and age-related brain diseases that will eventually lead to improved *non-animal* modelling of such conditions. Also, as this project is largely concerned with the

	effects of drugs on the brains of living organisms, little replacement is possible. For example, one of our aims is to investigate how experimental drugs affect the brain, by measuring changes before and after drug treatment on brain blood flow, which is linked to changes in brain activity. This cannot be replicated in cells in a dish for example, due to a lack of adequate models of brain blood circulation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By using neuro-imaging techniques in living animals to measure brain structure or function we can substantially reduce the number of animals needed. This is because these techniques allow continuous (repeated) monitoring and are non-invasive. This means we can follow the same animal over time. This is ideal because we can see what happens to the same animals before and after treatment and control for variation in response between animals. These methods can also be integrated with other approaches such as measurement of brain blood flow, metabolism or the behaviour of an animal. In this way we can acquire different, but complimentary measurements from the same animal. Combined, this approach greatly reduces the number of animals needed per experiment.
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 genetics of the rat and the mouse and the anatomy and physiology of their brains are well understood and validated. Our laboratory has extensive experience (>10 years) in the use of rodents in neuroimaging and behavioural experiments. Rodent models of brain diseases, including psychiatric and age-related disorders, which we intend to study, have already been optimised and characterised by multiple laboratories; therefore we will only use valid and proven models for testing new drugs to treat these disorders. For example, we will prioritise the use of rodents that carry identical or similar gene mutations that cause human psychiatric and age-related brain disorders as an ideal model system. Despite the use of multiple measurements we will not cause unnecessary suffering to the animals. The

	majority of our experiments are performed under anaesthesia, thus it is possible to measure several aspects of brain function simultaneously without causing additional discomfort and suffering. We will only use behavioural tests that are relevant and appropriate to the drug or the model under study. The minimum number of sessions required to produce sufficient data will be conducted, so that each animal experiences only a minimum of required testing. These behavioural assessments will only be undertaken when there is no existing data to demonstrate that a drug may be potentially useful to treat a given brain disorder. The behavioural tests we propose to use are well established and are not of substantial severity. Drugs will only be tested in an appropriate disease model, at an appropriate dose and route of administration, and only when it is predicted that the compound might be have a positive effect.

Project 16	**Physiology and pathophysiology of motor circuitry**	
Key Words (max. 5 words)	Zebrafish, electrophysiology, motor network	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our overarching aim is to better understand neural circuitry controlling vertebrate motor behaviour in normal and pathological diseases states. We will use the larval zebrafish, a small freshwater fish to meet this aim. Our programme of work will comprise the following elements: i) To determine how motor-related circuitry in the brain regulates the activity and output of spinal motor networks. ii) To understand early, circuit-level changes that are caused by disorders such as amyotrophic lateral sclerosis disease (ALS) and Parkinson's disease (PD). To undertake this study we will maintain stocks of genetically modified zebrafish. Two types of genetically modified fish will be used. First, we will keep fish that express genetically encoded reporter genes in subsets of motor-related neurons so that	

	they can be visually identified for electrophysiological study. Second, we will keep fish harbouring gene mutations known to cause ALS and PD 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)?	The expected benefits are twofold. First, our work will greatly improve our understanding of how neural networks in the brain and spinal cord interact to shape motor behaviour. Since motor-related circuitry in zebrafish is highly conserved with those of mammals we expect our findings to uncover general principles about how neural circuits interact to engender behavioural flexibility. Second, our work will identify early, presymptomatic defects associated with ALS and PD. Little is known of the defects that occur during pre-clinical stages of these diseases and we hope that our work will help identify new therapeutic targets for slowing or preventing disease progression.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will run for 5 years. During the course of the project, we will house approximately 1,200 genetically modified adult zebrafish that will be used to generate offspring for study.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will maintain adult zebrafish for breeding purposes only. These will be used to generate offspring that will be used for in vivo electrophysiology experiments. All electrophysiology experiments will be conducted on early stage zebrafish that are too young to fall under the Animals (Scientific Procedures) Act 1986. Thus, the only licensed procedure we will be required to undertake is breeding of genetically modified adults. The genetically modified lines used for study are expected to exhibit mild phenotypes. However, zebrafish lines harbouring genes that cause ALS and PD may develop motor phenotypes during latter stages of life. Any fish that exhibit clinical signs indicative of suffering (such as reduced of feeding, abnormal or impaired movement) will be immediately removed from the colony and killed via a Schedule 1 method. Lines that are known to develop an adverse phenotype, such as the degenerative conditions

	above, will be killed before these signs develop.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no alternatives to in vivo models: to understand the neural basis of vertebrate motor flexibility and how degenerative diseases impact motor-related circuits, intact systems must be used that are capable of exhibiting behaviourally-relevant output. Using the Frame.org website, we have attempted to find suitable alternative models for this project but have been unsuccessful. However, the pre-feeding fish we will use for experimental study are not considered sentient and thus are not protected under the Amina's (Scientific Procedures) Act 1986.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Adult fish will be maintained solely for breeding purposes. To minimise use of adult fish, we will breed GM zebrafish only when we require embryos for study. During intervening periods, males and females will be kept separate to avoid overbreeding. When GM embryos are required, GM zebrafish will be crossed and the offspring obtained for experimental 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.	When compared to traditional mammalian models, larval zebrafish have the potential to significantly reduce suffering and improve welfare of animals used for in vivo electrophysiology research. Current mammalian models carry significant ethical concerns because recording electrode implantation is a high-severity band procedure with potential to cause pain, suffering distress and lasting harm. By contrast, the pre-feeding zebrafish that will be used for electrophysiological study are not considered sentient and thus are not expected to suffer. Larvae will be euthanized on completion of electrophysiology experiments and so will not reach stages where suffering becomes a concern. Thus, the only regulated procedure required by our model is the breeding of protected adults for production of larvae. Therefore, when compared to equivalent mammalian models, severity limits are significantly reduced. As zebrafish are commonly used for bioscience

	research, established methods (see: http://science.rspca.org.uk/sciencegroup/researchanimals/ implementing3rs/ housingandcareaquaticspecies) can be employed to ensure optimum welfare of adults that are maintained for husbandry.

Project 17	**Sheep as a large animal models in health and disease**	
Key Words (max. 5 words)	Huntington disease, sheep, behaviour, electrophysiology	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of the project is to advance knowledge by determining the relationship between neurodegenerative processes and symptoms seen in Huntington's disease (HD) We will identify the roles of different regions in the brain during behaviours known to break down in HD, such as decision-making.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We will gain understanding of complex mammalian brains, particularly with respect to cognitive function in human neurological diseases such as Huntington disease. We will establish if it is possible to use sheep as an alternative large animal model to non-human primates for research into neurodegenerative diseases.	
What species and approximate numbers of animals do you expect to use over what period of time?	Sheep, approximately 300	

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 surgery needed for cannulation and instrumentation will cause discomfort. This should last only a few days. Some adverse effects may arise from lesions that model neurodegeneration, such as abnormal gait. These will be minimized by restricting lesions to small parts of the brain. Animals that undergo surgery or are part of drug treatment studies will be killed for pathological analysis. Normal animals used only for behavioural testing that are not needed for post mortem study may be re-homed at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Behaviour is too complex to study in a test-tube or a dish, because connected neurons in many parts of the brain are required. Adult neurons cannot function out of the brain. There are no alternatives to using animals for studying higher brain functions such as learning, memory and psychiatric aspects of behaviour because all pathways are interlinked and not fully understood so cannot be reproduced without a living animal. There are no biochemical readouts that can substitute for behavioural testing.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Pilot data will be used to perform power calculations to ensure that our studies are adequately powered to provide adequate data from using the minimum number of animals. For all studies, a control group will be used, but where possible, experiments will be done longitudinally so animals will serve as their own controls (by testing them before and after treatment data). This is particularly suitable for drug studies and will reduce the number of groups 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	We use large brained animals for our behavioural testing and electrophysiology in order to have an animal that is intermediate between rodents and humans in terms of brain size and complexity. A second species (in addition to rodents) is currently required for pre-clinical testing of new therapies. We believe that sheep are suitable for such use and represent a credible alternative to other more sentient

(harms) to the animals.	animals such as dogs or non-human primates. Sheep are gregarious farm animals and will be housed outdoors in flocks wherever possible. They will always be housed in close proximity to a companion animal. They will be trained to perform behavioural tasks voluntarily and will not be food or water restricted to encourage performance of such tasks. When surgery is performed on animals, a high standard of surgical technique will be employed, with pain relief and good aftercare and monitoring utilised as standard.

Project 18	**Understanding locomotor control mechanisms**	
Key Words (max. 5 words)	Locomotor circuit; development; neuroregeneration; electrophysiology	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The project aims to study how groups of cells (neurons) in the spinal cord control swimming activity and how they recover following injury. Different forms of movement, like walking and swimming, are controlled by neurons distributed throughout the brain and spinal cord, which form networks and produce precisely coordinated rhythmic activities. How these neurons are connected and how they control and modify movements is still poorly understood. We use *Xenopus* tadpoles and zebrafish larvae to address these questions. The swimming activity of these simple animals has many similarities to mammalian and human movements. We will monitor individual neuron activity and study how these neurons interact with each other and how their networks reconfigure during growth in order to produce more flexible movement. This study will generate data that help to understand fundamental mechanisms and common principles, which are much	

	more difficult to be revealed in mammals. Many simple animals, like *Xenopus* tadpole and zebrafish larvae, have the ability to repair the network controlling movement and regain its function after injury; such ability has been almost completely lost in mammals, including humans. Therefore these animals enable us to explore the process of function restoration in a permissive environment. We plan to monitor neuron activities over the course of recovery and to study how different classes of neuron response to the injury, and how they contribute to the functional restoration of the whole network. Many substances present in the network regulate both normal swimming activity and network repair following injury. Therefore we will study how these substances affect cell activity and network function, which can provide a better understanding of how the network operates in health and following 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 way in which the nervous system controls behaviour remains an unresolved biological question, and studying the operation of simple animal nervous systems is an important approach. Because basic mechanisms are likely to have been conserved during evolution, clues will be provided about the operation of more complex nervous systems where detailed study is difficult or not possible. We will reveal how the various components of the nervous system of simple animals act together to produce the whole integrated behavioural repertoire. Also the ways in which nervous systems are altered during development to accommodate changing behavioural needs is of fundamental importance and is best studied in relatively simple and rapidly developing models like *Xenopus* and zebrafish. The ability to repair the nervous system of these animals provides huge possibilities to test how the restoration can be achieved after injury, which will facilitate studies in mammals and even humans.
What species and approximate numbers of animals do you expect to use	We need zebrafish adult (*Danio rerio*) and *Xenopus laevis* toad for breeding. For the duration of the project (5 years) we estimate to use approximately

over what period of time?	3000 adult fish and 200 toads. Experiments will be performed only in embryos and larvae before free feeding: larval fish until 120 hours post fertilization and *Xenopus* tadpoles until stage 42, which are not 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?	Maintenance of transgenic zebrafish and hormone injection induced breeding of wild type *Xenopus* adults are both considered being a mild procedure. The severity limit of this PPL is Mild, and no adverse effects are expected; however, in their unlikely event, the advice of the named veterinary surgeon will be immediately sought and animals will, if deemed necessary, be killed humanely. All adult animals are used for breeding regularly. At the time of not providing fertilized eggs, they 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	In this project, we investigate the function of groups of neurons controlling swimming activity during early development and how they recover following injury. To fully understand how a nervous system works it must be examined in the context of a whole, living animal, and cannot be replaced by computer models. At present computer models are incomplete, and cannot replace animals used for neuroscience study. This project uses embryos and larvae of *Xenopus* and zebrafish and these offspring must be obtained from breeding of adult animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reduction in numbers of *Xenopus* toad is achieved by selecting good breeders for re-use over many years and maximising the use of each batch of embryos. The only alternative is to use 4 new adults per week to get fertilized eggs, which would significantly increase the number of animals used. By rearing fertilized eggs at a range of temperatures, each batch of eggs is able to provide animals for five days, and therefore it reduces the number of hormone injection. The oldest zebrafish larvae used in the project are 5 days post fertilization before they are protected under

	the Act. Eggs from a good breeding can be used for at least 3 days. Up to 100 pairs of zebrafish adults per year are needed to breed in order to maintain the colony.
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.	*Xenopus* tadpoles and zebrafish larvae are ideal animal models to study how movement (i.e. swimming) can be generated and modified by groups of neurons. They have much simpler structures comparing to mammals, but still possess many hallmarks in how they operate. Understanding what happens in simple animals can facilitate studies on more complex systems, and even humans. Both animals have the ability to repair their nervous systems following injury; therefore, these animals provide the possibility to reveal how groups of neurons recover and how they can produce swimming activity again after injury, which is almost not possible by using mammalian models. Genetically modified zebrafish can help us target at specific neuron types embedded in their nervous system. **Photos and tank numbers** are used to identify each *Xenopus* adult**;** each tank holds a small group of animals to help identify them. Toads are injected with hormone on a rotational basis; the number of animals in the colony ensures that no individual is injected twice in three months. Zebrafish adults are used for breeding no more than once per week. Refinement used will involve environmental enrichment of the housing for adult toads; regular handling of the same animals to reduce fear/stress; refinement of the injection procedure to minimise pain and distress.

Project 19	**Mechanisms underlying Rett syndrome and other autism spectrum disorders**	
Key Words (max. 5 words)	Sensory processing, Rett syndrome, EEG, neurons, autism	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	An increasing number of devastating autism spectrum disorders including Rett syndrome are associated with genetic mutations or genomic rearrangements. Identifying the mechanisms through which these different genetic alterations lead to the shared and distinct behavioural manifestations will greatly enhance our understanding of how the brain functions in health and disease. This project is aimed at understanding the molecular, cellular, and neural network alterations that ultimately lead to the age-dependent manifestation of behavioural changes in autism spectrum disorders. We will begin by investigating changes that occur in Rett syndrome. These findings will then be used to understand whether other autism spectrum disorders caused by different genetic changes lead to similar alterations in brain function.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The results from this project will provide new insights into the pathogenesis of autism spectrum disorders. Additionally, these data may provide new potential strategies for the treatment of these disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5-year time period of the project we expect to use 2,500 mice.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of procedures to be performed are classified at a 'non-recovery' level of severity. A small number of procedures are classified as moderate and may lead to temporary stress in affected animals. At the end of procedures, 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	We need to use animals in order to gain access and manipulate small areas of brain tissue in a way that is not ethical in humans. However, we are expanding our capability to use tissue from human patients to replace animal use as much as possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical power analyses are used to ensure that the suitable number of mice are used for each study with variation in experimental values determined from published peer-reviewed literature
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 many advantages to using the mouse as a model organism for the proposed research. One of the most important of these is their striking similarity to humans in anatomy, physiology, and genetics. Over 95% of the mouse genome is similar to our own, making mouse genetic research particularly applicable to human disease. In addition, our ability to directly manipulate the mouse genome provides an incredibly powerful tool to model specific diseases for which the causative gene is known. For

	example, the manipulation of genes involved in human Rett syndrome has allowed for the creation of many mouse models that recapitulate the symptoms of Rett syndrome, greatly enhancing our ability to find new and effective treatments for this devastating disorder. Indeed, it has been show using these mouse models that Rett-like symptoms in mice are reversible. Any potential harm to the animal will be minimised by strict adherence to approved procedure methods and overall high quality of animal husbandry.

Project 20	**Regulation of body glucose and energy levels by the brain**	
Key Words (max. 5 words)	Diabetes, obesity, glucose, energy, brain	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of this programme of work is to understand how special regions in the brain can monitor whole body glucose and energy levels, and why in certain conditions such as diabetes and obesity these brain regions fail to work properly	
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 know very little about how the brain monitors whole body glucose and energy levels and why problems in special parts of the brain contribute to the development of diabetes, obesity and even low blood sugar levels (hypoglycaemia). If we get a better understanding of this we may be able to develop new treatments for these conditions which are becoming very common in our society	
What species and approximate numbers of animals do you expect to use over what period of time?	We will study rodents (mouse and rat), and expect to study a maximum of 2500 animals over the duration of the project.	
In the context of what you	Adverse effects depend on the studies being	

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?	performed and mainly relate to surgery or the development of diabetes, very much as we see in humans. Diabetes, as in human subjects is monitored by checking blood sugar levels and treated with insulin when needed. Surgical procedures such as the insertion of a catheter into an artery or vein are of moderate severity and performed under general anaesthesia. Pain killers are used routinely and antibiotics as required. When animals have completed all the in vivo studies they are humanely euthanized.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Blood sugar (glucose) and fat (energy) levels are controlled in part by highly specialised nerve cells in the brain. While it is possible to study these nerve cells in isolation, the information we get from these studies is limited in 3 important ways: (i) The actual identity of the specialized nerves important in the glucose sensing is not known and so current cell culture systems are not optimal, (ii) Central glucose-sensing nerves are heavily regulated by other nerves, as well as by astrocytes (cells that surround nerves and control the immediate environment in which they exist) as has been shown, and these connections are missing in isolated cells, (iii) replicating the complex and variable conditions that occur in a disease state such as diabetes is extremely difficult to do in a petri dish and limits the applicability of these models to disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible we perform studies in cell systems first to ensure that a candidate gene or pathway is important to the things we want to study. Subsequently highly specialised techniques have been developed for measuring the animals response to challenges such as low or high glucose and these means we can compare much smaller groups of animals. Techniques have been developed that allow us to study the animal while awake and so we can conduct repeated tests in the same animal rather than using lots of groups

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rat/mouse: These are a well-studied models used frequently in metabolic research which helps when comparing results to other groups. Also because sugar is a critical fuel for the brain for the rodent as it is for the human, we find that rodents like humans become fat and develop diabetes when given too much food. Also they respond in the same way to both single and multiple episodes of low glucose as do humans with type 1 diabetes and so they are a good animal model to study. For instance it has been shown in people with type 1 diabetes that a drug called diazoxide that activates potassium channels on sugar sensing nerves can help improve their defence responses to low glucose. It was first showed that this was a possibility in rats. We minimize welfare costs by using highly trained staff to conduct all our studies who have now many years' experience in working with animals and using the techniques we employ. New staff are rigorously trained. All studies are carried out following recommended guidelines under the guidance of the local veterinary team and in a facility with highly trained staff

Project 21	**Developing in vivo Raman spectroscopy in mice**	
Key Words (max. 5 words)	Raman spectroscopy, neuromuscular disease	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Diseases that involve nerves and muscles, often collectively referred to as neuromuscular disorders, are traditionally diagnosed with a variety of investigations that will include a test called electromyography. In this a needle electrode is placed into the patients muscle and electrical signals from the muscle are collected and analysed. The signals can give clues to the underlying cause of a patients symptoms but the findings are rarely specific to a particular condition; that is, the same signal can be seen in a variety of conditions. To reach the final diagnosis further tests, including surgical procedures such as muscle biopsies are needed, which can delay diagnosis and treatment. The present study will apply a new method that uses a beam of light to analyse the composition of muscle. When the light is reflected back by tissue the amount of energy it has changes and this change can be used to work out what the tissue is composed of. This phenomenon is well known and is the reason why the sky is blue. Recent technological advances in the technology used to produce and capture the light beam have enabled people to start thinking about whether the effect can be used to diagnose human diseases. As Raman spectroscopy can detect very subtle changes in tissue, when applied to neuromuscular disorders it could speed up diagnosis and mean that invasive procedures like muscle biopsies are no longer needed.	

	This project will investigate whether the signals recorded from muscle using the light beam can be used to tell the difference between diseased and normal muscle.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	1). Development of a new technique for the diagnosis of neuromuscular disease that could replace more invasive diagnostic tests 2). Development of a new means of monitoring disease progression that can be used to see if treatments are working
What species and approximate numbers of animals do you expect to use over what period of time?	Mice with genetic mutations resulting in the development of human diseases affecting nerves and muscles will be used as well as non diease affected mice. Approximately 150 will be used over the lifetime of the license (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 effect of the recording procedure on muscle will be assessed as part of the study. Similar technology has been used to study the skin and cervix of humans and has not caused any adverse effects. It is possible that the recording will cause some scarring of the muscle, which might further impair the ability of the animals to move around. As difficulties in walking develop as part of the disease process we are used to monitoring for such effects. However this will not be part of the current investigations. Under this license the recordings will be performed whilst the mice are under anaesthesia and the mice will not be allowed to recover. This will allow us to assess if the technique works but also look for signs of damage at the recording site as a first stage towards developing this new technique further.

Project 22	**Mutations in matrix proteins and disease**	
Key Words (max. 5 words)	Stroke, vascular disease, collagen,	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Stroke is the most common cause of disability and every year 150,000 patients in the UK develop stroke. Stroke can be due to bleeding in the brain for which there is no treatment available. The cells within tissues of the human body are surrounded by a matrix that provides structural support to tissues. A major protein that makes up this matrix is called collagen IV. Small changes called mutations in this protein cause haemorrhaging in the brain, eye and kidney disease. This disease occurs in adults but also in young children and babies. While patients with these mutations are rare, we have identified that this protein may also play a role in stroke due to brain haemorrhaging in the general population. Our recent analysis has identified that these mutations lead to structural defects in the extracellular matrix and to a type of cell stress. There is no treatment for the disease due to collagen IV mutation and for brain haemorrhaging in general. This is in part due to our lack of understanding of how	

	these diseases develop. In this project we will investigate how mutations in collagen IV lead to disease. We will also investigate if we can modify the cell stress and other defects caused by these mutations to interfere with the development of the disease.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will further elucidate the role of the extracellular matrix and collagen proteins in vascular disease and human health. It will increase our understanding of how mutations in collagen IV lead to human disease including bleeding in the brain. Importantly, as collagen IV plays a role in common forms of stroke the gained knowledge may shed light on pathways leading to general vascular disease, a common cause of death. We will investigate if we can modify the development of disease caused by these mutations by modulating the deleterious effects of these mutations. This is important as this will help the development of treatments. Interestingly, we will investigate the efficacy of compounds, that have been approved for use in the clinical for other diseases, in targeting the effects of collagen IV mutations. If successful our experiments using these compounds will accelerate the development of treatments for diseases due to collagen mutations and stroke.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse 5800
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The vast majority of animals will be used for breeding and maintenance and analysis will be performed on tissues collected post mortem. A minority of animals will undergo in vivo phenotyping protocols such as measurement of blood pressure and magnetic resonance imaging (MRI) to analyse brain haemorrhaging. The phenotypes of animals may be altered through administration of substances or altered diets with the aim of ameliorating the disease and identifying its underlying pathways. The adverse

	effects to the animals include the development of eye, kidney and vascular disease due to the mutation. Other very rare adverse effects are associated with the use of anaesthesia and protocols have been optimised to minimise adverse effects such as correct dosing and close monitoring of animals.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our research makes extensive use of non-animal based research such as in vitro and cell culture experiments. However to date the development of brain haemorrhaging and disease due to collagen IV mutations can not be accurately modelled by a cell culture system. Thus animal based research is required to investigate how mutations affect whole body biology, how brain haemorrhaging develops and if molecular defects identified in cell culture contribute to disease development. In vitro and cell culture experiments will assess the cellular effects of mutations and will guide the use of animal based experiments. The examination whether certain pathways occur in disease requires analysis of the physiological situation. This is critical to increase our understanding of the mechanisms underlying the human diseases represented by these animal models**.**
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce the number of animals required we will coordinate animals studies and collect multiple tissues of each animal. Performing multiple phenotyping assays will reduce animal numbers and provide an accurate characterisation of co-occurring phenotypes within one animal. Power calculations alongside pilot studies will be undertaken to assess the number of animals required and minimise any potential stress. Statistical analysis, e.g. two way Anova, will be performed.
3. Refinement Explain the choice of species and why the animal model(s)	The identification of the human disease caused by collagen IV mutation was based on the analysis of mouse models with mutations in the same gene. This illustrates that mice are a very accurate model of the

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.	human disease and a powerful model to investigate how the disease develops and to analyse potential treatment strategies. In addition there is a wealth of genetic tools available to researchers and protocols are well established and standardised. The mice models have been previously generated and recreation of other animal models such as zebrafish can be more difficult due to species differences: the cardiovascular system of mice has a much higher homology than the zebrafish cardiovascular system to the human cardiovascular system. The use of well developed protocols and pilot studies minimises the welfare costs to the animals. All possible measures will be undertaken to minimise animal stress as it is well established that stress has a strong influence on cardiovascular parameters such as blood pressure.

Project 23	**Molecular mechanisms underlying neurogenesis and neurodegeneration**	
Key Words (max. 5 words)	Nervous system, neurodegeneration, mouse models	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	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 goal of this project is to identify new genes and molecular mechanisms that regulate the generation of neurons during brain development and that regulate neuronal dysfunction during neurodegeneration. In order to achieve this goal we will use mouse lines with specific genetic modifications that affect these processes. Defects in neurogenesis lead to epilepsy, autism and other common neurological disorders. To elucidate the complexity of neurogenesis we need to identify key genes and molecules that regulate the generation and differentiation of neurons. This knowledge will lead to breakthroughs in the understanding and treatment of neurological disease. Overall the project will not only identify important new mechanisms, but also identify potential therapeutic agents to treat neurodevelopmental and neurodegenerative diseases.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	As a group neurological diseases are the most complex and poorly understood. This project will elucidate the mechanisms relevant to several of these diseases. The project will benefit researchers in the fields of neurodevelopment and neurodegeneration by providing new knowledge that will help reveal how the brain develops and degenerates at the molecular level. In the long term this project will benefit patients with neurological disease and so society in general as it will identify new molecular therapeutic targets and compounds that modify the disease process
What species and approximate numbers of animals do you expect to use over what period of time?	The project will use the mouse (including genetically modified mice) as a model system as the mouse is the only mammalian system available for modelling complex brain defects and testing their behavioural consequences. All the manipulations and tests we will use are established protocols that are optimised such that the fewest animals are used (maximum of 2,500 over 5yrs) and distress or suffering minimised.
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 protocols we will use are mild and we expect very few adverse effects. Overall, the severity of this license is expected to be mild. 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	Much of the preceding work has been performed in Drosophila where we have identified new genes and molecular pathways that regulate neurogenesis and neurodegeneration. Where hypotheses can be tested in Drosophila we are and will continue to use this invertebrate model. We will use ex vivo cultures for some aspects of the work where appropriate, but these will not fully recapitulate the complexity and interconnected nature of the mammalian nervous system, which necessitates a live animal CNS model.
2. Reduction	Wherever possible multiple readouts will be analysed on each animal, so the number of animals used are

Explain how you will assure the use of minimum numbers of animals	minimised. We will also use the minimum number of animals possible to obtain statistically significant results in our experiments.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice have been developed as by far the most sophisticated mammalian model for gene manipulation and so are the most suitable model for investigating the function of the genes and pathways we are interested in. We intend to study the role of specific genes in neuronal development and neurodegeneration and so use of GM mice provide the best tool to achieve this goal. Mice used in this project will be subjected to minimal stress and many of the protocols only require breeding and maintenance of colonies followed by killing to obtain tissues for analysis.

Project 24	**New medicines for amyloid and C-reactive protein**	
Key Words (max. 5 words)	Amyloidosis; serum amyloid P component; monoclonal antibody; immunotherapy; drug development	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our previous work has identified and validated plasma proteins of the so called pentraxin family, serum amyloid P component (SAP) and C-reactive protein (CRP), as key participants in important human diseases for which adequate treatments are not yet available, including amyloidosis, Alzheimer's disease, type 2 diabetes, heart attacks, strokes and other cardiovascular diseases. We have invented new approaches to targeting these proteins to prevent and treat disease and are developing them as drugs in collaboration with one large pharmaceutical company and potentially other pharmaceutical companies.	
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)?	Improved understanding of the causes and mechanisms of important common and also rare human diseases informs better diagnosis, treatment and outcomes, reducing sickness, saving and prolonging lives. Furthermore, development and introduction of new treatments to alleviate hitherto	

	unmet medical needs will have major health and economic benefits for individuals and society. The present ongoing first clinical trial of the most advanced therapy we have invented and are developing is yielding extremely encouraging and unprecedentedly beneficial results in amyloidosis patients. The first clinical trial of our drug for Alzheimer's disease will start in 2016. The animal work now proposed aims to deliver additional new drugs for testing in patients with these and other diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	The large majority the currently proposed work will be in mice, using up to 1000 per year over the coming 5 years. A smaller number of rats may be used, up to 100 over the entire course of the project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The severity levels will range from mild (for maintenance of genetically modified mouse lines) to moderate. Many of the animals used will have either deliberately induced or genetically determined manifestations of models of the various human diseases we propose to treat, including systemic amyloidosis, diabetes and amyloidosis in the brain (e.g in Alzheimers). They will experience adverse effects from induction of disease, and from techniques used to monitor the effectiveness of the treatments. In the vast majority of animals, the clinical effects of the disease will be of mild severity, at most, though this may reach moderate severity for mice with induced diabetes. The vast majority of techniques to be performed will individually be of mild severity (e.g. injection, blood sampling, drug administration in water), but a significant proportion of the experimental animals will undergo a series of mild techniques; for this reason, such procedures are judged to be of moderate severity. A small number of animals wiLl undergo up to two surgical procedures (moderate severity). Most animals will be killed at the end to provide material which will be used to evaluate the effects of the treatments. Others (genetically altered animals) may be transferred to other projects authorised to use

	them. Some animals (bred and maintained to ensure the security of the genetically altered lines) will unavoidably be surplus to experimental requirements, and will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The initiation development and evolution of pathology causing clinical diseases in living organisms are extremely complex. Similarly the mode of action and efficacy of disease modifying therapeutic interventions are also very complex, as are the Pharmacokinetics (PK) and Pharmacodynamics (PD) of all therapeutic agents, including those to be studied here. There is no alternative to use of living animals to achieve the level of knowledge and understanding of these processes which is necessary for the new approaches to treatment which we have invented to be taken into clinical testing in humans. No existing or conceivable in vitro studies can possibly provide the necessary information and in viva animal testing will in any case be a legal and regulatory requirement for drug development. Prior to in vivo testing, In vitro and ex vivo screening will be used to select the best candidates according to their binding affinity to their intended target, (e.g. amyloid fibril precursors, amyloid fibrils, or C-Reactive Protein (CRP)). This project is the continuation of a program of work in which findings from experiments conducted in animals have provided. major benefits for human medicine, improving diagnosis, treatments and outcomes for very large numbers of patients worldwide. These outcome clearly demonstrate the validity and highly predictive characteristics of the models we propose to use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All experimental protocols are based on our extensive experience with the particular disease models under investigation in addition to well established scientific and pharma industry experience of drug development. This comprehensive expertise will guide the size of experimental groups so that the minimum numbers of animals are used. Our input on the variance and significance of critical parameters

	will enable our statistical adviser to perform robust power calculations informing experimental design to yield statistically and biologically significant results. The ARRIVE guidelines will be followed when possible and the studies will be appropriately blinded and randomised. We will manage our colonies of genetically altered mice to match as closely as possible the requirements for experiments, while ensuring the security of the colonies. Strategies for breeding of mice with complex genotypes will be optimised to minimise 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 shall mostly use mice, and a small number of rats. Mice are ideal for the present project because well developed and thoroughly characterised models exist for all of the pathological process and diseases to be studied, closely resembling their human counterparts. Genetically altered mice are also uniquely available, with deleted genes and/or human transgenes expressed, to provide for pivotal evaluation of the roles of particular proteins in pathology and/or as targets for drug therapy. Nothing even remotely comparable exists in any other species and all the many years of work leading to the present point when we are on the verge of further human trials have been conducted in these mouse models. We have available a variety of animal models of amyloidosis, including an inducible transgenic mice recently developed with NC3Rs funding, and can thus choose the most appropriate animals for each experiment to optimally balance animal welfare and scientific considerations. We are aware of certain limitations in the animal models. This allows us to design experiments within those limits and to investigate possible means to refine the models to increase their validity and reduce adverse effects. Rats will be used principally for PKJPD studies of the CRP inhibitor drugs and are uniquely appropriate because they have constitutively high baseline CRP values and are thus an excellent model for patients with major acute phase response increases in human CRP concentration. No humans with absence or even polymorphism of either SAP or CRP have been

	reported. In the absence of any such ‘experiment of Nature’, targeted deletion of the respective genes in mice is the only possible way to evaluate the normal physiological functions of these proteins in viva and thus the potential consequences of drug treatments which inhibit those functions. SAP and CRP knockout mice are healthy, fertile and have normal life spans in animal house conditions, so their use in the present essential experiments, precisely controlled to minimise suffering, is the most refined approach possible.

Project 25	**Brain Protection for Birth Asphyxia**	
Key Words (max. 5 words)	Birth asphyxia, brain protection, newborn, therapeutic hypothermia	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		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 now conclusive evidence of the benefit and safety of cooling by 3°C for 3 days for term infants with brain injury. Despite cooling therapy, however, more than 50% of treated infants still have an adverse outcome. Other therapies are needed to improve outcomes. Our preclinical model will be used to determine which other therapies are safe and most effective when combined with cooling. These data will be essential for phase II clinical brain protection trials in newborn infants.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits of this project are: • Advancement of Biological Knowledge • The information will have a high value to other pre-clinical scientists working in this field and to neonatologists interested in neuroprotection of the newborn and magnetic resonance biomarkers. • Treatment of ill-health and reduction in the burden of disability from cerebral palsy and	

	cognitive deficits Almost one quarter of all neonatal deaths worldwide (equivalent to I million deaths per year) are intrapartumrelated (birth asphyxia). Every year around 1 million survivors of ‘birth asphyxia” develop cerebral palsy, learning difficulties or other difficulties. In the UK, around 1000 babies suffer birth asphxyia. The financial and human costs to infants affected, their parents, professionals and wider society are enormous. Birth asphyxia which leads to neonatal encephalopathy results in serious consequences including death, cerebral palsy, epilepsy and other significant cognitive, developmental and behavioural problems. Already the widespread introduction of therapeutic hypothermia as a routine treatment of birth asphyxia in the UK has reduced the burden of disability; the number needed to treat to prevent one adverse outcome is 9. The translation of safe and effective therapies (such as melatonin) to augment brain protection from cooling will reduce the NNT further. This will lead to fewer babies developing cerebral palsy and cognitive problems as they grow up. These studies are likely to lead to significant advances and reduce the number of infants with adverse outcome from lack of oxygen around birth.
What species and approximate numbers of animals do you expect to use over what period of time?	Neonatal piglet, a maximum of 120 per year over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All animals are anaesthetised and insentient throughout the study and are terminated at the end of the study.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Optimising neuroprotection of the newborn requires the use of in vivo rather than in vitro models to take into account the influence of other organs, circulating factors and changes in cerebral perfusion. We are not aware of any alternative that does not use animals that would allow us to assess the safety and efficacy of therapies for birth asphyxia. A model is needed in which meticulous intensive care and temperature control can be maintained. Mathematical modelling could not replace the use of experimental animals. The body of knowledge about therapies and combinations of drugs is not sufficient to allow for a clinical trial in human infants without these pre-clinical studies in the piglet providing important, safety, dosing and efficacy data.
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.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Our model has particular strengths and provides a valuable scientific resource in which neural rescue interventions birth asphyxia can be tested. The similarity of anatomy, size and maturation to the human infant allows for regional histopathology and vulnerability of the brain to be assessed. The model allows for meticulous neonatal intensive care support for up to 60 hours enabling metabolic and temperature homeostasis to be maintained during this time. This ensures that the model provides data that is relevant to the human neonate who would be cared for with similar intensive care support. The model allows for brain magnetic resonance imaging and spectroscopy studies to be acquired at 24 and 48h; these data are similar to those acquired in babies and therefore give weight to the model's clinical translational relevance. General anaesthesia will be maintained and hence the animal will be insentient throughout the entire procedure.

Project 26	**Protein function in genetically altered and stroke mice**	
Key Words (max. 5 words)	Brain, Stroke, Brain compounds, Receptors	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	In the brain, nerve cells (neurons) exist in large networks and communicate with each other using chemical substances called neurotransmitters. There are two types of neurotransmitters: excitatory neurotransmitters, which cause neurons to fire electrical impulses, and inhibitory neurotransmitters, which make neurons less likely to fire. In normal brains, these two systems are carefully balanced, which allows neurons to function properly. However, after ischaemic stroke, brain cells release a high amount of the excitatory neurotransmitter, glutamate, and this excess glutamate damages and kills neurons. The objective of our studies is to investigate the role of neurotransmitter receptors and how these receptor signalling impact the intact mammalian organism as well as in ischaemic stroke.	
What are the potential benefits likely to derive from this	As a primary means of regulating the activity levels of neuronal networks in the central nervous system, the	

project (how science could be advanced or humans or animals could benefit from the project)?	study of inhibitory neurotransmitter receptor GABA receptor signalling is particularly relevant to human health. GABA receptor signalling controls the rhythmic activity of the brain, which is relevant to normal brain function. GABA receptors have also been implicated in the pathology of many human diseases such as ischemia, anxiety, depression, Alzheimer's disease, dementia, autism, schizophrenia, and epilepsy. The expected benefits of this project are an understanding of GABA receptors in brain function produce novel therapeutics in neurological psychiatric disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 7500 mice 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?	Animals produced under this protocol are not expected to exhibit any harmful phenotype. However, it is not possible to fully predict the nature or severity of any potential defect and for all types of mice there will be a careful monitoring for possible side effects. For ex vivo studies, we will prepare brain slices 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, thrombosis and infection are not exDected. but the animals will be carefully monitored and any problems promptly treated. For in vivo studies, we will perform technique called two- photon imaging to visualise cell morphology. This procedure will be done under terminal anaesthesia. MRI may be performed multiple times

	under anaesthesia to detect brain activity and structure. Several behavioural tests will be performed. Animals used for tail suspension test (TST) to study depression will be terminated immediately after the test. For TST, medical adhesive tape will be applied in a 3/4 of the distance from the base of the mouse tail. A cushioned surface will be used 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, elevated plus maze, T-maze, and object recognition). Blood sampling may be done in some animals. 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. Some animals will undergo surgery for the transient middle cerebral artery occlusion (tMCAO) to generate model of stroke. These procedures are well established and we will follow best advice in our pre- and post- surgical routines to ensure animals experience the least amount of pain possible, for example, providing adequate local analgesia during that will be administrated so that it is effective before the animal starts to wake from the surgery. We only intend to allow animals to survive for a maximum of 72h post-tMCAO, a time point enable us to visualise core infarct to compare a number of outcomes including pathological damage and functional impairment after drug treatments, and determine therapeutic time window of these drugs. During this 72h post-tMCAO, the following clinical signs will be present and the score sheets (completed 3-4 times daily or once a day depending on the clinical sign of animals) are designed to allow the monitoring and recording of such possible clinical signs including pain and distress: weight loss, subdued behaviour, minimal peer interaction, intermittent hunching, intermittent vocalisation when

	provoked, intermittent abnormal breathing pattern. This is the most widely used techniques have been used to assess pain. Any weight loss beyond 25%, which we anticipate to be 2-4%, will result in the animal being culled. If animals lose greater than 20% of weight, which will be seen in —20%, and animal displays more than two clinical signs (subdued behaviour, minimal peer interaction, intermittent hunching, intermittent vocalisation when provoked, intermittent abnormal breathing pattern) for longer than 24h then the animal will be culled. In addition, we keep our animals housed in groups and provide environment enrichment as this is important for animal well-being.
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 understand the role of neurotransmitter receptors in the normal brain function as well as in the diseased brain (stroke). It is impossible to replace animals completely in this type of study as functional neuronal circuits (connection of neurons between different brain regions) and live animals for behavioural tests, which link cellular mechanisms and behaviour, are necessary. The mouse is the bestcharacterised animal in molecular biology and genetics, and many transgenic animals and animal models of human diseases are available. A mouse model of ischaemic .stroke that we will use in this programme is well established, and are known to progress similar neurological phenotypes seen in human patients and they are the best to perform pre-clinical studies. We anticipate that 70% of our work will be conducted in in vitro and ex vivo, rather than in in vivo models.
2. Reduction Explain how you will assure the use of minimum numbers	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

of animals	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://powerandsamplesize.com/. Importantly, in vivo experiments will only be conducted when we 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.	Model for ischemic stroke: Several animal models for stroke have been established and allow the study of pathophysiological consequences or potential therapeutic interventions following stroke. We will use transient middle cerebral artery occlusion (tMCAO), a less severe alternative to the permanent MCAO, which involves a period of vessel occlusion (30-45mm) followed by reperfusion. We will artificially blocks a major blood vessel via surgical occlusion, which is similar to the one occurring in human stroke. This is the most frequently used procedure in experimental stroke research. It does not require craniotomy (removing piece of skull), which will avoid possible complications and will produce lesions limited to the brain region called cortex and striatum. The severity of ischaemic injury can be modelled by leaving the suture filament in place for a variable duration of time and 30-45mm is enough for us to produce infarct to study ischaemic stroke. To determine whether the experimental treatments can reduce ischaemic damage, an infarct must be created. The size of infarct must be sufficient to cause pathological and functional deficits. We aim to minimise mortality by humanely killing animals, which appear unlikely to recover. We anticipate that less than 10% of animals undergoing tMCAO have to be culled. In particular, any animals showing persistent barrel rolling, respiratory distress or loss of the righting reflex will be killed humanely. All tMCAO animals will be carefully and regularly monitored. Post-operative monitoring sheets will be completed and kept with the animals ensuring that they can be identified by any members of technical staff.

	The mouse studies of applicable possible to is the best mammalian species for transgenic nervous system function and is directly to human neurobiology and disease. It is test subjects to develop novel drugs to improve and advance human health. We will use mice as the least sentient species appropriate for this type of work, which involves scoring neurological behaviours in awake behaving animals and measuring brain damage.

Project 27	**Therapeutic strategies in developmental disorders**	
Key Words (max. 5 words)	Neurology, autism, intellectual disability, Rett syndrome	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Intellectual disability (ID) disorders in children commonly have a genetic cause and are notoriously resistant to treatment. In particular, there is an almost complete lack of therapeutic options to alleviate deficits in the core features that define cognitive function - the ability to learn, remember, concentrate and communicate. The outlook for the more serious ID disorders, which tend to be associated with obvious abnormalities of brain structure or the connections between nerve cells, remains relatively poor. However, there is greater optimism for disorders in which neuronal abnormalities and dysfunction are more subtle and potentially open to corrective therapies delivered during childhood or to adult patients. The aim of this work is to develop and test drug-based and gene-based therapies.	
What are the potential benefits likely to derive from this project (how science	The work aims to identify and test putative therapeutic options for intellectual disability disorders with a focus on a particular severe form – Rett syndrome. Any	

could be advanced or humans or animals could benefit from the project)?	effective treatment that could ameliorate the lifelong motor, cognitive and other neurological features of this and related disorders would bring life-changing benefit to patients and their families.
What species and approximate numbers of animals do you expect to use over what period of time?	The study is expected to run over 5 years and involve mice genetically modified to model intellectual disability disorders. A maximum of 2000 animals will be used.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Rett syndrome is a severe disorder and mice with the equivalent genetic impairment have a range of features ranging from impaired memory and ability to walk to breathing problems. The mouse model is considered to be slightly milder that the human condition and the overall level of severity for the project is considered moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Rett syndrome and related disorders result from dysfunction of the nervous system leading to behavioural and other effects. Whilst studies in cells can assess molecular pathology, animals are required to assess the effectiveness of potential therapies in correcting brain function.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We adopt a number of approaches to reduce use including the collection of ex vivo tissues which enable the testing of a number of drugs/therapies on a single animal. Where possible we can conduct pilot studies in cellular models and only move forward the more promising approaches into live animal work.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use mice as they can be genetically modified to model Rett syndrome and related models and it is possible to study the features which characterise the disorder in humans such as social behaviour and learining. The gene central to Rett syndrome does not exist in simple invertebrate model species. We have in place special measures to assess the progress of Rett syndrome like neurological effects and built in humane endpoints to avoid unnecessary suffering.

Project 28	**Sensory feedback in artificial limbs**
Key Words (max. 5 words)	Sensory, artificial limbs, stimulation, peripheral nerve
Expected duration of the project (yrs)	5 year(s) 0 month(s)

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

Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.
Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The goal of this project is to develop technologies that will enable the assistive devices to provide truly natural control through sensory feedback. To enable this level of feedback, we must meet two clear objectives: to generate artificial signals that mimic those of the natural arm and hand, and to provide a means of delivering those signals to the nervous

	system of a prosthesis user. The objective of this project is to develop a novel electrode that can connect to the nerves of the arm to send real-time information about temperature, pressure and force of a bionic arm back to the brain. The amount of information that can be delivered to the human non-invasively, e.g. with skin vibration, is very limited. With this low resolution it is very challenging to deliver more information, e.g. position of fingers with respect to each other or the temperature of the grasped object. On the other hand, current implantable technologies that have been tested in humans can offer only very limited function and their long term interaction with the nervous system is unknown. Therefore, they are removed from the body after short preliminary experiments. To achieve efficient interfacing with and direct transmission of information to the human nervous system a lot of work is still required. Research on delivering sensory feedback for prosthesis users has attracted a significant level of scientific and clinical interest. There are mainly uncertainties, e.g. how best to sense and convert information from the environment to a signal that the brain would understand; and how to transmit to evoke naturalistic sensation in the brain. In addition, technologies do not exist for targeted delivery of this information to the nervous system.
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 the end of this project, in addition to neural engineering knowledge gained by conducting a series of experiments, a number of novel enabling technologies with application to sensory feedback in assistive devices will be developed and tested. For example the development of flexible neural probes, will not only allow us to study the interaction of the nervous system with a flexible material, in terms of specificity of the stimulation, amount of electricity required to induce a response, of the biocompatibility, it will pave the way for future neural interfaces such as bionic hands.

	Using advanced prosthetic hands, that are equipped with sensory feedback, would help people to naturally reach out and pick up a glass, for example, whilst maintaining eye contact in a conversation, or pick up a grape without bruising it. This level of feedback will advance the field of prosthetics, provide enhanced function to prosthesis users and decrease the learning time involved when acquiring a new device. The technology that will be developed in this project will also have applications for patients with neurological conditions where reduced sensation is a factor.
What types and approximate numbers of animals do you expect to use and over what period of time?	Rat 80 animals 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?	50 animals (maximum, cohort 1) will be used to test a prototype multi-channel neural electrode and an electronic stimulation system. The entire procedure will be carried out under general anaesthesia and the animals do not recover. As such there is no adverse effect and the expected severity category for the first part of the project is Non-Recovery. A further 30 animals (maximum, cohort 2) will be used to monitor long-term (up to 12 months) effect of implantation of the prototype multi-channel neural probe and an implantable electronic system on the peripheral nervous system. The entire implantation procedure will be carried out under general anaesthesia. The animals recover after operation and enter a behavioural experiment that will give information on long term interaction of the electrode-tissue, any shift in stimulation threshold and finally whether it would be possible to deliver electrical pulses to the nervous system to cue an animal, e.g. to guide the animal to solve a maze. Post-surgical pain and discomfort due to penetration of the electrodes in the nerves is a potential adverse effect of this part of the experiment. The animals will be tested in a final non-recovery experiment under general anaesthesia. Following euthanasia, the animal nerve tissue will be

	sampled for further analysis. The expected severity category for the second part of the project is Moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-protected animal alternatives	In this project, initial evaluation of the novel electrodes will be carried out with computer simulations (*in silico*). In software, we can predict the mechanical interaction of the new electrodes with the tissue in term of force and movement. The development of novel electronics will bench-tested with data collected in previous experiments (*ex vivo*). Only upon success in controlled *ex vivo* experiments, we test the novel electronic system *in vivo* . In parallel with the animal studies in this project, we continue our human studies recording non-invasive electromyograms and delivering non-invasive (electrical and mechanical) sensory feedback to inform our animal experiments. However, the function of these devices when operating in a closed-loop with the brain can only be fully evaluated *in vivo.* Therefore, experiments in animals are essential to achieve the objectives of this project.
2. Reduction Explain how you will ensure the use of minimum numbers of animals	Once the experiment is designed we will run pilot studies with a few animals to ensure the protocols could lead to sensible results. We then move onto the main experiments with a larger population of animals. In each animal in cohort 1, several electrodes, for example multiple electrode arrays of different lengths, moveable microwires and cuff electrodes, will be implanted in both hind-limbs. The use of different electrode architectures allows the peripheral nervous system to be stimulated at different depths from the surface. The rich dataset from different electrodes will be pooled to allow appropriate statistical tests. The statistical power of comparing multiple datasets from the same subjects minimises the total number of animals required to demonstrate significance.

	Only if all novel technologies are successful (verified in animals of cohort 1) we will start the recovery experiments with animals in the second cohort. We will design the experiments such that the minimum number of animals will be used. To account for potential drop out for the experiment (due to infection or pain) we use statistical techniques for missing data.

Project 29	**Repairing the damaged spinal cord**
Key Words (max. 5 words)	spinal cord injury, axon regeneration, neuroprotection, scarring, cavitation
Expected duration of the project (yrs)	5 year(s) 0 month(s)

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

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

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objectives of this project are to determine changes that occur after injury to the spinal cord that provides the information highway from the entire body to the brain and back. We are particularly interested in learning how neurons deal with the injury that makes them vulnerable to death, the scar tissue that

	forms after injury and the lack of nerve regrowth that follows nerve injuries of this type. This will allow for a better understanding of the mechanisms of nerve injury and will help us to identify therapeutic drugs that will be used to protect nerve cells from death, dissolve scar tissue and promote nerves to re-grow.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The project will provide important data that will improve our understanding of the changes that occur after nerve injury and provide an insight into what is required to promote nerve cell survival, removal of scar tissue and promote nerve regeneration. This will underpin the discovery of novel therapeutic drugs that will be used to promote nerve cell survival, scar tissue removal and nerve re-growth
What types and approximate numbers of animals do you expect to use and over what period of time?	Rats: 1,900 Mice: 1,300 Over a period of 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?	Potential harm results from spinal cord injury, which will be created under general anaesthesia. We will use 2 different models of varying severity of harm. We will mostly use the lower severity case, where there are little adverse responses to the injury, to evaluate our potential therapies. We will then select the best compounds for therapies to promote nerve cell survival, scar removal and nerve regeneration in the more severe model because this represents the most common form of injury in humans, with manifestation of many of the potential effects of spinal cord injury in humans. For example, animals will display hindlimb weakness whilst bladder function will stop temporarily (just as in humans). There are clear guidelines in place in our facility to ensure that suffering in animals is minimised by either administration of pain-killers or termination of experiments. Bladders of animals in the more severe model will expressed manually three times a day until

	normal control of bladder function returns (normally 3-6 days). Soft mash will be provided on the floor of cages as well as injections of fluids and extensive care within the first three days after injury. We will remain vigilant for any adverse effects and will promptly provide pain relief or treatment if appropriate, or humanely kill the animal. Animals will undergo behavioural/functional tests to maximise data output prior to using Schedule 1 methods to kill animals or animals will be perfused with 4% paraformaldehyde under terminal anaesthesia for histological analyses.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-protected animal alternatives	There is no adequate substitute for using the *in vivo* models described in this application. Establishment of potential clinical relevance of regulatory molecules interacting in a dynamically changing CNS injury site can only be achieved in an animal model. A less sentient animal such as fish cannot be used since they spontaneously regenerate their spinal axons after injury and achieve complete recovery of function. Therefore, rats and mice are our prototypic laboratory animals and have been rigorously characterised by ourselves for the spinal cord injury paradigms and shown to be representative of the human condition by others. The tools for the project have all been prepared in relation to the models described herein and continuity of the study in these species will be essential for significant progress to be made in a timely and efficient manner.
2. Reduction Explain how you will ensure the use of minimum numbers of animals	Some of the end-point measurements (e.g. nerve regrowth, scar formation etc) may be essentially qualitative and for these we use 3-6 animals per treatment group. In most experiments with quantitative end-points, 6 animals are randomly assigned to each treatment group, a number calculated as the minimum required to provide statistically significant results. This has been determined on the basis of our previous experience

	with these procedures, the methods of analysis and after consultation with statisticians to calculate power.
3. Refinement Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The models selected closely resemble the features seen in humans after spinal cord injury. Most therapeutic agents are evaluated and optimised *in vitro* prior to *in vivo* application. We keep our experimental time points in longitudinal studies to a minimum and use archival control results where possible. Multiple analyses are conducted on all harvested tissues. We use the minimum number of interventions and minimal volumes for drug delivery during experiments and continually seek methods to reduce these by studying alternative drug delivery strategies. These refinement steps significantly reduce animal usage and severity.