Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 19

Projects with a primary purpose of: Translational and Applied Research – Human Nervous and Mental Disorders

Project Titles and keywords

1. **Developing therapies for perinatal diseases**
    - Lack of oxygen, brain haemorrhage

2. **Neurovascular function in health and disease**
    - Brain, Blood vessels, Alzheimer's Disease, energy, pericyte

3. **Assessment of Blood Brain Barrier Transporters**
    - Drug Discovery; Blood Brain Barrier

4. **Modulating Autophagy tct Tackle Huntington's Disease**
    - Autophagy, cell death, Huntington's Disease

5. **Translational pharmacology for drug discovery**
    - Pharmacology, drug, discovery

6. **Electrophysiological studies of neurodegenerative disorders**
    - Neurodegeneration, drug discovery, electrophysiology, in vivo

7. **Anaesthesia and the Developing Brain**
    - Anaesthesia, Neurodegeneration, Neuro-apoptosis, Developing brain

8. **Gene transcription events mediating brain and behaviour**
    - Epigenetics; post-transcriptional modification; imprinted genes; behaviour; brain

9. **Examining the role of mitochondria in neurodegeneration and vascular disorders**
    - Alzheimers, blood vessels, mitochondria, neurodegeneration

10. **Using Zebrafish to understand disease and develop therapies**
    - zebrafish, development, disability, therapy, mechanism

11. **Pharmacodynamics and pharmacokinetics of novel compounds**
    - Drug discovery, receptor, pharmacokinetic, pharmacodynamic

12. **MK2 as a regulator of inflammation in Alzheimer's**
    - Alzheimer's, Inflammation, hippocampus, synaptic plasticity, behaviour.

13. **Neural signalling and disease in rodents**
    - Motoneurone, mechanosensation, MND, hypertension

14. **Repair in neurodegenerative disease and injury**
    - Central Nervous System, Nerve cells, Neurodegenerative diseases, Regeneration

15. **Role of D1R-D3R heteromers in L-DOPA-induced dyskinesias**
    - Parkinson's disease, Dyskinesia, Dopamine, Heteromers, Glutamate receptors

16. **Maintenance and regeneration of the nervous system**
    - avulsion, neurodegeneration

17. **Determination of CNS efficacy and safety pharmacology**
    - Central nervous system, behaviour, safety

18. **Zebrafish and medaka as models for the study of psychiatric disease and age-related cognitive decline**
    - zebrafish, behaviour, addiction, genetic modification, ageing

19. **Early life exposure to drugs and environmental complexity and neuropsychiatric disorder**
    - Genetics, Nature/nurture, Environmental complexity

20. **Neurological disorders: mechanisms and therapies**
    - behaviour, cognition, brain, neurological disorder

21. **Analgesic potential of Retargeted SNARE Proteases**
    - Analgesia, Pain, Neuropathic, Post-operative, Inflammatory

22. **CNS neurotransmitter action in health and disease**
    - Brain, Neurodevelopmental Disorders, Neurodegeneration, Neuron, Glia

23. **Improving the treatment of Parkinson's disease and Parkinson's disease dementia**
    - Parkinson's disease, Dementia, Better treatments, Cell protection, Cell repair

24. **Neuropharmacology and therapeutics in addiction and other dopamine-associated disorders**
    - Addiction, Parkinson's disease, treatments, neurogenesis,

Project 1	**Developing therapies for perinatal diseases**	
Key Words (max. 5 words)	Lack of oxygen, brain haemorrhage	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The work conducted under this licence aims to develop treatments that will improve outcomes for children who suffer brain injury at or around the time of birth. Such injuries occur as a result of disruption to the blood supply, which starves the baby of oxygen. Whilst the initial cause of the injury is understood its subsequent impact on the developing brain is unclear. This lack of basic understanding is hindering the development of effective treatments. This study will investigate how brain injuries at birth develop in the immature brain and evaluate a number of treatments aimed at minimising the extent 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)?	Through the evaluation of treatment aimed at minimising the damage caused to the brain by oxygen starvation we will identify ways to improve the outcome for the numerous children and adults who suffer the long-term consequences of such injuries	
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and Pigs. Over 5 years, we expect to use approximately 2874 rats and newbom pigs 400.	
In the context of what you propose to do to the animals,	1] Expected adverse effects Our studies will involve understanding the evolution of	

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	brain injury occurring secondary to lack of oxygen and blood supply, and bleeding within the brain. We aim to test the drugs that will minimise the brain injury. We will use rat pup and piglet models of brain injury. Under anaesthesia, animals will undergo interruption of blood supply to the brain either by surgical technique or reducing the BP and allowing the animals to breathe low concentration of oxygen. Some animals will undergo administration of blood into the brain under anaesthesia using cannula. Some animals will be exposed to infection by injecting the membrane protein of a germ. Treatments that could potentially reduce brain injury and to be tested in the animal models involve modulating the temperature of the animals, allowing animals to breathe gases, administering drugs orally, as an injection into the brain, vein, muscle or abdomen. It is possible that post-surgical complications such as bleeding or infection may occasionally arise or the animals may experience postoperative pain. Administering injections and performing behavioural tests may cause transient discomfort or stress. Some animals exposed to severe lack of oxygen or blood supply may die while under anaesthesia and the surviving animals may develop limb weakness or paralysis secondary to the brain injury. The likely/expected level of severity for these procedures is moderate. We know when this is likely to occur and will monitor the rats closely over the critical stages. At the end all animals will be killed by a schedule I method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The evolution of brain lesions at the cellular level at different intervals from the original insult cannot be studied in non-animal alternatives. Moreover the new therapies to treat human beings has to be tested in a model that is representative of the human situation and gives the opportunity to measure both the beneficial and harmful effects, before it can be tested on humans in clinical trials. Regrettably there are no non-animal alternatives, which can satisfy the above requirements.
2. Reduction Explain how you will assure the use of minimum numbers	We will use appropriate statistical techniques to calculate the minimum numbers of animals required to answer the research question. We use animals belonging to one type to reduce the variability in the

of animals	study design and hence can use fewer animals. We have developed a model that can produce different seventies of brain injury; thereby the research question can be stratified in the different severities of brain injury. We are constantly seeking to reduce the variability In the model to enable us to use fewer animals. We will employ imaging techniques, once they become available, to identify the animals developing brain injury at the earliest stage to minimise the number of animals needed.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will be using immature rodents at an age, when the brain development resembles newborn babies. The newborn pig has the brain maturation similar to newborn babies. The studies from newborn pig offers detailed information about how the brain and heart recovers in the acute situation, which cannot be obtained from the rats. The previous studies from these models have advanced the knowledge about the development of brain injury in newborn babies. The brain protective therapies developed from these models have gone on to be used in the newborn babies around the world currently. The surgical procedures used to induce brain injury are performed under anaesthesia and well-tolerated. Anaesthesia will be maintained at a suitable depth to avoid the animal feeling pain. Our team is well experienced and the animals will undergo the procedures in the shortest possible duration that will enable us to meet the scientific objectives. Aseptic operating procedures and appropriate surgical dressing will be used to reduce the chances of infection. Any procedure-related distress or pain is actively sought and treated with medications. The least severe route of administration will be used to give drugs or substances. If substances or drugs have not been given to animals before1 we will perform pilot studies. The behavioural tests are generally well-tolerated. Exposure to these tests will be kept to the minimum necessary to obtain the essential data. All animals will receive the highest possible standard of care. Technical support team in a purpose-built husbandry unit looks after the animals. We will be actively seeking veterinary advice, when it is needed. We will be always looking at ways of reducing harm to experimental animals.

Project 2	**Neurovascular function in health and disease**	
Key Words (max. 5 words)	Brain, Blood vessels, Alzheimer's Disease, energy, pericyte	
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)	Brain activity uses lots of energy, and is fuelled by oxygen and glucose, carried in the blood. To match energy demand with supply, active brain cells signal to nearby blood vessels to dilate, increasing the energy supplied to the active brain region. This process is called neurovascular coupling. The blood changes can be studied in humans using non-invasive brain scanning, so are often used as a surrogate measure of brain activity. The size of the blood flow increase that happens when brain activity increases seems to vary across brain regions and brain states, but we don't understand how this happens, and whether it matters for how the brain functions in these different conditions. This variation is a problem, however, for scientists who use blood signals as an indication of brain activity. Also, in several disorders, such as Alzheimer's disease or obesity, neurovascular coupling is impaired. This decrease in energy supplied to active brain regions might cause or exacerbate damage to brain cells that occurs in these conditions. This project will investigate how and why neurovascular coupling varies in healthy animals and how it goes wrong in disease states in the following	

	ways: ***1)*** It will study how brain chemicals that are released in different brain states (e.g. arousal) affect the balance between the brain's energy supply and demand. ***2)*** It will compare neurovascular coupling in two different brain areas, cortex and hippocampus, to see if variations in how the brain matches blood supply with demand may effect how sensitive these regions are to disease. ***3)*** It will determine if Alzheimer's disease could be triggered by a decrease in the ability of the brain's blood vessels to increase oxygen supply. ***4)*** It will determine how infection, psychoactive drug exposure or high fat diet, which all increase inflammation in the body and the brain, affect neurovascular function, brain function and behaviour.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This research will reveal how we fuel our brain activity, and whether our brain function is limited by the amount of blood supply it receives. This is important for understanding normal brain function but also for understanding how our brains go wrong in different disease conditions. If we understand how decreases in the brain's energy supply occur, and how this damages neurons, we might be able to interfere with these pathways to stop conditions such as Alzheimer's disease from progressing.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use mice. Approximately 3000 will be used over a 5-year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The genetically-altered mice usually show no adverse effects, particularly at the ages used in our experiments. Some animals undergo surgery to allow us to implant a window through which we can image their brains. They may require post-operative pain-killers but are usually fully-recovered and alert a few minutes after the procedure. After recovery from surgery, animals are gradually habituated to the experimental equipment, on which they have their head fixed in place but can run on a ball. They will be rewarded, for example with sucrose, and stress will be minimised by accustoming them gradually to the apparatus, but

	nevertheless, this will be somewhat stressful for the mice. Some animals will have surgery that briefly reduces blood flow to their brains. In the long run, this won't obviously affect their well-being (though they may feel some discomfort recovering from surgery, which will be treated with pain killers), but may produce memory deficits over a number of weeks. Some animals will be given a substance to mimic a brief infection, which will make them feel unwell for 48h (like having a cold). Some animals will be fed a high fat diet to mimic the development of human obesity. This won't be enough to produce full metabolic syndrome (diabetes) but will produce persistent weight-gain and, we predict, mild cognitive dysfunction. Some animals will be exposed to intermittent psychoactive drug treatment (e.g., morphine or amphetamine) to examined acute and chronic effects of centrally acting therapeutic agents. Doses and treatment used are expected to produce adverse physical consequences (e.g., dependence syndrome) but only mild to moderate changes in cognitive function. At the end of all experiments animals will be humanely killed and where applicable tissues collected and analysed. If animals are suffering for any reason before the end of the experiment and do not respond to treatment, they will also be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cultured cells are not appropriate for this work, as neurovascular cells behave very differently in culture than in the intact animal, and the spatial organisation of the cell types is lost in culture but is critical to the functioning of the system. A lot of my work is done using brain slices, where these spatial relationships are preserved, but which avoid using a living animal. I also use computer models frequently to simulate the processes underlying the observations I make from my experiments. Nevertheless, studying the relationship between brain activity and blood flow requires that, at least for some experiments, the blood supply to the brain is intact – i.e. the animal is

	alive.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Multiple data are collected from each animal, both while they are alive and then by labelling cells after their death. This reduces the number of experiments required and allows (where possible) within-subjects comparisons to be made, increasing the statistical power of our experiments, so that the smallest number of animals can be used to generate statistically reliable results. Where possible, controls will be shared between groups or 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 a similar neurovascular system to humans and can be bred to express proteins that allow us to see different cell types and the amount of brain activity. They are also small enough to be able to image their brains under a microscope. The experimental and disease models have been designed to provide robust data while minimising animal suffering. Firstly, they use surgeries from which the animals rapidly recover. Secondly, they study the onset of disease processes. This allows the key triggers to be identified before multiple other processes go wrong, and also means that at these early stages, animal well-being is barely affected. Animal welfare is monitored throughout the experiments, and animals are humanely killed where necessary.

Project 3	**Assessment of Blood Brain Barrier Transporters**	
Key Words (max. 5 words)	Drug Discovery; Blood Brain Barrier	
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)	The prime goal of this project is to investigate and identify novel carrier transporters and mechanisms for transport of biological and small molecule compounds across the blood brain barrier for the treatment of centrally mediated diseases including Alzheimer's Disease and chronic neuropathic pain (pain resulting from nerve 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 treatment of many diseases e.g. Alzheimer's disease is compromised by the fact that the majority of drugs are prevented from accessing the brain due to the presence of the blood brain barrier, a barrier designed to protect the brain. By developing molecules which can transport a cargo of drug across into the brain we will be able to help patients with these diseases.	
What species and approximate numbers of animals do you expect to use over what period of time?	Over the five year period of the licence we expect to use 1500 mice and 500 rats. A typical study will comprise 5 or 6 groups of between 7 and 10 per group (depending on species)	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of	Most studies performed under this licence will involve partial tying of the sciatic nerve (the main nerve in the leg) followed by investigation of the effects of treatment on the subsequent pain produced. Previous experience has shown that little or no adverse events are seen	

severity? What will happen to the animals at the end?	with the exception of wound opening caused by the animals licking the site of surgery. This occurs in, less than 5% of the animals. As the animals will have undergone surgery the level of severity will be considered as moderate. At the end of studies all animals will be killed using humane methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Compounds will have been tested in in-vitro assays prior to in-vivo use if possible but the in-vitro assays are not that predictive of brain penetrance due to the complexity of the blood brain barrier. For this reason in-vivo studies are required.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will engage with statisticians before studies commence to make sure we are using the correct number of animals - too many would waste animals but use of too few would mean that studies may need to be repeated.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodents have well developed blood brain barriers and appear to use similar mechanisms to allow molecules to cross. The models we have chosen include those which we believe show the best scientific rationale and have shown positive effects in work carried out under other licences (PPL 80/2443) and will provide us with most information. The end-points chosen are known to be driven centrally and are inhibited by molecules which cross the blood brain barrier. We will use the most refined techniques available to meet our experimental goals. Where appropriate analgesia and anaesthesia will be used unless there is a significant reason not to i.e. use of will interfere with end points.

Project 4	**Modulating Autophagy tct Tackle Huntington's Disease**	
Key Words (max. 5 words)	Autophagy, cell death, Huntington's Disease	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	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)	Huntington's Disease (HD) is a devastating dementia disease. The alteration in a gene called huntingtin. (HTT) leads to changed HTT protein and its toxic aggregates, which in turn cause brain cell death and FID. Strong evidence has shown that eel lular "self-eating "process, termed autophagy, counteracts dementia diseases such as HD, because autophagy clears up protein aggregates in cells, thereby removing their toxicity to cells. As such, defects in autophagy give rise to various dementia diseases. Bim is a critical protein that causes cell death in various tissues including those in brains. Recently we found that Bim also suppresses autophagy in addition to its cell death induction. In this project, we will test if enhancing autophagy (by using Bimnull and relevant genetic altered mice, and chemical agents) alleviates HD. This project may lead to the discoveries of important drug targets and therapeutic agents for HD.	
What are the potential	Previously, we observed that reduction of Bim	

benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	decreased toxic HTT protein aggregates and cell death in lID cell models. Bim are involved in autophagy inhibition and cell death induction, both of which are relevant to HD. These suggest that Bim may be an important driver for ND progression. Importantly, we identified a portion of Bun (Bim-P) capable of enhancing autophagy and reducing toxic HTT protein aggregates in HD cells. We will test if Bim contributes to ND progression and examine the efficacy of new agents such as Bim-P in treating HD using lID mouse models. This project may yield new agents to improve lID. As lID and other dementia diseases, such as Alzheimer Disease and Parkinson's Disease, share common processes in cellular level, this work may also potentially be beneficial to these diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, 700 per year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In case of mouse breeding, Bim or autophagy-relevant gene modification will not cause adverse effects to animals, thus these animals will not undergo harsh consequences as a result of the gene modifications. In lID therapy study, drug treatment is not expected to exceed moderate level of severity. ND transgenic mice are expected to develop motor deficit over ageing. The deficit will be closely monitored. In case the deficit causes severe harsh consequences to animals, they will be humanely killed immediately. All animals are killed on completion of planned procedures set in the protocols.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In case of mouse breeding, Bim or autophagy-relevant gene modification will not cause adverse effects to animals, thus these animals will not undergo harsh consequences as a result of the gene modifications. In lID therapy study, drug treatment is not expected to exceed moderate level of severity. ND transgenic mice are expected to develop motor deficit over ageing. The deficit will be closely monitored. In case the deficit causes severe harsh consequences to animals, they

	will be humanely killed immediately. All animals are killed on completion of planned procedures set in the protocols.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will plan appropriate breeding strategy to avoid/minimise the production of unwanted mice. We will decrease the numbers of animals by using tissues for multiple analyses. We will conduct literature search and/or preliminary study with a small number of animals to identify the best doses and routes of administration prior to larger-scale treatment. Based on the preliminary study and literature search, minimal group size that achieves statistical significance will be established.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	As humans and mice share almost all of biological properties in anatomy and physiology, studying HD with relevant mouse models will yield valid and important knowledge for us to tackle human HD. Therefore, we choose mouse as our study model in this project. Most of our genetic manipulation and chemical treatment will not cause lasting pain and distress to animal, and the protocols in the licence are set at moderate limit. In case the severity exceeds moderate level, the animals will be humanely killed immediately. The following measures are included to minimise animal suffering. Effective record keeping will be appropriately maintained. An overview of welfare assessment protocols will be carried out regularly. Records will be made with full details of the origin of each strain, the nature of mutation, any expected harmful effects and specific husbandry requirements. These measures will ensure that any potential welfare issues are easily picked up, and that potential sufferings are well predicted and dealt with. Given that mice can be traumatised by sudden and rough manual handling, mice will be carefully habituated by being gently held in hands and lifted out of the cage, when manual handling is needed (e.g. behavioural tests).

	Animals with signs of suffering (e.g. reduced activity, weight loss) will receive extra care, e.g. soft chow (wet mash) and plenty fluids will be provided on cage floor, and soft bedding will be supplied. During drug treatment, in case animals have signs of pain (e.g. vocalization, restlessness, rough hair coat, reluctance to move), stop eating/drinking, treatment will be stopped, and NVS will be consulted immediately. We will conduct literature search to identif' the doses and routes of administration prior to the treatment. Should the literature be unavailable, pilot study with small numbers of animals to identifS' the best doses and routes of administration prior to larger-scale treatment. Analgesia and anaesthesia will be used to minimise the pain and distress to animals when invasive and potentially painful procedures are performed. Anaesthesia will be applied when appropriate, and analgesics will be used. Additional care to prevent postoperative hypothermia will be given to anaesthetised animals during their recovery.

Project 5	**Translational pharmacology for drug discovery**	
Key Words (max. 5 words)	Pharmacology, drug, discovery	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The burden on patients, carers and society of disorders such as Alzheimer's disease, chronic pain and inflammatory conditions is immense and growing with an increasingly ageing population. Unfortunately, current treatments in all of these areas have substantial limitations in terms of the level of effectiveness provided and/or the undesirable side effects caused. The development of new safe and effective medicines are an important facet of how society approaches such unmet medical need. The aims of this project are to continue our efforts to help facilitate and optimise the advancement of potential new medicines being developed by other drug discovery scientists (e.g. pharmaceutical & biotechnology companies, academic institutions) for chronic disorders of the brain and inflammation. This will be in the form of providing robust evidence from pre-clinical rodent models of likely therapeutic benefit in the clinic, an indication of the levels of drug in blood required to produce such benefit, and a recommendation for the types of patient and clinical	

	outcome measures most suited to the new treatment. The models and technologies that will be used have been refined over many years and have strong translational relevance to the human condition.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The pre-clinical evidence produced by this project will help identify the best new drugs for progression into human clinical trials and is expected to reduce the currently high number of failures observed in the clinic. Importantly, the scientific translational approach being taken has previously been successful in advancing several new drugs that have proven to have some clinical benefit in Alzheimer's disease and various chronic pain conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 years of the proposed licence we estimate that we will use: Rat: 15000 Mouse: 5000 Gerbil: 500
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	A proportion of the techniques used under this licence are minimally invasive and therefore classified as mild. Some animals may have undergone procedure that will cause some pain and discomfort, but will be kept to the minimum possible and these will be classified as moderate. We anticipate only a small number of animals may show adverse effects and where do so they will be culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	A range of chronic disorders are covered by this licence, including cognitive deficits associated with Alzheimer's Disease and a range of painful (e.g. osteoarthritis) and inflammatory conditions (e.g. rheumatoid arthritis) and neuropathic conditions (diabetic neuropathy). These are all conditions where the whole organism (i.e. intact nervous system) is required in order to measure a cognitive or painful response. No in vitro systems are in existence that can

	replicate the whole functioning organism.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals will be kept to the minimum required through good experimental design. For most experiments, sample sizes have been set using power analysis, generally using a significance level of 5%, a power of 80-90%, to detect a difference between groups of 25%. For most procedures numbers of animals per group will be in the 8-12 range depending on the protocol in use. We will continue to monitor group sizes and modify as appropriate based on their analysis. Most experiments will involve parallel groups, though in some instances a cross-over design may be used if deemed appropriate. Substances can be administered using a cross-over design, whereby each animal receives all treatments and acts as its own control e.g. where animals have been surgically prepared for EEG or trained to perform a task such as touch screen. Within animal comparisons, are less variable than between animal comparisons, so this will allow the use of smaller groups of animals, to consistently achieve statistical significance.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	**Choice of species** The majority of experiments carried out under this project licence will be in rats and mice. Rats are widely used to provide data in drug discovery because there is a large body of data describing some of the similarities and differences between rat and human physiology. Mice are sometimes used for example in situations where antibodies optimised for use in humans retain their affinity for the equivalent mechanistic target in mouse but not in rat. We may use genetically modified mice, usually this is because no specific inhibitors for the mechanism of interest have yet to become available for the target in question. **Choice of models** The models described in this licence have been

	extensively used to identify candidate drug molecules, and are of the lowest severity that will allow decision making data to be obtained and where possible a human correlate exists. **Minimisation of suffering** Telemetry devices maybe implanted to measures physiological parameters such as body temperatures, blood pressure & heat rate, this will minimise the stress that maybe experienced with repeated measures such as rectal probe & tail cuff, eliminates the requirement for restraint/tethering and allows the continuous collection of data without the need for any manipulation. Therefore the benefit of the surgical implantation will improve the overall lifetime experience of the animal compared to repeated procedures. STZ injection produces neuropathy by evoking typical symptoms of diabetes and therefore, animals will drink more than usual and this will be taken into consideration during the husbandry care. 2 % sucrose is added to the drinking water to help avoid the initial hyperglycaemia, and animals will stay group housed to help maintain body temperatures. All animals undergoing nerve injury for the induction of neuropathic pain, are placed onto an environmental enrichment protocol, where from arrival day enrichment is changed on a Monday (castle) Wednesday (house) and Friday (tubes), to help reduce the incidents of autotomy. For all pain protocols, we will continue to encourage the use of spontaneous behaviours! non-invasive endpoints to reduce pain and suffering experienced by the animal, such as weight bearing, burrowing, paw volume and any other more naturalistic behaviours.

Project 6	**Electrophysiological studies of neurodegenerative disorders**	
Key Words (max. 5 words)	Neurodegeneration, drug discovery, electrophysiology, in vivo	
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
	X	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)	Exploration of the changes in brain circuitry and function that are affected in neurodegenerative disorders and how novel therapeutics can affect these changes.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The work will lead to improved understanding of the biological pathways leading neu rodegeneration and the creation of improved in vivo measures that better reflect the disease states e.g ad and pd. We will test new drugs that, if successful, could advance into clinical trials and may lead to new drugs for treating these diseases. This would allow people to be more independent, live longer and healthier lives and this should also reduce care burden and nursing home costs.	
What species and approximate numbers of animals do you expect to use	Rats and mice will be used for this work over a period of 5 years. Approximately 860 rats and 800 mice are likely to be used annually.	

over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Since we are studying consequences of neurodegeneration there may be specific expected adverse effects in some of the rodent models used. For example weight loss and poor tolerance to surgical intervention are more likely to occur in mouse models of Alzheimer's Disease, and motor symptoms may occur in models of Parkinson's disease. Adverse effects that might result from the surgical procedures used, or treatment of the animal with novel uncharacterized drugs, will be minimised by utilizing highly skilled individuals in these procedures and predicting beforehand as much as is possible the nature of any material administered or known phenotype prior to treatment. Animals exhibiting symptoms that impact their welfare will be closely monitored and euthanized if necessary. Previous work has demonstrated that the experiments proposed here will provide directly translatable and reproducible markers in subsequent human clinical trials. Animals will be humanely euthanized at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	While in vitro work can support many of the processes leading up to identification of potent and active molecules, due to the complex nature of the brain circuitry involved in controlling function and the level of interactions between different regions of the brain it is necessary to undertake the research proposed here using animals. This would be confined to rats and mice, which together provide a highly predictive method of demonstrating that these potential novel medications will perform as predicted in humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This work will involve the minimal number of animals needed to achieve a statistically significant estimate of the mean and variance of key parameters. A professional statistician will help in determining statistical power and reproducibility, thus minimizing animal numbers.
3. Refinement	The rat is predominantly used because of the large amount of information known about the biological basis

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.	of its behaviour. Mice may also be used when, for example, the target in those species more closely resembles the human form. Mice might also be required when a target protein has been under or over-expressed or where a mouse model of a disease exists.

Project 7	**Anaesthesia and the Developing Brain**	
Key Words (max. 5 words)	Anaesthesia, Neurodegeneration, Neuro-apoptosis, Developing 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
	√	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)	Millions of babies and young children require unpleasant and/or painful medical or surgical procedures to be performed during early life. There is great concern however because laboratory studies have shown an association between all of the commonly used anaesthetic gases with damage to cells in the developing brain and long-term problems with brain function. This project will primarily aim to show whether combining these gases with xenon, a weak anaesthetic gas not currently used in clinical practice and shown to have protective properties, can produce equally effective but safer anaesthesia. Secondly, this project will aim to study the mechanisms of any observed neuroprotective effect of these mixtures by comparing the effect of the gases alone and in combination with xenon on the physiology of the animals.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	The primary potential benefit relates to the value of the results to clinicians, particularly paediatric anaesthetists, in possibly identifying a new anaesthetic gas mixture that is effective and safe. The secondary potential benefit relates to new	

project)?	knowledge about the mechanisms underlying effective and toxicity of anaesthetics gases in developing brain. The aim is to publish the findings in academic journals and this information is likely to be of interest to pre-clinical scientists interested in anaesthetic-induced neurotoxicity as well as the broader scientific community who use anaesthetic agents in immature rodents.
What species and approximate numbers of animals do you expect to use over what period of time?	It is expected that the studies will use 850 rats and 1700 mice over the five year duration of the project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The expected level of severity for the animals in protocols is of a mild level. Animals will be humanely culled at the end of the proposed studies.
Application of the 3Rs	
1. **Replacement** State why you need to use animals and why you cannot use non-animal alternatives	The purpose of this study is to determine whether co-administration of xenon reduces the detrimental effects of anaesthetics on the developing brain. It is not ethical to randomise healthy human subjects to receive anaesthesia during early life, therefore an effective model system is needed to determine proof of principle. The mechanism underlying the toxic effects of inhaled anaesthetics and protective effects of xenon are poorly understood and this study aims to accurately quantify the physiological effect of the anaesthetics, alone and mixed with xenon, on the cardiovascular, respiratory and brain systems and this can only be performed in an in-vivo model. Consequently there is no alternative to the use of animals for these studies.
2. **Reduction** Explain how you will assure the use of minimum numbers of animals	For each of the proposed studies we will use power calculations to define the minimum numbers of animals needed for each experiment using pilot data. Whenever possible (and particularly for definitive experiments) experimenters will be blinded to treatment allocation and animals will be randomly assigned (by blocking where appropriate) to study groups. Multivariable analysis will maximise the information

3. **Refinement** Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	While rats and mice have both been used in models of anaesthesia-induced neurotoxicity, the rat has been used most frequently, specifically within the first two weeks of life and most frequently on postnatal day seven. The new method to define the doses used in this study was established in immature rats in order to provide information to optimise these models and may differ in mice. Therefore, immature rats will be used in this study. However, this project will also aim to establish this in genetically modified mice to allow analysis of the individual effect of specific receptors in the cells. The outcome severity from all experiments will be minor with an anticipated adverse event rate of <1%. A step wise approach to assessing the toxic effects of anaesthetic exposure will be used: Indeed, the aim is to use low anaesthetic doses defined using a single, rather than repeated, stimulus of lower intensity. The result is much less physiological disturbance and the perceived experience for the animal is the induction of sleep.

Project 8	**Gene transcription events mediating brain and behaviour**	
Key Words (max. 5 words)	**Epigenetics; post-transcriptional modification; imprinted genes; behaviour; brain**	
Expected duration of the project (yrs)	**5**	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our genes encode a range of molecules that are essential for development and normal function. We now know that in addition what these genes encode, when they are active and how much product they produce, is also critical. The process of gene activation, or expression, is known as transcription. Gene transcription is a highly regulated process; whether a gene is on or off, or even poised to be activated, is controlled by what is known are epigenetic mechanisms. Even once a gene has been expressed, producing an RNA copy of its DNA, this RNA molecule can also be further modified by what is known as post-transcriptional modification. We now know that these processes often go wrong in neurodevelopmental disorders. For instance, the epigenetic regulation of gene expression (transcription) may be altered due to a number of factors, such as a change in the DNA code of a regulatory region of a gene, or the complete loss of key regulatory proteins. Gene transcription may also be affected by the environment, such as the *in utero* environment, which in turn may lead to brain behavioural change. Our work aims to explore the relevance and importance of these processes by examining what	

	happens functionally when we alter some well-defined processes. These include looking at the regulation of genes in response to changes in the maternal environment and examining the effects of loss of key neurodevelopmental disorder candidate genes, known to encode gene transcription regulators.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This work will 1) Provide a better understanding of how epigenetic mechanisms and post-transcriptional modification contributes brain and behavioural outcomes 2) Investigate the biological role of known genetic risk factors for a number of neurodevelopmental disorders 3) Identify factors (genetic/lifestyle) that may alter gene expression and influence neurodevelopment, adult behaviour and predisposition to neuropsychiatric illness.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse – 750 per annum Rat – 70 per annum for 3 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will use measures of behaviour and cognition to assess the consequence of changes in gene transcription in rodents. These gene transcription changes will be induced by genetic alteration in mice. The majority of these genetically altered (GA) mouse lines are classified as “mild”, with a minority classified as “moderate” due to the survivability issues. In addition, we aim to examine the consequences of altering the maternal environment, in particular maternal diet, on gene expression in the offspring brain. This will be examined in both GA and non-GA mice. Finally, we will also monitor and modify gene expression in rats through surgery; this is also classified as “moderate”, however, the numbers here are relatively low. Some animals bred under the aegis of this licence may be transfered to other licences. However, although some animals may move between protocols on this licence, at the end of the protocol, the majority of animals will be culled.
Application of the 3Rs	
1. Replacement	It is necessary to perform whole animal studies to

State why you need to use animals and why you cannot use non-animal alternatives	achieve the experimental aims, since in all instances integrated physiological systems are affected. Behaviour is an emergent property of brain function, involving co-ordinated activity both within and external to the CNS. It is also not possible to model pregnancy using cell lines or any other *in vitro* system. Pregnancy and placental development are features of mammals generally not observed in other vertebrates. Consequently it is not feasible to use a non-mammalian model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will apply power calculation to all our studies. We will breed our transgenes on specific strain backgrounds to reduce genetic variability. We will cryopreserve lines we are not actively using.
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 chosen as an experimental model since their physiology is well studied, they are genetically modifiable and they are the lowest vertebrate group appropriate for studying fetal growth and pregnancy. Rats are chosen as an experimental model since their physiology is also well studied. Rats are the species of choice for assaying the behavioural effects of manipulations of the CNS, as they are larger than mice, and are more amenable to surgical techniques and infusions can be more spatially restricted and therefore more refined. We have a great deal of experience in performing studies of brain and behaviour, and have refined these greatly in order to minimise the harms experienced by the animals.

<table>
<tr><td>Project 9</td><td colspan="2">Examining the role of mitochondria in neurodegeneration and vascular disorders</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Alzheimers, blood vessels, mitochondria, neurodegeneration</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>√</td><td>Basic research</td></tr>
<tr><td>√</td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
<tr><td></td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
<tr><td></td><td>Preservation of species</td></tr>
<tr><td></td><td>Higher education or training</td></tr>
<tr><td></td><td>Forensic enquiries</td></tr>
<tr><td>√</td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">A common factor in neurodegenerative and cardiovascular diseases is the dysfunction of mitochondria - small components vital for the function of all cells. These small mitochondria primarily provide energy but also influence cell signals and detoxify some potentially-harmful chemicals. Mitochondria move within cells to sites of need, which is particularly important for the highly energy-consuming cells of the brain. Toxic or stressful events often damage mitochondria - damage that can accrue with age and contribute to, or even cause, cell damage or death.

One potential cause of stress to brain cells is a component of the blood that transports fats and cholesterol, called Apolipoprotein-E. ApoE circulates in the blood and is also present in the brain, particularly in the helper cells that transmit nutrients and clear waste. Dysfunctional ApoE can impair mitochondria in both brain cells and blood vessels, a factor that may contribute to the development of dementia.

ApoE is the main genetic risk factor for Alzheimer’s disease. Of the three human versions of Apo-E (2, 3</td></tr>
</table>

	or 4) inheriting only Apo-E4 increases the risk of Alzheimer's disease 20-fold. Apo-E4 also increases the risk of blood vessel dysfunction, before dementia starts, however it remains unclear whether the primary, disease-causing deficits occur in the blood vessels of the brain, or the brain cells that they supply. **Aims** This project will use rat models of Alzheimer's and cardiovascular diseases, allowing the animals to age in order to determine the critical steps in disease development. Alterations in the function of cells and their mitochondria will be examined in the various cells in the brain (nerves, helper-cells and blood vessels), to better understand how treatments and preventative strategies should be focussed. Dietary modifications that exacerbate blood vessel dysfunction (high-fat plus high-carbohydrate) will be contrasted with those that are reportedly neuroprotective (high-fat but low-carbohydrate). Exercise is reported to provide neuroprotection via the mitochondria and as such will be examined for its influence on mitochondria, brain cells (including blood vessels in the brain) and disease progression.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project examines how and when brain cell function starts to become impaired in Alzheimer's disease, using a model of the most common risk factors. Early alterations will be characterised and quantified to reveal changes that precede cell death. Current treatment options for the growing Alzheimer's disease-population are limited. This research will establish experimental platforms for evaluation of new treatment and prevention strategies e.g., minimising neurodegeneration by maintaining mitochondrial function. The project will explore how alterations in diet or exercise could be used as strategies to treat, delay or even prevent Alzheimer's disease-like dementias.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use new rat models of Alzheimer's disease that either have normal-, human-E4- or no Apo-E (latter two are genetically-altered strains). An estimated total of 1000 animals will be used over the 5-years of the project (approximately one third each of normal-control, Apo-E knock-out and human-ApoE4-knock-in).
In the context of what you	Rats will be allowed to age for up to 2 years in this

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?	project, with some given optional access to an exercise wheel and some given variations in their diet (altered fat/carbohydrate content). During this time the animals' cognitive and cardiovascular functions may be tested (by maze/recognition tests and ultrasound of the heart respectively). Finally, the animals will be humanely euthanised and tissue removed to allow age-associated changes to be examined both in brain tissue and intact blood vessels. The severity limit for the whole project is Mild, with the actual severity expected to be sub-threshold for the majority of animals. Expected adverse effects of this project are: - ApoE knockout rats can have increased fatty deposits in their blood vessels, leading to an increased risk of stroke. Cardiovascular function will be monitored to restrict severity of adverse effects to Mild. - ApoE4 knock-in rats are expected to develop cognitive deficits akin to human dementia, including decreases in brain volume and working memory and potentially increased aggression. It is anticipated that such deficits should not impair normal function. - The animals will also be maintained for up to 2 years to allow age-associated deficits to develop. Again, the maximal severity is Mild, additional animal husbandry care will be provided to increase comfort where necessary. - Modifications to the animals' diets may result in changes in weight, with the potential for obesity and also for decreased palatability. As the ApoE knockout rats already have elevated blood cholesterol, any alteration in the diet of these rats will be started as a pilot trial, with animals monitored daily for visual signs of adverse effects and weekly for blood sugar/lipid alterations, ensuring that severity does not exceed Mild. - Cardiovascular function will be tested by echocardiogram (heart ultrasound), carried out under general gaseous anaesthesia. This has the potential to cause cardiorespiratory depression and decreased blood pressure, if any such signs are observed and recovery from anaesthesia will rapidly allowed. - Blood sampling may result in temporary Mild discomfort, which will be minimised by the use of

	topical analgesics. The animals will be humanely-killed at the end of the project, either by Schedule 1 methods or under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal tissue is necessary to provide the complex mixture of cells that are all involved in the development of Alzheimer's Disease. This includes the interplay between the microscopic blood vessels in the brain and the surrounding nerve cells and helper cells. This project will also examine how lifestyle alterations such as modifications to diet and exercise, affect the onset of Alzheimer's Disease. Such systemic changes cannot accurately be studied using non-animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Minimal animal numbers will be used by taking samples of intact tissue (both brain tissue and blood vessels) and maintaining them in the laboratory. This will allow for informative, longitudinal studies and will maximise the experimental output from each animal.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	This project will use rat models of cardiovascular and Alzheimer's Diseases. These are new rat models designed to closely resemble human disease progression, which will allow imaging of changes to cell function during the onset, progression and potential offset of the disease by dietary or exercise modifications. Animal suffering will be minimised throughout wherever possible, for example by use of local analgesics. None of the protocols in this project exceed Mild severity.

Project 10	**Using Zebrafish to understand disease and develop therapies.**	
Key Words (max. 5 words)	zebrafish, development, disability, therapy, mechanism	
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)	This project aims to increase our understanding of diseases, provide disease models for therapeutic discovery and development, and investigate the processes and molecules involved in vertebrate development. The project focuses on rare inherited diseases, which together affect about one in every 10 people, even though each disease has an incidence of less than 1 in 2000 people. They frequently cause premature death in childhood and the vast majority have no approved treatments. We will also study diseases that have a more complex origin, in which environmental factors contribute significantly. There is a huge need to find treatments for both inherited and complex diseases. To help us find treatments, we model the disease in fish and investigate those fish to find out which aspects of the disease we should be targeting with treatment. As many of the diseases we study affect children, and because we need to understand the function of proteins in the whole animal, externally developing embryos are best suited for our studies.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We expect to increase our understanding of several diseases, predominantly neurodegenerative diseases that affect children, and move closer to a treatment. For example our drug discovery and testing studies, enabled by the large number of zebrafish embryos we can generate and the small quantities of compound needed for treating embryos and larvae, will speed up the selection of the most promising compounds for testing on mammalian models.
What species and approximate numbers of animals do you expect to use over what period of time?	We use the zebrafish as our experimental model. The embryos develop externally from the mother and do not become free-feeding and regulated by the Home Office until 5 days post-fertilisation. The diseases we model predominantly affect embryos at unregulated stages so these studies are not regulated. However, we require a Project Licence as we use genetically-altered zebrafish (which themselves do not have the disease), and we need to generate and chàracterise some new disease models and we do not know at what age they will show the disease. At regulated ages, we expect to generate 8000 genetically altered zebrafish that are older than 5 days post-fertilisation over a 5 year period. We already know that these adult fish will not be harmed by their genetic-alteration. Up to 2500 would undergo mild procedures with good husbandry to reduce the number of animals used overall and to enable identification of genetically altered fish.
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 maximum severity we expect is moderate. When we generate new disease models, we will prevent severe harm by limiting the number of fish and the maximum length of time it can suffer. Suffering will be limited and maximum information gained by culling followed by tissue analysis. We will also perform a mild procedure — removal of part of the fin for genotyping and extracting sperm and eggs for in vitro fertilisation. These protocols will be performed under anaesthetic. Analgesia will be given to fish from which fin tissue is taken. Hence, discomfort is expected to be at a low level and transient, and the fish are expected to make a full recovery.
Application of the 3Rs	
1. Replacement State why you need to use	Vertebrate development requires the co-ordinated growth, differentiation and movement of diverse tissues in time and space. While it is possible to

animals and why you cannot use non-animal alternatives	model simple aspects of development in vitro, it is impossible to model the complex events that occur between tissues to form a fully functional embryo. Furthermore, pathogenic processes during disease, and attempts to treat them, involve many cell types and tissues and this cannot be recreated in vitro. It is for these reasons we must undertake experiments upon animals. Our species of choice is the zebrafish as zebrafish are unregulated up to 5 days post-fertilisation and most of our experiments are performed on these unregulated animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To ensure minimum numbers are used, we will genotype fish and only keep those required. We will also store sperm when a genetically-altered line is not in active use and use in vitro fertilisation to rederive the line.
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 the zebrafish model as a substantive body of work shows that developmental processes are highly conserved between vertebrates and because it is the vertebrate model with the lowest neurophysiological sensitivity. Thus, data derived from our models can be extrapolated to humans and other animals. The protocols to be used are all standard methods in zebrafish research. With the exception of the minor surgery of fin tissues for genotyping which we hope to replace with swabbing in future, all of the protocols are non-surgical. When generating new disease models, we will use a small number of pioneer fish to set humane endpoints for further fish of the same genotype, and we will limit the length of time that fish will experience the disease. Latest knowledge on analgesia will be applied.

Project 11	**Pharmacodynamics and pharmacokinetics of novel compounds**	
Key Words (max. 5 words)	Drug discovery, receptor, pharmacokinetic, pharmacodynamic	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	There remains a large unmet need for new medicines to treat a large variety of disorders. However, drug discovery efforts within the UK have been hit particularly hard by the reduced investment in Research and Development by major pharmaceutical companies. We aim to exploit the UKs world renowned basic science and drug discovery expertise by translating our increased understanding of disease processes in areas such as cancer and neuroscience into new drugs. Although great advances have been made in computational (in silico) aspects of protein (drug target) structure and drug design that have reduced the usage of animals in the drug discovery process, there nevertheless still remain large gaps in our knowledge that necessitate in vivo testing in rodent species. The key aspects of the drug discovery process that this license address are: 1) how rapidly is the drug broken down (metabolised) by a live animal? This is a question applicable to all disease areas and is	

	important because there is little point in developing a drug which is rapidly broken down in animal species (and therefore by extrapolation man) since such drug will need to be administered multiple times a day, which is very inconvenient; and 2) to what extent does a drug designed to treat diseases of the brain actually get into the brain and interact with the protein of interest (so-called “target engagement”)? A secondary aim of this license is to understand the biochemical changes that occur in animal models of a disease or following the application of certain drugs.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits of this project are the identification of improved treatments for patients suffering from a variety of different forms of cancer as well patients with disorders of the central nervous system, including Alzheimer’s, Parkinson’s and Huntington’s diseases, schizophrenia, anxiety, depression, epilepsy. Additional indirect benefits of new drugs include a reduction in emotional strain of families and loved ones as well as a reduction in financial costs to care-givers and society in general. Aside from the drug discovery aspects, studies of disease mechanisms that are also covered by this Project License may provide insights into the disease mechanisms of, for example, cancer and disorders of the brain.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats = 5000 Mice = 2500 These animals will be used over a 5-year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The adverse events from the procedures themselves are expected to be mild. However, given the nature of the studies to be conducted (i.e., the in vivo testing of completely new drugs that have not previously been tested in animals), then it is possible that more severe adverse effects (e.g. lack of mobility and/or grooming) may be observed and therefore animals will be monitored closely and if adverse events are observed then animals will be humanely killed. At the end of each of the studies covered by the Protocols described in this license, animals will be killed and where

	appropriate, tissues (e.g., brain, blood) will be harvested for subsequent biochemical and/or bioanalytical analyses.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although in vitro (test-tube) studies of the mechanisms that break down drugs are possible (and will be an integral part of our generic drug discovery efforts), their predictive validity is variable and accordingly there are as yet no in vitro assays or computational models that can simulate the complexities of the in vivo system. Furthermore, there is also no current substitute for in vivo assays to determine if a drug can get into the brain.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The primary objective of the studies described in this project are to select and prioritize compounds suitable for further studies and to screen-out those that are not. Accordingly, we will use sufficient animals to permit us to make reliable judgments as to whether the in vivo properties are good, bad or intermediate rather than to power our studies (i.e., increase the group size) to demonstrate that the in vivo properties of compounds are statistically significantly different from one another.
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 species of choice will be the rat since any data that are generated as part of this project are then consistent with subsequent safety and toxicity studies that are required by the regulatory bodies to be conducted in rat. However, where necessary, we will use mice to generate data that are consistent with other studies showing the effects (efficacy) or side-effects of drugs in mice (e.g., efficacy studies in particular strains of transgenic mice). As regards animal welfare, pre-meetings between researchers and animal care staff will take place to evaluate and put in place monitoring systems to identify potential welfare issues arising from specific protocols. Environmental enriched housing is provided to all of the animals throughout these procedures.

Project 12	**MK2 as a regulator of inflammation in Alzheimer's**	
Key Words (max. 5 words)	Alzheimer's, Inflammation, hippocampus, synaptic plasticity, behaviour.	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The purpose of this project is to investigate the importance of inflammatory factors in Alzheimer's disease. We plan to determine if reducing the production of inflammatory factors could rescue the memory deficits observed in Alzheimer's.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits of this project in terms of basic science will be to investigate the molecular mechanisms underlying the importance of inflammatory factors in Alzheimer's disease and their effect on memory. As well as furthering our understanding of the effect of inflammatory factors in Alzheimer's and memory this project could also identify potential therapeutic targets for Alzheimer's disease.	
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using transgenic mice in this project. Over the 5 years we expect to use a maximum of 3,000 mice.	
In the context of what you	All protocols are moderate or mild in severity and the	

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?	only expected adverse effect is the Alzheimer's phenotype in the APPswePSIdE9 mice which can have a shortened life span At the end of the project the animals will be culled and will be used for biochemistry and electrophysiology experiments to further our understanding of the molecular mechanisms behind any in vivo changes we have observed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The function of the brain can currently only be studied using the brains of animals or humans as computer models are inadequate as our knowledge of brain function is too rudimentary to generate realistic models. Cell culture systems are inadequate because they do not preserve the functional architecture of intact brain circuits. Studies in humans are impossible because they would be invasive and require genetic modifications. This leaves the use of animals, specifically mice, as the best option.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Breeding will be carefully monitored to keep numbers to the minimum. Animals will be used on behavioral testing and then the same animals will be used for biochemical analysis. Statistical power calculations have been used to find the minimum number of animals to give us a conclusive answer.
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 Alzheimer's disease animal model chosen has been widely used in previous Alzheimer's research and is a well characterized model. The model has been chosen to maximize the numbers of relevant offspring. The welfare of mice will be maintained throughout the project by daily inspection of mice for general signs of ill health. The behavioral tests used have been designed to minimize the amount of stress caused.

Project 13	**Neural signalling and disease in rodents**	
Key Words (max. 5 words)	Motoneurone, mechanosensation, MND, hypertension	
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)	Motor and stretch-sensitive sensory neurones are essential for controlling movement. We have uncovered mechanisms the body uses to regulate the sensitivity of these neurones that signal to and from the muscles. This project explores how these sensitivity controls work and if they can be exploited as novel drug targets to ameliorate the symptoms of two major diseases: Motor neurone disease (MND) and high blood pressure (hypertension). Both are conditions that substantially threaten human life expectancy and quality of life. There are no effective treatments for MND, while hypertension is poorly controlled globally. New drug targets would reduce weakness in MND and offer radical new treatments for hypertension, a major risk factor for cardiovascular disease.	
What are the potential benefits likely to derive from this project (how science could be	The project will: 1) increase our understanding of the motor and sensory nervous systems.	

advanced or humans or animals could benefit from the project)?	2) identify and test the therapeutic potential of targeting nerve terminal sensitivity regulation for treating symptoms of two major diseases with substantial unmet need. 3)
What species and approximate numbers of animals do you expect to use over what period of time?	Rats (100), Mice (2100) 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?	GA rats will experience late-onset genetically-induced hypertension only. Previous research shows they experience no overt behavioural or psychological stress at the ages to be studied. GA mice will develop MND symptoms, with mild (90%) to moderate (10%) disturbances of motor control, weakness and fatigue during normal behaviour and/or on exercise. Wild-type animals will not cross any severity levels. All animals will be killed by Schedule 1 methods
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The normal function, development and disease processes in nerve cells of interest cannot be reproduced in culture and currently can only develop in the intact nervous system *in vivo*. Only the most primitive imitations can be produced in culture. Indeed, in mechanosensation research, we believe uncritical acceptance of data from culture systems has misled science in these areas. Thus, 90% of all planned work will use ex vivo tissues.
2. Reduction Explain how you will assure the use of minimum numbers of animals	1. All protocols use the minimum number of animals to give statistically robust results, determined by >30 years' experience of experimental design, good practice and statistical advice from institutional statistical services. 2. We have developed simple disease models from wild-type tissues to reduce genetically altered animal use as far as possible.

	3. We typically require small group sizes (3-6 animals per group) for our experiments by obtaining multiple identical tissue samples per animal where possible (e.g. muscles from both left and right limbs), optimising experimental design (e.g. paired pre-drug vs with-drug statistical testing) to minimise between-sample variability, plus use of power calculations recommended following consultation with statistical experts.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats and mice are the best available model for investigating normal motor and sensory terminal properties at the level focussed on in this proposal, and are used extensively for genetic modelling of diseases. Standard protocols, methods and reagents are optimised for them and genetic lines and technologies to execute this programme of work are also available. 'Lower' animals, e.g. invertebrates, do not reproduce the features of MND, mammalian mechanosensation or hypertension at the level where our work is focussed. Our experimental design uses predominantly wild-type rodents and avoids regulated procedures as far as possible; indeed, we have developed new assays here to replace their use even further. Where disease and genetic models are required, we focus on achieving the lowest severity possible, and our trial therapies are designed to relieve those symptoms. Finally, animal welfare is carefully and frequently monitored throughout experiments, with well-established scoring scales and humane end-points used throughout.

Project 14	**Repair in neurodegenerative disease and injury**	
Key Words (max. 5 words)	Central Nervous System, Nerve cells, Neurodegenerative diseases, Regeneration	
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)	Neurodegenerative disorders such as Alzheimer's disease (AD), motor neurone disease (MND), frontotemporal dementia (FTD) and progressive multiple sclerosis (MS) represent a major public health threat. In the UK alone over 1 million people suffer with an untreatable neurodegenerative disease. These are devastating progressive diseases that are uniformly without treatments and often fatal. The absence of any effective therapies reflects to a large extent our poor understanding of the underlying cause(s) of these diseases. In order to develop novel therapies – <u>the ultimate goal of our studies</u> – we must first improve our understanding of the "why" and "how" these diseases *start* and then *spread*. This knowledge will enable first development and then testing of targeted potential therapies which may well include novel stem cell based interventions.	
What are the potential benefits	The benefits from this project are several and include;	

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	advancing scientific knowledge about what might trigger these diseases; how these diseases spread; whether stem cell based therapies are effective in slowing or even stopping disease; evaluating whether novel drugs can slow, stop or reverse disease. Building on these findings we will then be ideally placed from our linked human clinical studies to accelerate clinical testing in human trials of promising therapeutic candidates. Collectively all new knowledge gained in these studies will be shared and disseminated with the scientific community, patient led charity groups and the public. This will be done through scientific presentations, published papers and public engagement events.
What species and approximate numbers of animals do you expect to use over what period of time?	Exclusively rodents, predominantly mice and some rats. Over the 5 year period we would approximately expect to use 10000-12000 rodents
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?	Due to the nature of neurodegenerative diseases we will be modelling including multiple sclerosis and dementias the animals will mimic the human conditions and adverse effects can include disabilities and behavioural changes similar to the human disease such as paralysis, bladder issues and memory problems. Therefore there will be adverse effects on some the animals in these models, however none of these adverse effects are any worse than observed in people suffering neurodegenerative. These effects are necessary in order to meaningfully study, model as well as crucially test potential new treatments of relevance to humans. It is important to note that some of these diseases have an average life expectancy of considerably less than 5 years (eg MND). Therefore the potential benefits of any advances/therapies to come out of our work are also hugely significant for people suffering from these incurable and fatal neurodegenerative diseases. These adverse effects will be limited by the use of careful endpoints during the studies and in some cases the animals are killed and tissues collected

	before any clinical signs are seen.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Due to this complexity of these neurodegenerative diseases it is impossible to accurately model them outside of a complex biological system, i.e. in animals. Only mammals have a complex nervous system and sufficiently developed immune-system to readily compare to humans. We are investigating the central nervous system and as of yet no-one fully understands how the CNS will react to a given situation. Furthermore the impact of other systems (blood and circulation) and situations (infections and stress) are also so complex and no-one fully understands how these complex systems interact. Therefore, with our present knowledge it is simply not possible to undertake these studies exclusively with computer simulation or even in vitro cell culture models. However, cell culture models can and are used including human stem cells and post-mortem material to inform animal experiments. Therefore we gain information from the human disease, test our theories in cells and investigate a potential therapy on these cells before progressing onto any animal models once we have information that shows us that there is a potential for a positive result.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will maintain our rodent colonies using as few animals as possible to keep them viable but not being wasteful. For example in transgenic colonies often wildtype mice, that do not carry any mutation/genetic insert are born and in several situations we can take these animals and use them to help maintain our wildtype breeding colonies and thus utilise as many animals and decrease wastefulness that way. For experiments we will use the lowest number of animals required to give meaningful, statistically relevant results. Also by utilising as much of the tissue as possible – for example separating the brain into the 2 hemispheres we can sometimes double the amount of material obtained for several different methods of analysis thus decreasing the numbers of animals required. We aim to maximise the use of

	animals as much as possible with multiple data capture from the same animals. Therefore in a single experiment we can capture behavioural, pathological, genomic (RNA) as well as protein data on the same animal. This will include collection and 'banking' of tissue from ex-breeder animals as well as collecting central nervous tissue from other animals that can be used to generate some of our disease models. Furthermore by having strong collaborations we can share tissue around the world and therefore decrease the number of animals generated and wasted.
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 aim to identify and modify disease progression in several animal models that mirror human neurodegenerative diseases including MS, AD, and MND. These will include the development and maintenance of genetically altered animals, where a gene that is shown to have a role in the human disease is modified in the animal to mimic disease and so allow us to investigate disease progression as well as treatments. We have significant experience with these models and have over time refined our endpoints and strategies to reduce adverse effects wherever possible – for example by discovering early loss of neurones in new areas of the brain that are not responsible for a known disability we can utilise animals before they develop signs of disability such as loss of hind limb function but still be able to use their brains to investigate neuronal loss. Other methods to create animal models that mimic the human diseases will include the use of compounds, drugs and/or proteins to create localised and known consequences of these diseases such as the loss of the protective myelin sheath around nerve cells (a known cause of MS), or the loss of specific populations of neurones (such as motor neurones in MND or cortical neurones in FTD). Also by utilising certain mice that show neuronal loss due to a protein that is seen to occur in many different neurodegenerative diseases (in our case tau, known to be involved in not only Alzheimer's but also motor neurone disease, frontotemporal dementia and even seen in MS) any benefits we observe could

	be transferred to a much larger group of diseases and therefore potentially impact many more people suffering from these diseases. We have many years of experience working with these models of neurodegeneration and thus by utilising our expertise we can increase the information and potential benefits we can obtain from an animal whilst in tandem decrease the impacts on individual animals and therefore minimise the welfare costs.

Project 15	**Role of D1R-D3R heteromers in L-DOPA-induced dyskinesias**	
Key Words (max. 5 words)	Parkinson’s disease, Dyskinesia, Dopamine, Heteromers, Glutamate receptors	
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
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	A part of the brain that is essential for all manifestations of Parkinson's disease is called striatum. It is controlled by inflow of impulses that release a substance called dopamine. Not all nerve cells in striatum are the same: there are two subtypes with contrasting properties, particularly how they react to dopamine. The main problem of Parkinson's disease is that the source of dopamine is lost. Nerve cells communicate and transmit information across structures called synapses. The sending nerve cell (presynaptic) relays the information by releasing chemical transmitters. The receiving cell (postsynaptic) detects that signal by specialized receptor proteins present on its body or fine extensions called dendrites. At the points of contact, dendrites have bud-like protrusions called dendritic spines that possess molecular machinery necessary to process the signal. Different types of receptors in striatal spines respond to dopamine. Normally they	

	stand apart, but can also aggregate into combined, higher-order structures called heteromers. Almost nothing is, however, known whether the dopamine receptors behave differently when they form heteromers, or about the consequences that heteromers may have in Parkinson's disease. This is important because it could tell us why patients' brains make wrong calculations and send wrong signals that result in unwanted movements. To answer all these questions, we will use mice with altered genes that allow us to tell between subtypes of nerve cells in striatum, even allowing us to see when heteromers are present in them. Using special methods, we will be able to track dopamine receptor heteromers and see if they come close to other receptors in spines. Then, we will record activity of striatal nerve cells. To achieve all this, we will combine a powerful confocal microscopy to see tiny details within nerve cells and top-notch electrophysiology to record synaptic currents in them.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Many people with Parkinson's disease do not respond well to therapy with a widely-used drug called L-DOPA, having uncontrollable body movements that make them feel ashamed or even fall and get injured. We and other doctors think this occurs because proteins called dopamine receptors associate in unusual structures called heteromers in nerve cells. However, no one knows how and where heteromers are formed, whether they affect other receptors and if it actually makes people feel bad. We are the first to show that there really are many more heteromers in brains of animals with experimental Parkinson's disease put on human therapy. Understanding what heteromers do will help us try to find the way to prevent them from overtaking control over striatum. This will help us devise a new strategy in fight against Parkinson's disease and its devastating consequences.
What species and approximate numbers of animals do you expect to use	Mouse, 240 animals of the final strain and 40 animals of each of the starting strains, i.e. 320 in total over 60 months.

over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals will be genetically modified, but this should cause no adverse effect by itself. They will undergo injections and behavioural testing before being killed humanely. Their tissues will be used after death for the imaging and electrophysiological studies. Anaesthesia and analgesia will be used as necessary and any animal experiencing an unexpected adverse effect will be treated as advised by the NVS or 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	Currently, there is no satisfactory alternative model for investigation of the mechanisms of synaptic changes in Parkinson's disease models that does not require the use of brain tissue acutely removed from animals. The project is intended to result in development of the new transgenic mouse strains. I will make extensive use of the transgenic mouse strains engineered to evaluate the role of dopamine receptors in striatal synaptic transmission. Therefore, this requires maintaining viable breeding colonies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Using the preliminary data, we have used validated the statistical procedures to calculate the minimal number of animals necessary to produce meaningful data, without compromising the scientific validity of the study. In addition, I will share the tissues with other groups to ensure that neuronal and non-neuronal tissue from the animals is used to the fullest extent possible.
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 chose mice as the species widely used in transgenic animal design, while also simultaneously validated as the species of choice by current scientific literature in procedures directed at evoking experimental Parkinsonism and abnormal involuntary movements (AIMs). Further, there is a wealth of correlative studies between mouse and human which indicate that the results gained by the animal use are translatable.

(harms) to the animals.	All of the procedures I propose a) are validated in current scientific literature b) will be performed according to the relevant legislature and c) will be performed by trained staff. Animal suffering will be minimised by the use of anaesthesia and analgesia and by implementing a clinical score chart that measures the well-being of the animals. Mice will be monitored on a daily basis and any animal that shows signs of adverse or unexpected responses, depending on the severity, either advice will be sought from the local NACWO and/or NVS or the mouse will be culled immediately to limit any additional discomfort.

Project 16	**Maintenance and regeneration of the nervous system**	
Key Words (max. 5 words)	avulsion, neurodegeneration,	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	There are no known treatment for loss of axons or restoring function neurons in the diseased/injured CNS. The aim of this project is to identify receptor pathways that are involved in these processes and hence small molecules that can stimulate them back to normality as a therapeutic.	
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 identify pathways that can lead to the development of drugs that can be used to treat spinal cord injuries and neurodegenerative diseases.	
What species and approximate numbers of animals do you expect to use over what period of time?	We will use approximately 300 mice and 700 rats. Rats and mice will be the only animals used in this project.	
In the context of what you propose to do to the animals,	The key procedures being applied in this project involve the induction of injury to either the central or	

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	peripheral nervous system or looking at neuronal survival in rodent models of neurodegeneration such as Alzheimer's disease. All surgical procedures will be carried out under general anaesthesia and animals will also receive postoperative medication to minimise any potential discomfort. This will often be followed by therapeutic intervention and various methods of assessing functional recovery, in particular the assessment of various functions whilst the animals perform behavioural tasks and the assessment of nervous tissue function by directly or indirectly recording the electrical activity of these tissues. Following injury, animals will show varying levels of impairment depending on the severity of the injury they have received. Impairment to forelimb sensory/motor function will be particularly obvious, all animals will display some form of paralysis at early post-injury stages. General well-being of the animals will recover quickly following injury (within the first week) and in the vast majority of cases this will be followed by dramatic functional improvements such that animals will be able to support body-weight on their affected limbs by 2 weeks post-injury. At the end of the studies, animals will undergo euthanasia and their tissues will be used for further analysis of treatment effects; making maximal use of tissue will reduce the number of animals.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Many of our studies involve the assessment of novel therapeutics with the aim of finding a therapy that could improve functional outcomes in patients with spinal cord or peripheral nerve injuries. Such therapies will clearly be required to show robust efficacy in relevant preclinical models before clinical testing could be considered. There is no alternative system that can model the integrated actions and the complex pathology of the injured nervous system. Therefore, it is necessary to undertake much of our work in animals.
2. Reduction	Animal numbers will be kept to a minimum by carefully

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

Project 17	**Determination of CNS efficacy and safety pharmacology**	
Key Words (max. 5 words)	Central nervous system, behaviour, safety	
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
	X	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)	Many compounds that interact with the central nervous system (brain/spinal cord) are known to produce profound effects even when the individual is exposed to very small doses, It is therefore important, where possible to produce drugs and other chemicals that are well tolerated and as free as possible from such side effects. Much of the work conducted under this Licence will be concerned with side effect profiling with the ultimate aim of minimising side effects. This Licence also allows for efficacy testing that will, for example, assess potential useful drugs affecting CNS disorders. Such conditions/disease states often affect large numbers of people eg. Epilepsy, Alzheimer' Disease, multiple sclerosis.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could	Governments require (and the public expects) that substances we are exposed to are safe or their hazards are well understood. It is an internationally mandated legal requirement. Regulatory approval is required to allow drugs to be tested in human or	

benefit from the project)?	veterinary trials, or for chemicals, agrochemicals to be marketed. Novel drugs may be developed with reduced or limited CNS side effects or a side effect profile that may be better tolerated than currently marketed products. Alternatively, novel drugs that produce beneficial effects via actions on the CNS system may also be assessed and developed as part of this licence.
What species and approximate numbers of animals do you expect to use over what period of time?	The species and anticipated usage over the lifetime of the Licence (5 years) are below: Rat: 13,500 Mouse: 7,000 Guinea pig: 1,500
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Early studies are performed on the basis of limited information and there may be uncertainty regarding the severity of the response. Most animals are expected to experience no more than mild transient effects such as weight loss or changes in demeanour. A small percentage of animals may show more significant adverse effects indicating moderate severity eg. a very small number of animals may potentially experience severe adverse effects were it not for humane end-points (early intervention or humane euthanasia) to prevent unnecessary suffering. Animals in surgical studies may experience some adverse post-operative effects similar to those experienced by human patients, however, supportive treatments are given to eliminate or minimise these and appropriate humane endpoints are again applied. All surgical procedures are performed under anaesthesia, with pain relief and/or antibiotic cover provided during and after, as appropriate. Most animals having procedures conducted under this Licence will undergo behavioural testing and historically, using this test, the vast majority of animals undergo nothing more than clinical signs within the mild severity categorisation. On study completion, some animals may be re-used in

	other studies, but most animals are humanely killed using an appropriate method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although non-animal (lab bench or computer based) studies can provide useful supporting data to limit and decrease the number animal studies, meaningful and reliable evaluation can only be comprehensively achieved in studies using intact animals where all the intricate systems in operation within the intact CNS can interact with each other. For this reason, in vitro and ex-vivo test systems in isolation remain inadequate alone. Use of in-vivo animal models remains a mandatory legal requirement; currently, for many of the study types in this project, there is no scientifically, ethically or legally acceptable non-animal alternative available.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A logical tiered/sequential approach is generally adopted. Information is reviewed to decide whether testing is appropriate and ethically acceptable and the studies in a program are designed to achieve the desired scientific endpoints with the least risk of pain, suffering, distress or lasting harm to the animals. The numbers of animals used are kept to the minimum commensurate with meeting study objectives and regulatory requirements and further input from statisticians used where appropriate, to ensure robustness and relevance of the scientific data produced. Where study designs allow, common controls may be used whereby a number of test substances under investigation may be tested and where comparison against a control is required, a single control may be used against which all the test substance groups may be compared thereby reducing the total number of animals required for testing.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	This project uses rats, mice and rarely guinea pigs. The animal models described in this Licence are considered to be the most refined as consideration has been given to the methods being the least invasive to

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	the animal whilst maximising the likelihood of generating quality scientific data that will answer the requirements of the piece of work being conducted. All animals are monitored for signs of any adverse effects on their health or wellbeing, and to prevent unnecessary suffering, early humane end-points are applied under appropriate veterinary guidance (e.g. modification/withdrawal of treatment with the test item, provision of palliative or therapeutic treatments, or humane killing of affected animals). Wherever possible, experimental samples are collected under anaesthesia or post mortem to minimise any potential suffering. In some circumstances safety markers will also be collected from the animals maximizing the data from individual studies. Maximising data decreases use of further animals and collecting samples post mortem or from terminally anaesthetised animals, minimises suffering. The use of biomarkers has proliferated greatly over recent years and continues to do so and therefore many studies that used to be conducted using disease models have been refined. In such cases, assessment of biomarkers in the blood of animals has replaced the need for the animal to experience the full condition/disease.

Project 18	Zebrafish and medaka as models for the study of psychiatric disease and age-related cognitive decline
Key Words	zebrafish, behaviour, addiction, genetic modification, ageing
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

Psychiatric or behavioural disease including addictions and age-related cognitive decline is a major concern for modern society with huge financial and social cost. This project aims to identify genes contributing to the etiology of psychiatric disease and cognitive decline and the processes affected. Further, the project explores the molecular basis of gene:environment interactions and tests the hypothesis that effects of environmental impact can be transmitted across generations by epigenetic mechanisms.

Specific objectives:

1: Identify genes influencing behaviours associated with psychiatric disease or age-related cognitive decline.

2: Examine the developmental and cellular mode of action of gene variants and environmental factors influencing behavioural phenotypes

3: Explore gene: environment interactions influencing etiology of disease phenotype

4: Test the hypothesis that effects of environmental impact can be transmitted across generations by epigenetic mechanism

5: Identify small molecules as potential treatments for psychiatric disease

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

This project will increase understanding of the aetiology of psychiatric disease and, in the long term, aid the development of novel and, potentially, personalised treatments.

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

The project uses zebrafish and Medaka (small Japanese fish). We expect to use up to 10000 animals over a period of 5 years

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

This is a behavioural analysis project. Adverse effects include mild stress as a result of single housing and behavioural assessments and mild toxicity as a result of drug exposure. Animals will be killed at the end of the procedures or, where animals have suffered no more than mild severity procedures and are not suffering or likely to suffer, they may be maintained for breeding purposes.

Application of the 3Rs

Replacement

It is not possible to study complex behavioural processes such as those associated with psychiatric disease and addiction with out the use of animals. Zebrafish and Medaka are non-mammalian vertebrates that have been shown to have a translationally relevant behavioural repertoire and conserved drug reward response to addictive drugs. Thus they are the vertebrates with the lowest neurophysiological sensitivity likely to yield results relevant to the human condition

Invertebrates such as Drosophila and C.elegans are other popular model systems for genetic analyses. However, despite being useful models for the analysis of the acute effects of drugs of abuse assays for psychiatric disease phenotypes and drug preference have yet to be established in these species.

Futhermore invertebrate brains do not show the same level of complexity as vertebrates and it is not clear whether the neuronal networks established to be involved in addiction and human behavioural disease are present in invertebrates. Thus findings from Drosophila and C. elegans are less likely to be relevant to human

biology than findings from studies on vertebrates such as zebrafish and Medaka where the conservation of neuronal networks has been established.

Reduction

Results from past work from our lab and elsewhere will be used to conduct statistical power tests to calculate the fewest number of animals required in order to achieve our objectives. Where animals have suffered no more than mild severity during the procedures and are not suffering or likely to suffer they will be maintained for breeding purposes.

To reduce the number of animals used we have established a collaboration with other institutes whereby they provide adults or embryos from their genetic modification programmes thus reducing the number of new mutagenized/ transgenic animals generated.

Refinement

It is not possible to study complex behavioural processes such as those associated with psychiatric disease and addiction with out the use of animals. The fish species used in this project (Zebrafish and Medaka) are non-mammalian vertebrates that have been shown to have a translationally relevant behavioural repertoire and conserved drug reward response to addictive drugs. Zebrafish and Medaka have been extensively used as a genetic model for the study of development and thus a large number of resources including transgenic lines and mutants are available. Large scale mutagenesis screens are more feasible in these species than in rat or mouse. Thus they are the vertebrates with the lowest neurophysiological sensitivity likely to yield results relevant to the human condition

We primarily use appetitive learning paradigms as our means of assessing behavioural phenotypes. Appetitive paradigms can be considered less severe that those using aversive learning. In fish adverse stimuli can be as mild as a knock on the side of the tank or sight of a predator as opposed to en electric shock that is commonly used in mammals.

We will only use electric shock as an adverse stimulus on rare occasions where the use of alternatives fails.

Mutant families shall only be kept if there is no sign of morphological abnormality and no sign of distress in the adults.

Within our facility we have 2 experienced fish technicians who regularly monitor the health of the fish and a fully trained animal welfare officer. They are on hand to remove animals from the project if they are suffering any pain or distress, or show any harmful abnormalities.

Project 19	Early life exposure to drugs and environmental complexity and neuropsychiatric disorder
Key Words	Genetics, Nature/nurture, Environmental complexity
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

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

Understanding the genetics of psychiatric disorder is essential if we are to produce individually tailored treatments for patients. Although we are making some progress with this, currently very little is known about the way that the genetic differences interact with environmental challenges during early development to produce psychiatric disorders. For example, this includes exposure to environmental challenges, such as impoverished or low-stimulus environment, but also drugs or chemicals, stress or other life challenges. The main objective of this project therefore is to test directly how differences in certain genes that are associated with individual differences in susceptibility to psychiatric disorder can be modified by environmental conditions; in particular, the complexity of the environment.

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 long-term benefits of this research are very clear: with a better understanding of the genetic basis of disease, we can tailor treatments to individuals thus increasing the efficacy. The short-term benefits that will result from this study, however, are: (i) characterisation of genes that can be modified by the environment to produce behavioural differences linked to psychiatric disease (ii) advancing our

knowledge of the mechanisms (cellular and neuronal) that mediate the links between genotype and environmental challenges (iii) advancing our knowledge of environmental conditions that affect behavioural responses in zebrafish will have the potential to increase welfare in laboratory-kept zebrafish in general.

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

This study will use the zebrafish as a model species. In total, over the 5-years of this project, we estimate that we will use 25,000 zebrafish.

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

The majority of the procedures that are planned to be used in this project are expected to be ‘mild’ severity. These will include breeding of genetically altered fish; exposure to environmental challenges; exposure to drugs, including ethanol, via immersion; behavioural test of anxiety; behavioural test of learning; injection of drugs. None of the above procedures are associated with a high likelihood of adverse effects (max 1%). All animals will be monitored closely throughout the procedures and safeguarding procedures are in place for every protocol to ensure severity remains mild. One procedure, manually removing eggs from female fish, may be of moderate severity in some animals owing to the physical trauma associated with the procedure. Again, safeguards are in place to ensure severity is minimised. At the end of the procedures, some animals will be retuned to breeding stock and some will be killed humanely.

Application of the 3Rs

Replacement

The use of animals for studying neuropsychiatric disorder is critical in order to understand further the complex interactions of genetics and environment. This would not be possible with humans as it would be impossible to gain sufficient information about environmental factors, and unethical to manipulate these variables. In addition, in order to examine gene x environment interactions, there are no current non-animal alternatives available.

Reduction

In order to minimize the numbers of animals used in thus project we have adopted two specific strategies

1) We are pre-selecting the fish we will use in our project based on their having mutations in genes associated with human neuropsychiatric disorder or from previous experiments with fish, some from previous PPLs.

2) We have calculated the minimum numbers of animals required for each separate experiment to ensure that we are able to detect any differences between our animals. This is crucial to avoid under-powered experiments.

Refinement

We believe that the zebrafish is a refined model for this work on account of it being the best system for carrying out forward genetic screens, as well as providing a balance of simplicity and complexity for a vertebrate system. There are a number of additional refinements that we are implementing in this project:

1. Using fish as opposed to mammals in this project will allow for a specific quantification of embryonic genetic effect, something that would not be possible with a mammal.
2. Many fish can be kept in a smaller area than mammals, thus allowing for enrichment to be used in a more effective way.
3. The fish will be housed according to new guidelines provided by ASRU on gold-standard zebrafish husbandry.

Project 20	Neurological disorders: mechanisms and therapies
Key Words	behaviour, cognition, brain, neurological disorder
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

Sufferers of neurological disorders frequently have disruptions in their genes. We are beginning to discover which genes these are, but we currently lack understanding of how these gene disruptions result in abnormal behaviours. The overall aim of the project is to understand how genetic disruptions linked to neurological disorders affect brain function and behaviour.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

The potential benefits of this project are two-told. (i) Scientific knowledge of how genetic disruptions linked to neurological disorders affect brain function and behaviour will be advanced. This will inform scientists who are interested in the molecular mechanisms of neurological disorders, as well as those interested in the normal function of the brain. (ii) Humans can benefit in the longer term by the insights provided by this work. Current treatments for neurological disorders are at best only partially effective or may produce serious side-effects in some patients. By finding out more about the causes of neurological disorders in animal models, this project will aid the development of better treatments.

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

The project will use mice, some of which will have genetic alterations relevant to human neurological disorders. It is predicted that no more than 3000 mice will be used within the protocols of this project over 5 years.

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

No procedure is expected to exceed a ‘Moderate’ severity rating. Behavioural testing will be performed as a battery (i.e. each animal will be subjected to several different experiments); the total number of experiments and the number of experiments with aversive stimuli that any one animal will receive will be limited. The administration of certain drugs to modify behaviour will be carefully monitored for any unexpected reactions; the choice of drug will be informed by previously published studies and checked with the vet. The surgery to implant mini-osmotic pumps is well established and should not cause any lasting pain or distress. However, to improve post-operative outcomes, appropriate anaesthesia and analgesia will be used in consultation with the vet. Careful post-operative monitoring will be made to ensure no suffering. At the end of the procedures, animals will be killed by either a Schedule 1 or a non-Schedule 1 method or, if appropriate, the animals will be used for further breeding.

Application of the 3Rs

Replacement

The project aims to understand how genetic abnormalities related to human neurological disorders alter brain structure and function. In particular, we will focus on behavioural outcomes. It is not possible to perform these experiments in humans; access to human brain tissue is very limited and there are strict limitations upon invasive approaches in clinical studies with human subjects. Behaviour can only be studied in living organisms, so experiments using neural cells derived from neurological patients would not be suitable because they do not exhibit behaviours. Computer models are not appropriate because there is insufficient information available on how the brain functions.

Reduction

We will ensure reduction by writing a protocol for each experiment, which will include statistically designed group sizes (by power calculations) and by searching the literature to ensure that experiments are not unnecessarily duplicated. Breeding protocols will be designed to ensure that only the required number of animals is bred, to minimise wastage.

To avoid the necessity of breeding new cohorts for each behavioural test, each cohort will be subjected to a battery of tests, rather than a single test.

To avoid the necessity of maintaining animals solely for conventional breeding, animals that have been subject to non-invasive behavioural tests may be maintained for conventional breeding.

Refinement

We will be using mice, which have a similar genetic makeup and neuroanatomy to humans. There are many behavioural tests that allow us to assess neurological disorder-related behaviours in rodents, something that cannot be done in lower model systems.

We are undertaking a series of experiments that have been shown by others not to cause suffering. Before all experiments, the rodents will be handled to reduce the stress of human interactions. Behavioural experiments in general do not cause pain and suffering, but suitable time intervals between experiments will be given, and there will be a limit on the use of aversive stimuli given to any one animal. For surgical procedures, suitable anaesthesia and analgesia will be administered in consultation with the vet; any sign of suffering will be discussed with the vet for immediate advice.

Project 21	Analgesic potential of Retargeted SNARE Proteases
Key Words	Analgesia, Pain, Neuropathic, Post-operative, Inflammatory
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

My research is devoted to the neurobiology of pain. Around 12% of adults suffer from severe, disabling chronic pain which occurs due to nerve damage, cancer chemotherapy or inflammation. At present, the use of drugs and/or injections to treat chronic pain is based on imperfect evidence, is rarely curative and often limited by intolerable side 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 project)?

A key feature of my approach is the use of retargeted proteases called RSPs to selectively silence specific types of neurons for prolonged periods of time. My laboratory recently developed RSPs which have a selective action. Specifically, several of my products target central neurons but not neuromuscular junctions. This feature makes RSPs more attractive in treating various chronic neuronal disorders since neuronal silencing can be achieved without muscle paralysis. A naturally occurring neurotoxin which uses such protease has recently been approved for treatment of migraine. However, the fear of muscle paralysis restricts the use of

native neurotoxin for pain management. I plan to evaluate safe RSP products in various pain models with a view to take them to clinical practice as soon as possible.

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

I plan to use approximately 1800 rats and 900 mice over the course of the 5 year project.

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

Clearly, conducting research into pain necessitates that the animals should experience a degree of temporary and measurable pain, within mild to moderate severity limits. If animals will show signs of distress (persistent hunching, abnormal breathing pattern, weight loss) they will be humanely killed. In the very rare cases where animals will react badly to new molecules they will be also humanely killed. The majority of animals will complete the planned study and following culling their tissues will be analysed to better understand mechanisms of RSP-induced analgesia.

Application of the 3Rs

Replacement

Chronic pain is a dynamic process that requires an intact nervous system and results in behavioural changes that can only be measured in sufficient and reproducible detail in an animal.

Reduction

We use cell cultures to test our new RSPs and only promising molecules are then tested in animals for their analgesic properties. Each experiment involving animals is designed to involve the minimal number required which satisfies statistical requirement.

Refinement

Testing of the analgesic potential of RSPs on rats was chosen because they represent well-established models in analgesic research. Mice, on the other hand, offer the possibility of gaining scientific insights into pain mechanisms through using genetically-altered animals. All three models of pain will be generated as described in literature in separate animals which will then be treated by injections of chosen RSPs in a single limb. The rat is the model of choice for pain research based on publications due to their placid nature and relatively large size of their paws. Given the importance of examining the mechanisms of pain transmission, the use of mice will allow us to study relevant transgenic strains in the future.

Our models of pain have been extensively studied and validated, and severity limits have been clearly established (mild to moderate). The models are:

1. Post-operative pain (paw incision);

2. Inflammatory pain (paw inflammation);

3. Neuropathic pain (spared nerve injury, SNI, and chemotherapy-induced neuropathic pain, CIPN).

These models have been chosen in collaboration with specialists in pain management and research to solve important clinical problems that are relevant to patients with unmet needs. Inflammatory, neuropathic and post-operative pain conditions affect large numbers of humans and there are no long-acting drugs to alleviate chronic pain.

The experimental design will follow contemporary best practice guidelines for the welfare of experimental rodents. For example, the administration of substances will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm.

Project 22	CNS neurotransmitter action in health and disease.
Key Words	Brain, Neurodevelopmental Disorders, Neurodegeneration, Neuron, Glia
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

Our overarching objective is to determine how chemical and electrical signalling in the brain is altered in diseases or disorders which affect [either its development or cause its degeneration. To do this we will use mice and rats which have been genetically modified to model such diseases. We think that the best way to understand how these diseases lead to changes in brain signalling is to take a multidisciplinary approach and analyse gene expression, record electrical signals from different types of brain cells, conduct biochemistry and perform high resolution imaging to determine how dysfunctional signalling manifests itself in the disease state. Our experiments are aimed and have the ability to detect very small changes in gene expression, electrical signals, protein expression and changes in cell morphologies which will allow us to test hypotheses about the extent to which these changes are causal to disease and which, if reversed, could lead to amelioration of the dysfunction present. We need to compare these properties in cells from normal animals and animals which have been genetically modified in order to mimic the dysfunction that occurs in human disease. We think that although the actual genetic errors which cause such diseases are diverse that different diseases result in shared changes in brain signalling. If this is the case, then it might be possible that therapeutic interventions which ameliorate or reverse dysfunction in one disease may be effective in another.

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 seeks to increase our fundamental understanding of electrical and chemical signalling in the brain. To achieve this, we need to use animal tissue to understand how signalling works normally and how in rodent models of autism spectrum disorder and intellectual disability this is altered. We will analyse the extent to which expression of genes which encode key proteins needed for electrical and chemical signalling are altered in our disease models. A potential benefit of our work is that we will identify novel targets for drug action and which ultimately could lead to the development of new therapies to treat brain dysfunction in humans. For example, if we identify a protein being involved in changes in signalling we can manipulate its activity the gene which encodes the protein to determine the extent to which this reproduces the disease phenotype. Since it is estimated that around 30% of the population will experience some form of “mental illness” in their lifetime it is critical that such targets are identified in order that interventions can be tested to see whether they ameliorate disease/dysfunction. Moreover our experiments are designed to determine whether genetically diverse diseases result in similar changes in brain signalling. This has the potential benefit that, in the future, similar therapies may be used to treat different diseases.

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

It is estimated that approximately 12,700 mice and 17.250 rats will be required over the 5 years of this licence to complete the research aims 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 levels of severity? What will happen to the animals at the end?

In order to carry out our work we need to use animals (mice and rats) which have been genetically modified (GM) so that they model what happens in humans with the diseases we are interested in studying. Most of the work will require us to take brain tissue from GM animals; animals will be killed humanely to allow us to collect tissue for experiments. In other experiments we will administer substances either to disrupt signalling pathways or to test whether the substance has the ability to correct the dysfunctional signalling that is present in our disease models. Substances will be administered in concentrations that have been used by other researchers and which have been shown not to cause adverse side-effects. For substances which are new or have not been tested before we will start by using low concentrations and then increase doses to determine the most optimal amount to administer. For some experiments we need to carry out manipulations to alter the level of brain activity prior to collection brain tissue; this can be done by rearing animals in the dark or by inducing terminal general anaesthesia. Each of these manipulations is not considered to have adverse side-effects. Finally, in a small subset of experiments we

will expose animals to high decibel sound. This reveals an increase in the susceptibility to seizures caused by loud noises and which can be present in humans with certain types of neurodevelopmental disorders. This procedure is classified as having a moderate level of severity but our protocol only allows us to do this once and all animals will be humanely killed following the procedure.

Application of the 3Rs

Replacement

Complementary research in our lab uses expression of receptors, ion channels and transporters in cell culture systems . Such studies are extremely valuable in assessing pharmacological and structure-function properties of these proteins. Nevertheless such systems do not and cannot replicate the properties of the specialized points of communication (termed “synapses”) found in brains. Humans share >97% genetic identity with mice and rats and as such these rodents are models with which we can carry out experiments to understand how the brain works. Non-animal alternatives are extremely useful to getting us “part of the way” but ultimately we need to study the mammalian brain and mice and rats are the lowest order mammal in which this can be done reproducibly while having the ability to perform genetic manipulations that allow us to create structural validity of disease.

Reduction

It is essential that the number of animals we use allows us to collect data that are robust. We have many years’ of experience in performing experiments using animal tissue and this allows us to make predictions, using Power calculations and previous data about the anticipated effect size we are likely to see and how variable that effect will be. Using this information we can estimate the numbers of experiments we need to carry out. Our experience indicates that the effects sizes we anticipate require between 8 – 10 animals to ensure statistical robustness. Good experimental design means that we assess drug effects alongside non-drug treatments or control tissue with that obtained from genetically modified animals. We encourage, where possible, that experimenters share tissue from the same animal which will reduce the overall number of animals required for experimentation. We seek advice from statisticians where appropriate when planning experimental designs.

Refinement

Mice, together with rats, represent the most commonly used mammal to study central nervous system function. While genetically-modified (GM) mice have been models of choice for more than two decades, recent advances in transgenic technologies, together with the larger brain size of the rat and the increased behavioural repertoire they display means that is it now possible to conduct studies of brain signalling and its dysfunction. Such studies allow for cross-species comparisons which is essential when one considers the potential for translating

findings from ‘basic’ research to potential therapeutics. The majority of the GM mice and rats we study display few overt phenotypes The majority of the GM mice and rats we study display few visible signs in terms of alterations in their physical features, development, behaviour or their ability to thrive. Indeed it is only with sort of detailed experiments we propose to carry out that deficits in brain signalling are revealed. Some of the pre-clinical models we use are classified as have a “moderate” phenotype as the mutation has the potential to cause harm if the animal was to live for a long time. However, we closely monitor our GM animals and if they show signs of distress they are killed humanely. For our experiments we use animals before these phenotypes develop. The animals in these studies will be cared for by trained staff within a well-resourced and well-equipped modern animal facility that contains individually ventilated cages (IVCs) and barrier systems to maintain specific pathogen-free (SPF) status/health.

Project 23	Improving the treatment of Parkinson's disease and Parkinson's disease dementia
Key Words	Parkinson's disease, Dementia, Better treatments, Cell protection, Cell repair
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

Parkinson's disease is an age-related neurodegenerative disorder that affects around 1% of people over 60. In the UK, some 127,000 people are living with Parkinson's. The impaired movement in Parkinson's results from loss of brain cells that produce the chemical dopamine which is essential for proper control of movements. Current treatments for Parkinson's are far from ideal. Firstly, they do not address the progressive cell loss so symptoms gradually worsen over time. Secondly, the need for higher doses of medication leads to disabling side effects of excessive, uncontrolled involuntary movements. Finally, it is increasingly apparent that people with Parkinson's do not only have motor problems; they also have other 'non-motor' symptoms like depression, anxiety, pain and dementia that lessen their quality of life. Sadly, we still do not know how to tackle these symptoms effectively as the reasons behind their emergence during the course of the disease are not fully understood.

In light of this current situation, there are some clear unmet clinical needs for improving the life of people with Parkinson's

1). To find drugs that can slow down, halt or repair the damage to cells in the brain of people with Parkinson's.

2). To find ways to provide relief of motor symptoms while not evoking disabling side-effects or to find ways to reduce the frequency or severity of these.

3). To understand more about, and find ways to better address the non-motor signs associated with Parkinson's.

The work covered in this licence will tackle aspects related to all three of these unmet clinical needs. Specifically, we will look for new ways to reduce cell loss in the brain, or repair the damage already caused. We hope to find ways to avoid the side-effects of the current best drugs. We will also learn more about the non-motor aspects of Parkinson's and related dementia so that we are better placed to treat all aspects of this 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 has the potential to find new treatment strategies that will improve the quality of life of people living with Parkinson's disease. This could be through better control of their wide-ranging symptoms or through provision of a medicine that can slow down progress of their disease, or better still, halt it in its tracks and repair the damage already caused.

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

We will use around 2,000 adult rats or mice for this 5-year project.

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

Other than rare (<5%) post-surgical complications such as stitches coming undone, adverse events are not expected from these studies. Refinements will nevertheless be made where better techniques or procedures come to light. All the studies fall within the mild to moderate level of severity. At the end of the studies, the majority of animals will be killed and their brains analysed to provide a wealth of valuable information about the mechanisms underlying the different symptoms and cell loss in these disease models and the actions the potential treatments have had to resolve these.

Application of the 3Rs

Replacement

Around a third of our studies do occur without animal studies, using computer database work, cell culture and brain slice preparations. Through such studies we are able to pre-select the most promising strategies to proceed through to the animal models. There are some invertebrate (fly and worm) models of Parkinson's disease available to researchers. These are helpful when learning more about the genetics of

the disease. However, they are not suitable for our purposes as while they show some moderate cell loss, their symptom profile is very weak. Furthermore, only a small proportion of people with Parkinson's (<10%) have a genetic basis to their disease so modelling the genetic cases in these lower organisms does not reflect the situation in humans.

Reduction

We will apply statistical design at the outset to ensure only the minimum numbers required are used. Where possible, crossover designs will be used so that multiple treatments may be examined in the one group of animals.

Refinement

The complexity of the human brain and neural connections comprising the systems that control the motor and non-motor symptoms mean a realistic picture of problems associated with the development and treatment of Parkinson's and related dementia can only be obtained in whole organisms. While the MPTP-treated primate is the most clinically-relevant model of all, its use is not justified, or necessary in early pre-clinical studies of the kind we plan to undertake. In this case, rats and mice are the most appropriate species. They display the appropriate brain chemistry, clinical signs and inducible Parkinson's like pathology. The rodent models we use are mostly constructed using chemicals that mirror the events happening in the brain in Parkinson's (events like oxidative stress and inflammation). As such, they represent a much more valid model of the disease than the invertebrate genetic models to address our purpose.

Appropriate use of analgesics and anaesthetics plus consultation with the veterinary surgeon at the first sign of any unexpected event will ensure our animals are exposed to the minimal suffering possible.

Project 24	**Neuropharmacology and therapeutics in addiction and other dopamine-associated disorders**	
Key Words (max. 5 words)	Addiction, Parkinson's disease, treatments, neurogenesis,	
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)	It is often mistakenly assumed that drug abusers lack moral principles or willpower and that they could stop using drugs simply by choosing to change their behaviour. This is not so. Through research into the biological and psychological causes of addiction, we know more about how drugs affect the brain than ever before, and we have learned that drug addiction can be successfully treated to help people stop abusing drugs and live better lives. Psychosocial and behavioural treatments are important for promoting recovery and prevent recidivism, but the success of these programs has been limited so far. This is where neuroscience and experimental research can make a difference. First, it is important to understand the biological causes of addiction and how drugs change the brain. Drugs produce persistent molecular and biochemical alterations in brain function, as well as changes in	

	how the neurocircuits are connected functionally. One of the objectives of this project is to learn more about such modifications. This knowledge is important. It is the first step in the attempt to develop new forms of treatment. Second, understanding the psychological factors and behaviour that predispose to addiction is also critical, especially from the prevention perspective. This project will also focus on these important aspects. Third, there are no specific medications to help drug users detoxify and recover more quickly from chronic drug abuse. A number of behavioural, metabolic and pharmacological interventions hold promise as future treatments for this condition, however their effectiveness has not been fully evaluated and their mechanism of actions are only partially known. Here, we propose to investigate new treatments that are mainly aimed to “normalize” dopamine transmission and increase brain neurogenesis. Fourth, dopamine transmission is also the key player in other disorders of the nervous system and some of the new medications we are planning to evaluate in addiction model could be highly effective in the treatment of other related conditions, especially the compounds targeting TAAR1. For example, treatments for Parkinson’s disease have limited efficacy and the search is under way for new pharmacotherapies. In this project we will aim to provide the first evidence of the potential benefits of TAAR1-based pharmacotherapies in an animal model of Parkinson’s disease.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The proposed project will advance our scientific knowledge and will pave the way for the development of real world treatments. This project will bring benefits to humans by: 1. Expanding our knowledge of the neurobiological and psychological causes of addiction and exploring ways in which addiction can be prevented and/or recovery facilitated. This project will shed light into the brain circuits that are altered by drug exposure and into the psychological and behavioural tendencies that are

	more closely associated with compulsive drug taking and addiction. In parallel, we aim to gather evidence that through the use of non-pharmacological interventions, such as physical exercise and diet, it may be possible to build up resistance against addiction and facilitate recovery from drug exposure. 2. Identifying new targets for medicinal development in addiction and their mechanism of action. Addiction is a difficult disorder to treat, a problem that stems from our lack of understanding of how drugs actually change the brain in the long-term. There are no specific medications to help drug users detoxify and recover more quickly from chronic drug abuse. We will use new pharmacological tools that may become future treatments in addiction and related disorders, exploring both their therapeutic efficacy in well validated models and their underlying mechanisms of action. These experiments are based on strong evidence we have recently published 3. Evaluating the potential of new treatments for Parkinson's disease. Some of the anti-addiction compounds that we propose to evaluate have clear potential to serve as medications in Parkinson's disease. Treatments for Parkinson's disease have limited efficacy and are associated with long-term complications. Thus the search is under way for new pharmacotherapies. Using an animal model of Parkinson's disease, this project will advance our knowledge in this area by conducting the first ever experiments with compounds targeting a recently discovered receptor.
What species and approximate numbers of animals do you expect to use over what period of time?	Rodents are the choice of species for this programme of work. We propose to use approximately 500 rats over the course of the project under a number of protocols.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	All procedures performed on the animals such as behavioural tests and administration of drugs, lesions and other non-pharmacological interventions shall not exceed the moderate severity level. Incidences of

level of severity? What will happen to the animals at the end?	adverse effects during surgical procedures or post-surgical procedure are very low (<1%). In addition, incidences of long-term adverse effects of the doses of drug/compound solutions used have a low incidence of adverse effects (<1%). However, the effects of drug withdrawal, although not immediately apparent in some cases, may be quite profound in some cases. Moreover, some of the treatments do lead to permanent motoric impairments, e.g. when neurotoxic substances causing irreversible motor effects are administered (model of Parkinson disease). At the end of experiments, animals will *be humanely killed by an appropriate regulated procedure.*
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Rodents are neurophysiologically less complex than other so-called “higher” species like primates. The kind of research question that we are trying to address, which mainly involves understanding addiction from the behavioural and biological perspectives, cannot be answered through the use of non-sentient systems such as cell culture or computational modelling.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will ensure that we use statistical design and analysis of data, including the use of power analysis to determine adequate sample sizes. The use of within-subject design protocols will enable us to minimise the number of animals used, as will using the animals in different protocols whenever possible. *In all cases when animals are exposed to several protocols, we will ensure that severity remains within moderate limits.*
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	The procedures being used have been refined over many years in rodents. We know a lot about rodent physiology and neuroanatomy and also the behaviour in response to drug treatments. Therefore there is a wealth of literature that we can draw upon in order to conduct these experiments, Careful monitoring of animals during all protocols by suitably trained and competent staff will also minimise any adverse effects

(harms) to the animals.	and welfare costs to the animals. We are committed to refining all protocols used in this project. Examples of measures will include the following: *(i) the drug / compound solution chosen for investigation will be drawn from previously published work and relevant literature and we will use these to inform us about the dose and frequency of administration; (ii) the dose and frequency selected will be those that result in minimal adverse effects.*