Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 12

Projects with a primary purpose of: Basic Research – Sensory Organs

Project Titles and keywords

1. **Signal detection in auditory cortex of the mouse**
    - Cancer, Genetics, Epigenetics

2. **Neuronal communication in fish**
    - Zebrafish, vision, olfaction, neuron, synapse

3. **Genetics and etiology of eye disease**
    - Blindness, cornea, lens, stem cells

4. **Cell to cell signalling in skin disorders and wound healing**
    - Skin inflammation, connexin, wound healing, epidermal dysplasia

5. **The circuit mechanisms of auditory processing**
    - hearing loss, ageing, tinnitus, auditory system

6. **Mapping the Functional Architecture of Vision**
    - Zebrafish, Topography, Retina, Vision, Mapping

7. **Studying the retinal response to depression**
    - retinal response to depression

8. **The retinal pigment epithelium in health and disease**
    - Retina, Diabetes, Age-related macular degeneration

9. **Understanding vision, developing therapies**
    - Retina, retinitis pigmentosa, sight, melanopsin, vision

10. **Strategies for the restoration of sight in retinal dystrophies.**
    - Retinal degeneration, stem cells, saffron, subretinal transplantation, intravitreal transplantation

11. **The neural basis of sound perception**
    - Hearing, sensory cortex, auditory cortex, multisensory

12. **Exploring the role of inflammation in common eye diseases**
    - Eye, blindness, inflammation

13. **Genes and environments that influence behaviour**
    - Genetics, behaviour, smell, intellectual disability

14. **Development of novel therapy strategies for skin diseases**

- Skin diseases, therapy, genetic disease

15. Developing gene therapy for glaucoma

- Glaucoma, gene therapy, IOP

16. Brain mechanisms of listening and learning

- auditory; hearing; mice; neurons; perception

17. Inflammatory Diseases of the Brain and Eye

- Multiple sclerosis; Uveitis, Inflammation, Vasculature

18. Lateral line and hair cell function in zebrafish

- Hair cells, zebrafish, Development, Neurons, Regeneration

19. Regulation of mesendoderm formation in vertebrates

- Gene regulation, zebrafish

Project 1	**Signal detection in auditory cortex of the mouse**		
Key Words (max. 5 words)	Brain, hearing, perception, deafness		
Expected duration of the project (yrs)	5		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X		Basic research
			Translational and applied research
			Regulatory use and routine production
			Protection of the natural environment in the interests of the health or welfare of humans or animals
			Preservation of species
			Higher education or training
			Forensic enquiries
	X		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of this project is to determine the mechanisms by which the brain detects and discriminates environmental sounds, and to establish how these mechanisms are disrupted in common forms of hearing loss so that we can design effective treatments and prevent early hearing loss in humans.		
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The brain plays a crucial role in the conscious perception of sounds and it is therefore vital to understand the mechanisms by which sound is processed by the brain which are currently poorly understood. We aim to provide doctors, engineers and biomedical workers with valuable information to help them with the development of therapies for hearing loss.		
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mostly mice (several thousand) and some rats (several hundred) over the 5 year course of the licence		
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the	We do not expect the majority of our animals to undergo anything beyond mild suffering. Animals will usually undergo a surgical procedure (always under general anaesthesia) and will be closely monitored and provided with pain relief before and after surgery. Animals will have restricted access to water, and may		

end?	experien ce thirst. In 5-10% of animals, hearing impairment and tinnitus will be induced. Thereafter, animals will learn to perform simple tasks for reward, that are mentally stimulating. Rarely, in less than 5% of cases, complications can occur including weight loss and failure to recover from anaesthesia. However, animals will be constantly and closely supervised by trained individuals. If any animal is deemed to be suffering, they will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Brain research cannot be carried out without recording from real brains, that are involved in performing real behaviours. Animal use is unavoidable because brain slices lack the real sensory inputs from the outside world and connections from other parts of the brain.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use computer modelling to analyse our data and make precise predictions. We use principles of experimental design and analysis to get meaningful data from minimal numbers of animals. We also record from large numbers of brain cells at the same time – reducing the overall number of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the best animal model because their brains are very similar to human brains, they can be easily trained to carry out recordable tasks, and we can use genetic technologies to mimic human diseases. Animals are constantly and closely supervised by trained individuals and advice sought from the veterinary team if there is any cause for concern. If any animal is deemed to be suffering, it will be humanely killed. Overall, we use anaesthesia, analgesia, and humane endpoints to limit suffering.

Project 2	**Neuronal communication in fish**	
Key Words (max. 5 words)	Zebrafish, vision, olfaction, neuron, synapse	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our aim is to understand how nerve cells transmit information at their specialized connections called synapses. The specific context in which we study this question is when the fish uses its senses, and especially the senses of vision and smell. In order to understand how synapses in the retina and brain of zebrafish transmit and process sensory information it will be necessary to visualize patterns of neural and synaptic activity as the fish responds to visual or chemical stimuli. Our general project plan is to make genetically altered zebrafish expressing fluorescent proteins that emit light when the synaptic connections are activated. This will allow us to investigate the function of synapses between different types of neuron in the retina as they respond to defined visual stimuli. We will also investigate the activity of neurons that provide the final output from the retina to the higher visual centres in the brain.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We are pursuing this project because relatively little is known about the mechanisms by which neurons transmit sensory information across synapses. The mechanisms controlling neurotransmitter release at the synapse play a key role in determining what we can and cannot see or smell and we need to	

	understand these processes as they operate in the intact retinal circuit. This project will advance science in several ways. First, we will improve our understanding of the processes which transfer signals between nerve cells. Defects in these processes have been associated with important diseases, such as Alzheimer's and Parkinson's. Second, we will improve our understanding of the way visual and olfactory signals are processed in the brain.
What species and approximate numbers of animals do you expect to use over what period of time?	Zebrafish (6,000 over 5 years)
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Procedures to be applied involve breeding and genetic manipulation of zebrafish (including the use of viruses and fin biopsy), as well as imaging *in vivo* imaging. This second procedure involves anaesthetizing the larval fish and immobilizing it in a gel. In some cases, we will also need to inject a protein that leads to paralysis and stops movements of the eye and/or body. The fish is then placed in a chamber under a microscope that uses a laser to image the fluorescence emitted by proteins that respond to electrical activity in neurons. No adverse effects have been observed so far. The most severe adverse effects that might arise are visually defective or blind fish, which will be killed by a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We must use animals for this project because we need to maintain the whole the retinal and brain intact and responsive to light and smells. It is not possible to carry out this work using cultured neurons that have been removed from the brain because these become disconnected from other neurons. But we will limit the use of animals by testing all the fluorescent proteins that we use in cultured neurons to make sure they will yield useful results when used in fish in vivo.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use the minimum number of animals for our experiments. Animals used for imaging experiments will be killed by Schedule 1 procedures and, where possible, the retina of these animals removed for use in other experiments.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We choose to use zebrafish because this has become a very widely used experimental animal across the world, providing a great deal of shared expertise on the husbandry and experimental manipulation. The retina of zebrafish functions very similarly to that in mammals, providing an excellent alternative in which to study many aspects of nervous system function. We minimize welfare costs in a number of ways: environmental enrichment in all tanks by addition of plant-like features; use of anaesthetics wherever possible; when collecting tissue of one type from adults, care is taken to also collect other types of tissue from the same animal (eg. eyes and brain) thus minimising the number of adults used; stress in animals used for *in vivo* experiments is monitored by assessing changes in heart rate and terminating the experiment if it rises too high or falls too low; at the end of in vivo imaging animals are killed by killed by an appropriate method of euthanasia and the retina used for other experiments whenever possible.

Project 3	**Genetics and etiology of eye disease.**	
Key Words (max. 5 words)	Blindness, cornea, lens, stem cells	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The purpose of the project is to understand and develop therapies for degenerative diseases of the eye. The primary focus is the transparent structures at the anterior of the eye – the lens and cornea – which together focus light onto the retina. Lens opacity (cataract) is one of the commonest causes of poor sight in the world. Cataract operations are commonplace but a significant proportion (over 50%) of patients exhibit some form of secondary opacification as the remaining lens epithelium overgrows to fill the gap left by the removed crystalline lens. The genes responsible for this growth will be investigated in this project, together with strategies for preventing overgrowth. Opacity of the front surface of the eye, the cornea, is a major cause of blindness worldwide, following infection, corneal transplant, laser eye surgery, injury or genetic disease. Significant questions remain unanswered about the response of the cornea to injury, including the role and location of stem cells the pattern of cell migration across the cornea, and whether these patterns of migration are permanently disrupted by corneal damage. This project will address	

	these unknowns.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	In cases where, for example, people suffer long term loss of sight due to corneal damage not being repaired after laser eye surgery, corneal transplant, injury or infectious disease such as trachoma current clinical strategies for therapy are either exhausted or off only a temporary amelioration. This is because the questions of stem cell location and activity, and the response of the cornea to damage have not been fully understood. Resolving these questions will offer new routes to therapy, and this project will investigate the role of some potential therapeutics on the cornea. Secondary opacification of the lens after cataract operation may lead to the need for a second operation, and is most likely to affect children. Better knowledge of the genes that control lens cell overgrowth after cataract operation, and the drugs that could be used to prevent it, will avoid subsequent operations. Furthermore, the scientific questions being addressed are central to basic research into cell growth, tissue regeneration, and stem cell activity in mammalian growth and development, which will be applicable to other tissues and organs.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will use mice. It is estimated that about 4000 mice will be used over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the 4000 mice will be used only in breeding colonies and will be humanely killed for tissue analysis and cell culture. Mice will be carrying genetic alterations however it is expected that these will have no or only mild effect on the animal. Mice may receive cell labelling substance or DNA, but this is expected to have no adverse effect. Mice may undergo surgery - corneal transplant or lens removal - that recapitulates similar operations performed in patients to correct defects in vision. These operations are carried out under general anaesthesia and cause little or no pain to mice. Mice recover within 2 hours. In order to study this more closely mice may be imaged using MRI or PET techniques but this will be under general anaesthetic. Mice will be killed humanely and their tissues analysed

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals need to be used as a source of tissue of the correct genetic status for in vitro experiment. There are no cell lines that fully recapitulate the behaviour and three dimensional arrangement of ocular cells in vivo. Long term studies of lens or corneal regeneration after injury cannot be performed in vitro because we want to follow up the mice for 6 months or more, whereas culture systems for these tissues keep them intact for a few days at best.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We reduce animal numbers by 1) Modelling cell behaviour in silico to reduce the number of animal experiments. 2) Performing trial and pilot investigations to assay the level of effect produced by genetic or pharmacological manipulations and then using those pilot data to accurately estimate the minimum numbers of animals required to achieve statistical significance. 3) Using cell lines and in vitro techniques where possible. 4) Careful experimental design to maximise the amount of scientific information from each animal.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are used because: 1) As a mammalian species their physiology and anatomy is comparable to human. 2) They are the only species for which the required genetically altered lines are available. Refinement occurs because most experiments are performed on tissue taken from mice humanely killed with no additional intervention. Surgical procedures are only performed by or under direct supervision of experienced human ophthalmic surgeons on animals when there is no alternative to address the important questions designed above and when there is a clear scientific advance to be made. Surgical and analytical techniques used will be the ones that are known from prior experience to cause little or no pain or distress to the animals, and all animals are monitored daily for health status. The named Veterinary Surgeon will advise re anaesthesia and any required analgesic regime. Any animal deviating unexpectedly from normal health status that cannot be effectively treated will be humanely killed.

Project 4	**Cell to cell signalling in skin disorders and wound healing**	
Key Words (max. 5 words)	Skin inflammation, connexin, wound healing, epidermal dysplasia	
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)	Maintenance and renewal of healthy skin is critical for survival. The overall aim of the project is to gain a deeper understanding of the role of specific proteins that enable cells to communicate and co-ordinate their activities to maintain healthy skin. During diabetic wound healing, a range of Genodermatological disorders and inflammatory skin conditions including psoriasis, the function and expression of these proteins are altered and they emerge as prime therapeutic targets. Transgenic mice that harbour mutations in these proteins give excellent models of the human disease states. Using skin cells isolated from mice, in vitro organotypic skin models representing intact skin have been used to probe the role of the faulty protein in the renewal of the skin. Such models use small numbers of animals to perform extensive in vitro experiments. In parallel human living skin equivalent models are often used. Together these models have helped identify a range of highly specific reagents that target these cell to cell communication proteins, reduce inflammation and improve wound closure events. Biomathematical modelling approaches also help to map modes of cellular responses. Maintaining breeding colonies of genetically altered mice is	

	therefore critical. To ultimately understand the role of these proteins in inflammatory skin conditions and assess the therapeutic potential of specific reagents it is now necessary to use a limited number of animals in in vivo studies to represent skin inflammation or wounding. Experiments will be optimally designed and tissue end products and cellular explants will be used in a wide range of biochemical, genetical and cell biology assays. Although animal models are required the majority of work uses skin cells isolated from the animal, thus from relatively few animals we can obtain enough material for extensive analysis. Human biopsy material is also used when available, however, this is difficult to obtain from aged diabetic patients or those harbouring distressful hyperkeratoic conditions.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The outcome of this work will be published in peer review, high impacting research journals to disseminate knowledge. Successful outcome may result in moving forward to clinical trials to improve wound healing events and resolve inflammation that would benefit a wide range of patients. Further, understanding the molecular mechanisms underlying skin disorders where these proteins are de-regulated will benefit the patients and may lead to novel theraoies and clinical intervention.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice Up to 1000 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?	We propose the following procedures on mice: I. Breeding and maintenance of genetically altered mice (mild procedure) ii. Tape stripping of the skin — similar to application of an Elastoplast to remove the upper layers of the skin (an area of -1 cm2). This will induce mechanical stress to the skin and result in skin inflammation. It is a non-invasive, mild procedure. iii. Punch biopsy and wound healing. This is a moderate procedure where a small wound (‘-0.5mm2) is introduced on the back of the animal. Topical treatments, proven by in vitro cell based assays to reduce inflammation and improve wound closure, will be applied. These procedures are all standard applications. The animals will be carefully monitored for adverse side

	effects, such as skin irritation, pain and infection. Advice from the named veterinary surgeon will be sought as required. At the end of the procedures animals will be killed by a humane method (as regulated by the Home Office)
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have in place ethics to obtain human skin biopsy material from normal, diabetic and psoriasis patients. From this tissue we can isolate and maintain keratinocytes (the main cells that make up the outerlayer of the skin) and fibroblasts (involved in wound closure and deeper skin layers). This resource is highly valuable and requires patient donor consent. Cells isolated from neonatal mice are also versatile tools and together have informed the cell signalling pathways and help identify novel compounds that can modify cellular events. However, the skin is a complex organ not just made up of individual cell types. Therefore studying integrated events cannot always be assessed in ex-vivo organotypic systems and limited animal studies are required.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All our experimental procedures are validated initially by cell based assays performed in vitro. We also work closely with bio-mathematicians who have helped model the disease processes and cell signalling aspects related to our studies. This means that we only use animals when we have definitive end points to meet. All experiments using animals are carefully planned. The number of animals required is derived using a mathematical calculation to estimate the lowest group size that will achieve statistically significant results. In addition multiple organs and blood vessels will be derived from animals on completion of terminal procedures. These will be distributed among various other investigators within this institution who are researching the effects of similar chemical messengers on different disease pathogenesis. Thus we can maximise the information derived from each animal and reduce the number of animals used.
3. Refinement Explain the choice of species and why the animal model(s)	We have chosen to use mouse models as our studies focus on pre-clinical investigation. Mice have been widely used in study of skin disorders and wound healing. Using mice, tissue derived from them and

you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	human skin cells we can design our studies to achieve maximum information from them. Minimisation of animal suffering: • Animals will be housed in comfortable conditions with environmental enrichment. • Where possible animals will be group housed. However, animals may be housed singly for short periods of time in order to record their responses. In addition, male mice sometimes require to be housed singly due to in- cage fighting. • Diabetic animals will have their bedding changed frequently to compensate for any excess urination which may occur. • All procedures will be carried out by, or under the supervision of, an experienced competent person. • For all aspects of our work we will refer to the NC3Rs website for guidance (www.nc3rs.orci).

Project 5	**The circuit mechanisms of auditory processing**	
Key Words (max. 5 words)	hearing loss, ageing, tinnitus, auditory system	
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)	Hearing is not just one of the essential sensory systems, but is also critical for our social lives. Ageing typically impairs auditory function. Moreover, nearly 15% of people between 20 and 69 years old have high frequency hearing loss due to noise at work or in leisure activities. The number of young people with hearing loss has grown in the last decade (Shargorodsky et al 2010). *Hearing loss* Hearing loss affects 360 million people worldwide and it is estimated that this number will exceed 900 million in next decade (WHO, Fact sheet 2015). Hearing loss or deafness refers to conditions in which individuals are fully or partially unable to detect or perceive at least some frequencies of sound. Of several categories of hearing loss, *sensorineural* deafness is caused by a defect located anywhere along the auditory pathway, from the cochlear to the auditory cortex. The major causes of sensorineural hearing loss are long-term exposure to environmental noise, genetic factors, disease or illness, and exposure to ototoxic chemicals. At present, only prostheses including mainly hearing aids and cochlear implants are used in sensorineural	

	deafness. Although cochlear implants are the most successful neural prostheses to date, there are still limitations. For instance, cochlear implants require functional auditory nerves. Alternative prostheses, such as auditory brainstem implants and auditory midbrain implants, have been applied for patients without functional auditory nerves, but the recovery of hearing abilities is very limited This research project aims (1) to characterise the neural basis of normal hearing, (2) to characterise pathophysiology of hearing abnormality in animal models, and (3) to develop new strategies to improve and restore hearing.
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 contribute to advance the knowledge of both normal and abnormal hearing. Thereby, this project benefit patients who suffer from hearing impairments, such as hearing loss and tinnitus.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 300 rats and 14000 mice will be used over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of the animals are genetically modified and will experience sub-threshold to mild discomfort. Non-recovery animals will be anaesthetised throughout. Surgical procedures have inherent risks (e.g., infection, haemorrhage, post-surgical complications). The behavioural training and assessment under head restraint conditions may be stressful for animals. These procedures are moderate, all animals will be humanely killed at the end of the procedure for further investigation.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no feasible alternative that would entirely replace the use of a living animal in order to conduct behavioural experiments to assess hearing abilities, electrophysiological experiments to monitor neural activity *in vivo*, and brain stimulations to control brain activity.
2. Reduction Explain how you will assure the use of minimum numbers	The proposed experiments and data analysis will be carefully designed using rigorous statistical approaches. Advices from a statistician will be sought whenever necessary. In addition, using advanced

of animals	technologies, the number of animals to be used will be minimised by increasing data quality and quantity.
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 were chosen because the existing literature describes relevant methods and techniques in rats and because of the availability of genetic alterations in mice. We will minimise welfare costs to the animals by the use of experienced personnel and proven techniques and by use of aseptic technique.

Project 6	**Mapping the Functional Architecture of Vision**	
Key Words (max. 5 words)	Zebrafish, Topography, Retina, Vision, Mapping	
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)	Specific objectives of the proposed research are to: 1) map how information from neurons in the eye is delivered and organised in the brain - this has not been possible in any animal todate 2) Chart the dynamics of such maps and examine differential biological rules underlying their formation, precision, alignment and maintenance. 3) Define fundamental rules that underpin biological vision.	
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)?	Machine vision technology is common place in industry and is increasingly being applied to classify and interpret biological and medical images. Indeed, machine vision is also facilitating prosthetic retinal implants and computer aided smart vision. Zebrafish are visually adroit animals - understanding exactly how they encode the visual world will be inspired by and contribute to machine vision technologies. A reciprocal relationship facilitated by open access data sharing that will ultimately help reveal what the fish's eye tells the fish's brain.	
What species and approximate numbers of animals do you expect to use over what period of time?	Over five years: Zebrafish: 5000 Adults, which are used for the sole production of embryos and larvae. 10000 larvae, the majority of which will be used prior to the developmental stage at which they become protected by the act.	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For the vast majority of zebrafish they will live a normal life within the animal facility with no adverse effects and be used for breeding until they are humanely killed around 18-36 months of age. The majority of experiments will be performed on zebrafish larvae prior to the developmental stage at which they become protected by the act. In a subset (~20%) of larvae will be used at an age at which they become protected. These should experience no suffering as all protocols at these ages are non-invasive and non-harmful.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are examining how the brain processes visual information. The complex neural architecture required for processing sensory stimuli and driving behavioural output cannot be generated in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To prevent the need for severe procedures, we employ advanced genetic approaches with single cell resolution to determine outcomes. A suffering animal is less informative than one with a specific minor defect that can nevertheless be analysed in detail. For this reason we use zebrafish, whose optical clarity permits use to track the behaviour of defective circuits in an otherwise healthy animal. Where more severe experiments are essential, statistical methods, such as the resource equation, will be used to ensure that cohorts are the minimum number needed to give reliable results. Pilot experiments will assess the likely appropriate size of these cohorts.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Most of our experiments will be performed on zebrafish, both because they have experimental advantages and because they are regarded as less sentient than mammals. All invasive procedures involve the use of anaesthetics and pain relief as advised by veterinary staff.

Project 7	**Studying the retinal response to depression**	
Key Words (max. 5 words)	retinal response to depression	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objectives of this project are to further our scientific understanding of the retinal response to depression and antidepressant drugs. It is currently unknown to what extent this structure participates in the development of depression and the mode of action of anti-depressant 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)?	Major depressive common psychiatric condition and the leading cause of disability worldwide. Presently, both the scientific understanding of MDD and treatments available for this devastating condition are poor. This project will generate new data that furthers our scientific understanding of the fundamental neurobiology of depression. In particular, this project will inform us about changes to gene expression and protein levels within the retina, a potentially important site regulating the onset of depression. This work holds potential to identify new neural circuits involved in both the onset and treatment of depression. In addition to advancing scientific understanding in this area, the project will also generate new data that could lead to the future development of novel antidepressant druqs for the treatment of MDD.	

What species and approximate numbers of animals do you expect to use over what period of time?	During this project we will be using social isolation as a tool to induce depressive behaviour in mice. We expect to use approximately 600 mice over a five- year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	As this project studies depression, we have to induce a depressive-like state in mice. This will be achieved using the least stressful and most relevant method to the human condition (social isolation). The side effect of social isolation in mice is a subtle weight gain (mild severity), which does not adversely affect the animal's physical health. However, the procedure will produce depressive-like symptoms that can only be detected using behavioural tests. Socially isolated animals will be given anti-depressant drugs to reverse these symptoms and we expect no adverse effects from this treatment. All animals will be humanely killed at the end of this study and the expected level of severity of procedures is moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although we make use of in vitro (laboratory-based, non-animal) methodology in this project, there is currently no way to study the impact of depression on retinal gene expression without first inducing depression in living animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	During this project we will assure that the minimum number of animals is used in order to achieve our scientific aims. This will be done by conducting statistical power analysis prior to initiating experiments in order to ensure that we use only the minimum number of animals required to produce valid statistical comparisons between experimental groups.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the most extensively used species in depression research and as such there is a large and informative literature available. The social separation model of depressive behaviour has been validated in mice and provides a robust method for inducing depression in this species. This method also recapitulates two important precursors to the development of depression in humans: social stress and loneliness. As such, this model is more relevant to human patients than other models, which seek to induce depression using chronic physical stressors. It also manages to effectively induce depression without the added risk of physical harm to the animals that may occur using alternative approaches. As such, animal suffering has been minimised from the outset by our

	choice of model. We have also sought to minimise welfare costs by limiting the number of post-depression behavioural tests and not including recovery following studies of retinal function. In terms of the chronic administration of anti-depressant drugs, we have chosen to dose animals via their drinking water in order to avoid the stress induced by repeated injections. This method has also been shown to be the most effective for producing drug levels within the clinical range.

Project 8	**The retinal pigment epithelium in health and disease**	
Key Words (max. 5 words)	Retina, Diabetes, Age-related macular degeneration	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The project aims to investigate the retinal pigment epithelium (RPE) in health and disease. We are doing this project because there are several forms of blindness and vision loss that are caused by RPE cell death or dysfunction. Among these, age-related macular disease (AMD) is the most common form of vision loss in the elderly, affecting almost one in four of the population aged 70 or over. AMD results in loss of central vision, so tasks such as driving and reading become difficult, and eventually impossible. In addition, diabetes is believed to alter the properties of RPE cells in ways that may contribute to diabetic retinopathy. Through this project we aim to shed light on how and why RPE cells become defective, and in doing so, create new opportunities for the development of improved therapies.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits of this project will be a better understanding of how RPE cell defects lead to blindness, particularly with regard to AMD and diabetic retinopathy. Furthermore, it is possible that some of the animals we investigate will make useful models for the design and testing of new therapeutics that may be used to treat patients with these diseases.	
What species and approximate numbers of	Our overall plan is to use mice, in which we can delete or modulate gene expression in the RPE, and in a few	

animals do you expect to use over what period of time?	experiments rats. Rats and mice have been chosen for this work because of the many similarities between the rodent and human retina, and also because of the ability to manipulate gene expression in these species. We will investigate several disease-associated genes in this way, and then examine the consequences on various aspects of visual function and retinal health. Mice and rats have to be used for this work because the complex structure of the retina, in which layers of different cell types are physically associated with one another, cannot be reconstructed using cultured cells. However, we minimise the numbers of animals used by doing as much as possible on cultured RPE cells, and by careful management of animal breeding to avoid generating a surplus. Over the entire project we do not expect to use more than 3000 animals, of which 90% are likely to be mice and 10% rats.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For invasive procedures that are of moderate severity, such as electroretinography, exposure to atmosphere with varying oxygen concentration and retinal imaging, animals when necessary will be given either a local or general anaesthetic. Throughout all procedures we will closely monitor the animals for any signs of distress, as well as during recovery from anaesthesia. In some studies we will pharmacologically prevent insulin secretion in order to investigate how diabetes affects RPE cell function. Diabetic animals may experience similar adverse effects to diabetic humans, such as weight loss and excessive thirst. At the end of all experiments animals will killed by a Schedule 1 method, and in most instances tissues will be retrieved post mortem for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Much of our research does involve the use of in vitro systems such as cell culture, and this complements the animal work. However, in vitro assays cannot adequately model the complete array of molecular, cellular, physiological and behavioural interactions necessary to fully understand how genetic modifications result in changes in the RPE. This is because RPE activities are heavily influenced by the adjacent choriocapillaris and photoreceptors, in a configuration that cannot be recapitulated in vitro.
2. Reduction Explain how you will assure the use of minimum	Unnecessary production or import of genetically altered animals will be avoided by searching cryobanks and databases. The strain used for generating a new colony will be carefully considered to avoid producing

numbers of animals	unwanted mice. Animals will only be bred if we require them, and the breeding programme will be subject to regular review to optimally meet anticipated demand. Spare animals will be made available for use on other scientific projects. Breeding will be optimised, wherever possible, to produce only the genotype required e.g. homozygous breeding pairs. Cryopreservation of gametes and embryos to archive lines will avoid wastage from the need to maintain colonies by continuous breeding.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are universally used for work involving genetic alterations. The standard protocols, methods and reagents have been optimised for this species and there are acknowledged benefits from their use. Animal suffering will be minimized by careful monitoring during all protocols. In experiments using STZ early humane endpoints will be the potential development of adverse clinical signs such as polyuria, polydipsia, polyphagia, anorexia and weight loss. Any indication that animals are suffering unduly as a consequence of fasting will lead to a reduction in the fasting period to a minimum of 4 hours. A blood glucose level in excess of 30mmol/L and weight gain below 80% control will be judged to be humane endpoints. STZ is used here in place of a transgenic diabetic model as our aim is study the consequences of RPE gene function in diabetes. A transgenic diabetic model would necessitate the use of much greater numbers of animals due to the extensive crossing required to generate double knock-outs. The low dose protocol for STZ administration used here is the mildest appropriate for these studies because it rarely generates blood glucose levels in excess of 28mmol/L. Administering STZ in successive low doses minimises the risk of an adverse reaction to a single much larger dose, whilst ensuring a level of ß-cell ablation sufficient to induce diabetes. Although mice will be used for most diabetes studies, rats may be used occasionally, their larger size may make them a more suitable model if surgical interventions are required or if historical/background data are predominantly in this species.

Project 9	**Understanding vision, developing therapies**	
Key Words (max. 5 words)	Retina, retinitis pigmentosa, sight, melanopsin, vision	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	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 two related objectives. On the one hand, we want to understand how a newly discovered light sensing mechanism (melanopsin) and the long established rod and cone photoreceptors work together to allow us to see and to drive a range of subconscious responses to light. We understand quite a lot about how the retina and brain measure small differences in light intensity in order to help us see. However, in our everyday life the amount of light falling on our retina varies enormously over space and time. Our work to date tells us that those big differences in light intensity are in part measured by melanopsin. We now would like to determine how vision is influenced by changing light levels and how melanopsin helps us see. We would also like to know how melanopsin and the other photoreceptors adjust our biology in ways in which we are not even aware – especially keeping us alert and setting the phase of our biological clocks. As part of that work we will also determine what happens to these aspects of vision in diseases that affect the retina. This brings us to the second major objective, assessing ways of restoring vision in blindness. Loss of rod and cone photoreceptors is a common cause of blindness in people. We have recently shown that it is possible to recover vision in mice with an	

	equivalent condition by introducing a photosensitive protein into the retina that remains when rods and cones disappear. We now need to understand that therapy better. How well can it work? What limits its performance? Can we find alternatives that work even better?
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)?	Blindness caused by rod and cone loss is quite common (around 1:2500 people) and currently untreatable. If we can develop new therapies for this condition it will make a big difference to people with those conditions. Understanding how melanopsin vision works is also very important. In modern industrialised societies people spend much of their time under artificial light and viewing electronic visual displays. These differ fundamentally from what we experience outdoors. If we better understand how the visual system works we will be able to design these artificial systems better, improving our visual experience and ensuring that lights not only help us see but also have appropriate sub-conscious effects. Its increasingly clear that artificial light at night can have deleterious health effects, the same likely is true to a lesser extent during the day. It is unrealistic to expect people to stop using artificial light, but we can do our best to make sure that it is appropriately designed.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 10100 Mice and 300 Rhabdomys (African striped mouse) over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the mice will not suffer pain or distress because they will be used for breeding, observations of voluntary behaviour, or killed for tissue collection. The majority of the rest will experience transient mild distress associated with inducing and recovering from anaeasthesia, short term restriction of food and water, or swimming in a maze to find an escape platform. The highest severity level we expect would be moderate, and that might be reached in those animals undergoing recovery surgery to implant transponders or cranial electrodes or inject substances into the eye. The main concern here is post-operative pain during wound healing and we will alleviate that with analgesics. The relatively small number of mice undergoing recordings from electrodes implanted into their brain will have to get used to a small additional weight on their head

	associated with the electrode mounting and, transiently, the ultra-light weight wire we use to connect to the recording apparatus. All animals will be killed at the end of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Sight is an emergent property of the brain and retina that can only be studied in animals. It cannot be recreated with computer simulations, or tissues/cells grown in the lab. It is possible to study the process of photoreception outside animals and we will take advantage of this to replace animals as a first screen of potential improvements to our gene therapy. Only the strategies that prove most promising in vitro will be taken through to trial in mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Wherever possible we use electrodes that record simultaneously from many parts of the brain, which allows us to record a lot of data from a single animal and reduce the numbers used in total. We use statistical power calculations, randomise treatment allocations and analyse blind to treatment wherever possible in order to maximise efficiency.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have chosen mice because i.) they share many features of our own visual system; ii.) there is a large amount of baseline information in this species upon which to build; and iii.) there are a range of transgenic models that allow us to ask important questions. One way in which mice are unlike us is that they have rather few cone photoreceptors. There is an obvious need to also work in an animal with more cones. The conventional models would be cats or primates. We have instead chosen an animal with lower sentient ability (an African mouse species, Rhabdomys pomilio), that is diurnal and has a cone rich retina and higher acuity. Over the course of my previous project licence, we introduced refinements to electrophysiological and behavioural experiments that ensure we do NOT have to train mice to recognise aversive stimuli (mild electric shock), or restrain conscious animals to achieve our objectives.

Project 10	**Strategies for the restoration of sight in retinal dystrophies**	
Key Words (max. 5 words)	Retinal degeneration, stem cells, saffron, subretinal transplantation, intravitreal transplantation	
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)	Photoreceptors (rods and cones) are the retinal cells in our eyes that transform light into electrical activity. There are cases where they do not function properly because of genetic mutations, and as a result, they begin to die, resulting in serious visual impairment and in extreme cases, complete blindness. These diseases are grouped under the common name “Outer retinal degeneration” (ORD). When photoreceptors have completely degenerated, one of the most promising therapeutic approaches is to replace them with new photoreceptors derived from stem cells, and the development of this approach is the main aim of this project. Our recent work has shown that it possible to make, in the lab, structures that resemble the human eye and that contain several eye parts (retina, cornea, lens) from human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC). We will work with mouse models of ORD and will investigate how the retinal cells generated from hESC and hiPSC can potentially lead to the restoration of vision in these animals. In parallel, we will investigate the effects of the natural spice saffron in slowing down the death of photoreceptors in these same mouse models. Saffron is known to protect photoreceptors from dying	

	through antioxidant and anti-inflammatory actions. ORD is also characterised by the proliferation of glial cells (support cells in the central nervous system), and it has even been suggested that the activation of this process precedes and induces photoreceptor death. Hence, we will investigate whether inhibiting the activation of glial cells with pharmacological agents (such as neurostatin) has a potentially beneficial effect on the progression of ORD. We estimate that the duration of this project will be five years.
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)?	ORD with photoreceptor loss is the final common pathway of irreversible visual loss in many diseases. Recent estimates indicate that the global number of people with sight loss is 285 million, of whom 39 million are blind. Although gene therapy strategies to delay visual loss are under development, this approach requires that photoreceptors remain alive, otherwise there is nothing to target with the replacement genes, and it is therefore ineffective following complete photoreceptor loss. Currently, there are no effective therapies to reverse the underlying pathological changes in ORD and, therefore, there is a requirement for new therapies to treat patients once visual loss is established. Cell transplantation therapy is an important option for patients suffering from currently incurable forms of blindness. The results from this work will allow a deeper understanding of the behaviour and biology of transplanted cells within the diseased retina so that the protocols and techniques can be optimized prior to clinical application in humans, improving visual outcome using this therapy. Moreover, this project will also provide important knowledge about the use of natural remedies such as saffron or gliosis inhibitors such as neurostatin in slowing down, or even stopping degenerative processes in ORD disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate using a maximum of 632 animals over the duration of this license.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	Genetically altered mice that develop retinal degeneration and blindness will be bred and maintained. Some of the mice will be immunosupressed with drugs, including use of

level of severity? What will happen to the animals at the end?	surgically implanted devices (in the eye), and will have stem cells or drugs injected into one eye under anaesthesia. They will be monitored for up to 16 weeks and then humanely killed and their tissues examined *in-vitro*. Risk of infection post transplantation will be minimised by using sterile instruments under aseptic conditions. For cell transplant procedures, post-procedure pain will be controlled using appropriate analgesics. Animals showing signs of poor recovery from surgery under anaesthesia will be closely monitored. Animals that are recovering will be carefully monitored and if they exhibit weight loss greater than 20% (compared with similarly operated cohorts), or show marked signs of distress, e.g. marked piloerection, dehydration, hunched appearance, subdued behavior, solitary—for more than one day, will be killed. Animals that will receive human stem cell transplants will be immuno-suppressed which may increase the risk of infections. This will be minimised by housing them in conditions appropriate for immunosuppressed recipients. Furthermore, injection of cyclosporine to pregnant dams may affect the development of pups, although this may be rare and sporadic. Affected pups will be humanely killed. To date there are very few studies describing the effects of cyclosporine on pups following injection of dams; the effects are described as due to poor crossing of cyclosporin through the placenta. The only notable impact is a transient decrease in thymus weight and cellularity if injections are performed every day from coitus to birth. All these changes disappear 30 days post weaning. There is no expected direct adverse effect of neurostatin, the gliosis inhibitor. The level of severity is moderate. The animals will be sacrificed at different time points with the longest procedure lasting four months.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will investigate the fate of photoreceptor progenitors (cells that will give rise to photoreceptors) derived from hESC/hiPSC following subretinal transplantation into several different murine models of ORD. Recent reports have shown that hESCs and hiPSC can be coerced to become, or 'differentiated' into retinal photoreceptors *in vitro* with good

	efficiency. Notwithstanding these advances, *in vitro* results cannot shed any light on the survival, engraftment and functionality of cells following transplantation into the degenerate retina. Hence, *in vivo* investigations are required to answer these questions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All attempts have been made to reduce the number of animals and procedures to minimise suffering yet achieve clinical and statistical significance. In each experiment, one eye will be injected with stem cell-derived retinal cells and the other eye will serve as a control, thus minimising the number of animals used. Each of the experimental groups comprise 9 animals based on power analysis.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	All experiments will be performed on genetically modified mouse models of ORD. These experiments cannot be performed on patients, but their outcome will provide extremely valuable new knowledge, paving the way for future clinical trials. The models we plan to use are well recognised for faithfully representing common human ORD disorders and therefore, they will provide important new knowledge about how to rescue or repair retinas with advanced ORD. The protocols have been designed to keep suffering to a minimum. Where subretinal and intravitreal transplants will be carried out, appropriate anaesthetics and analgesics will be administered pre- and post-operatively. Any loss of condition will indicate removal from the procedure and killing by a Schedule 1 method. Transplants will be performed using cells that have been differentiated at least for 30 days ensuring that virtually all stem cells have differentiated into retinal cells. However, a small risk of tumour formation exists from any remaining undifferentiated stem cells in the grafts, thus animals will be closely monitored for this possible occurrence. Animals that do not recover well from surgery or develop an intraocular tumour will be humanely killed. There is no expected direct adverse effect of neurostatin, the gliosis inhibitor.

Project 11	**The neural basis of sound perception**	
Key Words (max. 5 words)	Hearing, sensory cortex, auditory cortex, multisensory	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall purpose of this research program is to better understand how the sensory brain and particularly the auditory cortex, function to facilitate active listening. Listening, as distinct from hearing, is an active process shaped by our current goals and requirements. Our research therefore steps away from determining neural tuning in static laboratory situations and engages animals in complex naturalistic sensory tasks that mimic situations such as listening to one person in a busy restaurant. While we focus on auditory cortex we consider the influences of both auditory and visual stimuli within, and beyond, the classic auditory processing pathways. We will achieve our objectives by combining behavioural and electrophysiological investigations in auditory cortex with causal manipulations of neural activity.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This is basic research that will increase our understanding of how the brain (specifically auditory cortex) processes sensory signals and how what we see can influence what we hear. Since the principle complaint of listeners with even mild hearing loss is the ability to discriminate speech sounds in the presence of background noise an understanding of how the healthy brain achieves this task may allow us to build better signal processing devices for hearing aids and cochlear implants. Understanding when visual	

	information can enhance hearing may be of therapeutic benefit to listeners with hearing impairments by allowing us to develop training strategies that use visual processing to optimize listening abilities, This work may also provide benefits for technology: an understanding of how neural firing is decoded is fundamental in the design of any neural prosthesis or brain-computer interlace. Understanding how the brain encodes sounds such as speech in the presence of noise will aid the development of speech recognition software and speech processing algorithms.
What species and approximate numbers of animals do you expect to use over what period of time?	Ferret, 150 over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We propose to train animals in tasks that require that they discriminate auditory and/or visual stimuli. While the testing itself is not harmful (the sounds are not unpleasantly or dangerously loud) to motivate the animals we restrict the amount of water that animals have available in their home cages and reward them with water during testing. We carefully monitor the health of animals during periods of behavioural testing (and separate testing blocks with periods of unrestricted water) and ensure that their water consumption remains close to normal levels. We will record and/or manipulate neural activity in some animals. This requires a general anaesthetic and surgical procedure to implant devices to achieve this. Since the brain does not have any pain receptors implanted devices do not cause any discomfort (human patients being treated with deep brain stimulation have very similar electrodes chronically implanted) but there will likely be some pain after surgery which will be managed with postoperative painkillers. Recording itself simply requires connecting a wired or lightweight wireless connector during behavioural testing. Animals seem unperturbed by these connectors. The maximum severity is moderate although only for short periods of time for any animal. We will maintain the highest standards of welfare throughout and ensure that animals remain happy and healthy. Our experience has shown that animals who are undergoing behavioural testing, and/or have chronic implants are just as inquisitive and playful as those who have not undergone any procedures.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Studying the neuronal process of listening can only satisfactorily occur in an intact animal where we can make simultaneous measurements of perception and neural activity. Alternatives to in vivo animal work include tissue culture, but this lacks the complexity of an intact brain in terms of both the inputs and network dynamics, and computer modelling. Our work relies upon sophisticated computer modelling and simulations, but we can only obtain the data on which to perform such simulations from real neurons: the auditory brain is not well enough uhderstood to be simulated accurately on a computer. Whilst functional imaging studies in humans can tell us which areas of the brain are engaged in a particular behaviour, these methods lack the spatial and temporal resolution to provide information at the level of individual nerve cells, and therefore cannot tell us about the mechanism by which behaviourally-relevant signals are encoded in the brain or about the circuitry that underlies that processing.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible and appropriate we use computational methods or human behavioural testing or functional imaging in preference to animal experiments. Nevertheless, to understand the cellular mechanisms that shape auditory perception we still need to perform experiments on intact animals. Over the past 5 years both technological innovation and improved methods have allowed us to collect more data from each animal (with minimal or no additional cost to any individual animal) ensuring that we can use fewer individuals. Our work relies on computer modelling and simulations where possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We choose to use ferrets as our animal model due to their excellent low frequency hearing and amenability to behavioural training. Human hearing is critically dependent on low frequency sounds — speech, music and our ability to localise sounds all depend on frequencies lower than the hearing range of mice and rats, Our work focuses on auditory cortex and like humans (and unlike rodents) spatial hearing is crucially dependent on auditory cortex in ferrets. We place great emphasis on the welfare of the animals. Behavioural testing is integral to every aspect of our work and daily testing over a period of 1-3 years

	allows us to develop an understanding of each animal’s personality which in turn allows us to spot any early changes that might be indicative of a health or welfare concern. We carefully monitor their health and have developed methods (for example recording from freely moving animals rather than having to restrain them to make recordings) that allow us to perform our experiments with as little cost to the animal as possible. Our procedures themselves are developed specifically to minimise suffering, pain, distress or discomfort. This ensures that we maintain high standards for both our experimental methods and alsowelfare.

Project 12	**Exploring the role of inflammation in common eye diseases**	
Key Words (max. 5 words)	Eye, blindness, inflammation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to determine the role of ocular inflammation in common retinal diseases and to establish whether novel therapeutic targets have the potential to improve outcomes.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The primary objective of this project is to gain a greater insight into the factors controlling inflammation in the retina. We aim to use this information to identify new potential therapeutic targets and to test new treatment strategies to prevent, attenuate or reverse ocular diseases that have an inflammatory component, including some of the most common causes of blindness in the Western world, such as diabetic eye disease, age-related macu lar degeneration and uveitis. Current therapeutic approaches for treating these diseases offer some benefit but are often only partially effective and are frequently associated with serious side-effects. These diseases, therefore, continue to represent a significant socioeconomic burden in the UK and the industrialised world and consequently there continues to be an urgent need to identify novel targets and generate new therapeutic strategies. This project will use animal models of ocular inflammation to assess and test new treatment modalities emerging	

	from the biomedical mechanistic knowledge gained from the approaches outlined above.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice - 1200; Rats — 500; this project is expected to last five years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We aim to use a number of well-established models of eye disease in rodents to improve our understanding of disease and to test new therapeutic approaches. We do not employ severe models in our research and do not expect significant distress. We do not expect adverse events, but some animals may develop weight loss or disturbed behaviour as a result of injection with novel compounds. They will be closely observed for this and killed by a schedule 1 method if they develop. Animals will be killed at the end of the research.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use cell-culture systems wherever possible and plan to expand their usefulness during the course of this experimental programme. However, the complex nature of the pathogenesis of retinal (i.e. neuroinflammatory) diseases means that it is currently not possible to fully understand drug effects and mechanisms of disease in cell-culture systems. It is also difficult to obtain tissue from human diseases sufferers, as ocular biopsies generally result in visual loss, so are only performed in exceptional circumstances. Non-mammals do not have a sufficiently developed immune system to readily compare to humans. Rodents are the lowest species in which suitable autoimmune models are available which can be applied to human disease. Literature searches (including web searches of Altweb and FRAME) identify areas where cultured cells can be used, and we will use these wherever possible and learn how to expand their use if possible. However, there is no alternative to animal models that provide the necessary complexity to enable us to achieve all of our objectives. Well established and characterised mouse and rat models of disease will be used in this project, and have clinical and histopathological features similar to those observed in humans.
2. Reduction Explain how you will assure	For each experiment a protocol will be written following careful consideration of the total number of animals

the use of minimum numbers of animals	and group sizes required to realistically achieve the objectives based on both relevant publications and previous experience of the model used. This will include analysis of potential sources of bias, to enable the results to be as valid as possible. It will also involve the rigorous use of power calculations to ensure that that the number of animals per experiment to be kept to a minimum, whilst still generating the results that the project requires.
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 model and species:** The model chosen for the induction of ocular inflammation in mice and rats are very well established models that have been extensively characterised. We have extensive experience with these inflammatory model systems. **Minimising animal suffering:** This is our major concern. We do not employ severe models in our research. Of note, vision is also not the major sense by which small rodents perceive their environment, so the loss of sight does not affect their normal behaviour and feeding. However, we are aware that these experiments have the potential to cause suffering and have set tight criteria to detect and respond to signs of stress. Should such signs of distress be exhibited (e.g. rubbing the eyes with paws above that normally associated with self-grooming), affected animals will be killed by a schedule I method. We have set tight limits for signs of distress such as weight loss — we do not expect to see this, but have limited allowed reductions to 15% of initial body weight rather than the 20% often seen in similar protocols. Similarly, UK Laboratory Animal Science Association (LASA) guidance will be used to keep animal suffering to a minimum, and we have referenced these guidelines for drug doses, for example, to make sure that we use doses that obtain the experimental result required but whilst minimising suffering. Our main model can involve suffering, as it involves the injection of toxic substances. Problems occur in <1% of animals, but we will be vigilant for these and have a low threshold for killing affected animals before significant suffering develops. The PIL will monitor animals after disease has been induced at a gradually reducing frequency in order to pick up any specific adverse effects. This is in addition to the daily monitoring by the technicians that takes place for general health and welfare of the animals.

Project 13	**Genes and environments that influence behaviour**
Key Words	Genetics, behaviour, smell, intellectual disability
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):

This project aims to understand how genes (“nature”) and previous experiences (“nurture”) work together to explain the different behaviours individuals display. It will investigate if and how experiences in one individual can transmit across generations to influence the behaviour of offspring. Finally, it aims to understand how animal models of human brain disorders perform in behavioural tests after different experiences.

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

We need to better understand the biology of the brain to reveal how the range of human behaviour occurs, and to find out why behaviour differs in people with brain disorders. Although scientists have been studying the brain for many years, our understanding is still limited. This project hopes to identify both genes that alter behaviour, and learn how much different experiences can influence this behaviour. We anticipate that the genes uncovered by this project will be relevant to better understanding human behaviour. This will facilitate the development of future research paths, and perhaps new treatments and therapies to help people with behavioural conditions such as rare intellectually disability disorders or autism spectrum disorders.

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

Over the next 5 years, we will use up to 20000 mice (including genetically-modified) for breeding and up to 5000 mice for experimental procedures.

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?

Most of the genetically modified mice are not expected to have any adverse effects that would impact their welfare. The majority of mice used in these experiments will experience enriched environments, for example provided new smells or food types, which could result in mild and transient anxiety when first introduced. A minority may be exposed to moderately stressful environments, for example to the smell of predators, but only for a short period of time. At the end of the studies, the mice will be killed using humane methods. Blood and tissues may be collected from them for measuring the body's response to the environment.

Application of the 3Rs

Replacement

Behaviour is a complex process, involving many different parts of the brain interacting with the rest of the body. It also requires sensory organs responding to a stimulating environment.

In vitro technologies cannot adequately reproduce the full complexity needed for the intended studies. The current state of computer technologies is not powerful enough to model this complexity. Therefore it is necessary to use animals to study behaviour.

Reduction

Whenever possible we intend to use mouse colonies that already exist, using national and international repositories rather than create them ourselves. When new colonies are needed, we intend to use a service that has specialist expertise, which will reduce the number of animals used for generation.

The work is also carried out in a facility with strict environmental controls, so we reduce the variation in our mice and require fewer to find behavioural differences.

We intend to carefully select the human brain disorders we will model, preferring ones that are caused by a single copy of a mutated gene. This will mean we need to breed less animals to obtain enough of the right type for our experiments.

Refinement

Mice are a suitable species for this project because they have similar brains and sensory systems to humans and display many of the same behaviours. Mice also have very similar genomes to humans, so the majority of human genes we would like to study will exist in mouse. Mice also use their sense of smell to influence much of their behaviour. Smell is a good environmental stimulus to study. We can carefully control the odours a mouse can detect in their cages and use them as a tool to test memory or learning ability in models of intellectual disability. Mice also breed very quickly, so they are an ideal model for studying behaviours across generations.

Mice are social animals, so in this project they will be group housed, except for specific periods where single housing is required for scientific reasons (such as being able to control the food available to each mouse). The mice are provided with cardboard tunnels and nesting materials to facilitate normal behaviours. We use a sophisticated database system for tracking the tests the mice have had and for monitoring of health and welfare concerns. This allows live reporting on the condition of every mouse, so that if a mouse is found to have a health problem, swift decisions can be made about its welfare.

Project 14	**Development of novel therapy strategies for skin diseases**	
Key Words (max. 5 words)	Skin diseases, therapy, genetic disease	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	• To develop gene therapy strategies for rare genetic skin diseases • To develop a specific treatment for the common skin disease Eczema	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	In the human skin as the first barrier, plays a protective role against infection and water loss. In patients who have inherited faulty genes, the protective function of the skin is impaired or defective; therefore, they are vulnerable to infection, dehydration and other conditions secondary to the impaired skin. In some patients, the skin is very fragile and can causes blistering at places on the body that experience trauma, often the hands, feet, arms and legs, which usually results in scarring and skin cancer. All this can cause significant disability and affect the quality of patients' life. There are no curative treatments for these genetic skin conditions, the development of novel therapy is, therefore, urgent. This project is designed to develop new therapies for these diseases. If successful, our results will ultimately lead to treating patients. In addition, it would not only benefit patients that we are studying, but also benefit	

	those patients with other skin diseases as the technology of developed in this project could be extended to other serious skin disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice in the study and numbers of animals will be around 250 — 500 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We use genetically altered mice that can tolerate transplantation of human tissue. We will remove a piece of skin from the back of the mouse and graft a human skin sheet generated from human patient cells to make a skin disease model. In the model, gene corrected patient cells will be given by injection through vein or topical application on the skin. Following the treatment, the grafted human skin will be collected post mortem for analysis to reveal whether the treatments are effective. To reduce the stress and pain caused by the surgery, animals will be anaesthetised and will be given pain relief after the operation. • The animals will be killed under terminal anaesthesia at the end of studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	• To achieve a clinically relevant method for gene therapy or other skin therapies, it is essential to evaluate strategies in an appropriate model similar to humans. There is a cell based 3D skin equivalent model which can be used to assess the efficacy of transgene expression and function. However, the model cannot be used for observing a long-term effect as cells can only survive for 3 to 4 weeks in the model. • this model lacks the blood circulating system seen in the human body. • Our previous studies have shown that the grafts generated from human keratinocytes and grown on SCID mice resembled the original human skin morphology. We therefore have to choose the humanised skin graft model for our study.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To ensure the minimum number of animals is used in our experiments, we will revise and test vectors in cell models before moving on to animal models. In addition, we have carried out pilot studies of skin graft and carefully assessed experimental design. We will also share controls cross experiment groups or use internal controls. All this will ensure the use of

	minimum numbers of animals in every experiment.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice have been chosen as they have the lowest nerve sensitivity while still having a body envIronment with comparable complexity to the human. In addition, the use of therapy strategies that we have been developing on mice is highly representative of the procedure in humans. For these reasons, mice have been chosen for our study. We have carefully assessed experimental design and ensured our experiments to have the minimal impact on the animals to be used. We have also chosen protocols in our study that involve the least suffering for the animals. This includes the use of anaesthesia and analgesic prior- and post- operation and prophylactic antibiotic-therapy for grafted mice. In addition, animals will be monitored carefully during the experiment and particularly after surgery and injections. If there is a sign of stress or infection or dehydration with no improvement after treatments, the animals will be humanely killed.

Project 15	**Developing gene therapy for glaucoma**	
Key Words (max. 5 words)	Glaucoma, gene therapy, IOP	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to develop and assess the viability of gene therapy for the treatment of glaucoma, which is a major cause of blindness. The treatment targets genes which control the production of fluids that maintain the shape of the eye ball. In glaucoma overproduction of these fluid increase pressure within the eye resulting in damage to the retina that results in blindness. The gene therapy is aimed at reducing fluid production by switching off some of the genes responsible for this function, thereby lowering the pressure within the eye ball.	
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 could lead to a new form of human therapy for glaucoma, which is itself one of the leading causes of blindness worldwide. In addition the study is expected to contribute to a better understanding of how different cells function to maintain the correct pressure of fluid within the eye, which could in turn lead to the discovery of new targets for future drug development.	
What species and	We will only use laboratory-bred mice. We expect to	

approximate numbers of animals do you expect to use over what period of time?	use up to 800 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will inject the eyes of the mice with the experimental gene vector and measure its effect on the pressure within the eye. This will be done with a gentle, non-invasive, non-painful method, used commonly by opticians. In later studies glaucoma will be induced in mice to test the effectiveness of the treatments. This is performed by either injection or drops does not usually cause pain. All injections are done under general anaesthetic. Unfortunately at the end of the experiments all mice will be killed in order to obtain tissues to fully investigate the effectiveness of the treatment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To test the new therapy will require the use of animals as it is only possible to evaluate the effect of treatment on the pressure within the eyeball in a fully functioning eye. The pressure within the eye declines rapidly after death therefore the donated post-mortem human eyes cannot do this and there are no other cell culture or computer models that could replace the experimental conditions needed to evaluate the new treatment.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Every effort will be made to minimise the number of animals needed to undertake this work through good experimental design, and the use of the most appropriate statistical analysis. In addition preliminary studies conducted in cell culture will enable us to select the most appropriate gene targets prior to animal testing. The non-invasive techniques being used to determine eye pressure and evaluate the effectiveness of the treatment will enable us to maximise the data generated from each animal thereby minimising the number of animals needed. For the majority of studies treatment will be given to only one eye, thereby enabling each animal to act as its own control, thus greatly decreasing 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.	Mice are the most suitable animal to use, as they have been extensively used in the development of gene therapy and in the modelling of glaucoma. They are also the least sentient species suitable for the proposed study. Attention to minimising harm has been given through the design of the study and the techniques for assessing and monitoring all cause minimal and are widely used by opticians, consequently the animals are not expected to experience distress during monitoring. Wherever there a potential for an animal to experience pain this will be prevented by the use of a general anaesthetic.

Project 16	**Brain mechanisms of listening and learning**	
Key Words (max. 5 words)	auditory; hearing; mice; neurons; perception	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objectives of this project are: I. To determine how different parts of the auditory brain contribute to sound perception. II. To analyse how sound experience and learning shape auditory brain function. Ill. To understand the neural mechanisms of auditory temporal processing disorder and auditory brain abnormalities associated with diseases such as schizophrenia and autism. IV. To determine how hearing loss affects auditory brain function and increases susceptibility to pathologies such as tinnitus.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could	This project will advance scientific understanding by providing new information about the brain mechanisms of listening and learning, and the origins of complex hearing disorders. These insights may lead to development of new technologies and therapies for auditory pathologies such as tinnitus, central auditory	

benefit from the project)?	processing disorder, auditory hallucinations in schizophrenia, and acoustic hypersensitivity in autism.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use no more than 700 juvenile mice, 1300 adult mice, 400 adult guinea pigs, and 250 adult gerbils over five years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Approximately 50-70% of animals will be used only for breeding, terminal experiments, or pilot behavioural studies, and will therefore experience no more than mild discomfort (e.g., from ear-punching for genotyping or injection of terminal anaesthesia). The remaining 30-50% of animals will experience procedures of moderate severity, such as recovery from surgeries involving implantation of neural recording devices, and/or behavioural training involving food restriction. The most common moderate-severity adverse effects expected are post-operative discomfort which will be managed with analgesics given during and after surgery, and hunger from food restriction which will be managed with careful calibration of food intake and daily monitoring of body weight to ensure maintenance above 85% of the free-feeding weight. At the end of all experiments, the animals will be humanely euthanised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Brain mechanisms of listening and learning are not yet understood well enough to be simulated effectively in computer models, or replicated in cell culture. These brain mechanisms of hearing can only be studied in intact animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Data points in our experiments are brain recordings, not animals. We will minimise the number of animals required by maximising the number of brain recordings we obtain from each animal.
3. Refinement Explain the choice of species and why the animal model(s)	We will primarily use mice, which are arguably one of the least sentient species with a mammalian auditory brain. We will sometimes use guinea pigs or gerbils when the experiments require a species with a larger

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.	brain or lower frequency hearing. Most animals will experience no more than mild discomfort, as from an injection of anaesthetic. Some animals will undergo surgery with recovery, and will be given analgesics to ensure that any post-operative discomfort is minimised.

Project 17	**Inflammatory Diseases of the Brain and Eye**	
Key Words (max. 5 words)	Multiple sclerosis; Uveitis, Inflammation, Vasculature	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of this project is to gain a greater insight into the contribution played by the blood vessels of the brain and eye in the development of inflammatory diseases. Despite there already being considerable knowledge generated relating to this question, we still do not understand many of the mechanisms involved. This is exemplified by the fact that inflammatory diseases of the brain and eye cannot be cured and as such continue to represent a major unmet clinical need. Here we aim to investigate various unanswered questions regarding how white blood cells gain access to the brain and retina, what the consequences are of inflammation on vascular permeability and oedema, and how these pathogenic events can be attenuated through therapeutic intervention.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could	Inflammatory diseases of the brain and eye represent a major socioeconomic burden to society. Two such diseases are multiple sclerosis (MS) and uveitis that affect the brain and eye respectively. Thus, MS affects 2.5 million individuals world-wide and is the	

benefit from the project)?	commonest cause of disability in young adults. Typically it is diagnosed between 20 and 40 years of age with an estimated global prevalence of 30 people per 100,000 although in the USA and Europe the incidence is greater than this average. Apart from the enormous social cost, it has been estimated that the total annual fiscal cost of MS to Europe is of the order of 14.6 billion Euros. As with MS, uveitis is also an inflammatory disease but affects any part of the internal segment of the eye resulting in severe discomfort, impaired vision and in the worst-case, loss of sight. Posterior uveitis, which affects the retina, remains a significant cause of blindness in people of working age (mainly between 20 to 60 years old) with a prevalence as high as 155 people per 100,000. Of these, 35% of cases will suffer visual disability accounting for up to 25% of legal blindness in the USA and Europe. Similar to MS, posterior uveitis mainly affects people during the most active period of their working life and as such the cost to the patient and the economy is considerable. During the last decade there have been significant advances in our understanding of such diseases and in the development and application of new effective therapies. Nevertheless, many therapies are associated with incomplete penetrance across the patient spectrum and can be associated with deleterious side effects. This is especially problematic as these conditions usually take on a chronic course requiring long-term treatment. There remains, therefore, a clear unmet clinical need. To address this, new therapeutic approaches are needed but require a greater understanding of the biological mechanisms that orchestrate the disease process in order to identify novel targets. The aim of this project is to obtain a greater understanding of the disease process so that new therapeutic targets can be identified and new therapies tested.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use well-established rodent models of disease, both rats and mice, in this study. We anticipate that we will use approximately 400 rats and 4,725 mice during the 5 years of this licence (total

	5,125).
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?	Every effort has gone into reducing the need to use animals and to reduce suffering. We will employ an escalating go/no-go approach whereby we will only proceed if there is evidence that we need to move to a more complex system to provide further critical data. Accordingly, in the first instance we will gain as much information as possible from cultured cell lines. To validate key data emerging from this approach we will then employ primary cell cultures and tissue derived from culled animals as these provide a more representative and valid model system than cell lines. As the complexity of inflammation and the multiple interactions of different cell types cannot be replicated in reductionist in vitro model systems, it is imperative that to address our hypotheses fully we utilize animal models of disease. To reduce suffering we will, in most instances, undertake studies in the ocular inflammation model (moderate severity) as the severity level is less than in the brain inflammation model. Only when we have evidence that the preceding data warrants it, will we proceed to acute and then chronic models of brain inflammation (both severe). It is anticipated that using this approach will minimise the number of animals being used in severe procedures. This overall approach is designed to reduce animal usage and suffering whilst maximising the pathobiological information we obtain. During all animal procedures we will use robust monitoring to ensure that the animals fall within the permitted severity limit and do not exceed the allowed duration. Any animal that experiences adverse effects during the experiment will be treated or killed by a schedule I procedure. Upon completion of the permitted experiments all animals will be terminated through schedule I killing.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complexity of the vascular system we are investigating is part of a complex structure that is influenced by many cell types as well as its environment. This is impossible to recapitulate in more simple model systems. As much of our work as

	possible is conducted in in vitro and ex vivo systems. These provide us with the ability to control carefully the environment and answer fundamental questions. Once such proof of principle data has been acquired it is then necessary to establish whether this translates into the more complex in vivo environment.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Pilot experiments using in vitro culture systems whenever possible will be used to provide some data and reduce animal numbers. Animals will be bred on a need basis only. We will also minimise numbers through rigorous application of methodology to avoid experimental bias. Power calculations will be made in conjunction with a statistician to determine likely minimum numbers required to achieve 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.	We have chosen rodents (rats and mice) as they replicate most of the specialised features of the vasculature of the brain and eye. Some insight may be gained from studying non-mammals such as the zebrafish but in general these do not fully translate to humans. Rodents are the lowest species in which suitable models are available which can be applied to human disease. We are not aware of any alternative to animal models that provide the necessary complexity to enable us to achieve our objectives. Undertaking careful monitoring of all animals that have undergone procedures in order to evaluate welfare within the permitted limits will be a principle objective. We will apply standard established assessment methodology to minimise harm to the animals.

Project 18	Lateral line and hair cell function in zebrafish
Key Words	Hair cells, zebrafish, Development, Neurons, Regeneration
Expected duration of the project	5 year(s) 0 months

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

Purpose

Yes (a) basic research;

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

The aim of this programme of work is to define the critical physiological steps required for the maturation and function of sensory hair cells, which in mammals are present in the auditory and vestibular systems. Hair cells are specialized to detect either sound vibration or head movements. The work proposed under this licence is expected to improve our understanding of sensory system function by providing critical basic understanding of how these peripheral sensory hair cells are able to communicate with the brain.

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

Hearing is one of the key senses that allow humans and other vertebrates to acquire important information from the surrounding environment. When mammalian cochlear hair cells are damaged, they cannot be replaced and this is the reason why hearing loss is an irreversible process. Hearing loss is a very common disorder affecting 250 million people worldwide. Therefore, by determining the main elements required for hair cell function and development we will be able to support translational/clinical research, which is directly aimed at developing a cures for hearing loss, as well as progress the technical development of hearing aids, including cochlear implants. The scientific advance generated by the proposed study thus offers a great social benefit since it would contribute, in the long term, to decreasing the number of people with permanent hearing loss. Humans rely on sensory information such as sound, vision and smell for day-to-day living. In order for the brain to perceive this information,

sensory cells from the ear, eye and nose have to detect and process stimuli from the outside world in the form of electrical signals and rapidly transfer them to nerve fibres via special connections called synapses. Despite the crucial role of these synapses in sensory perception we do not fully understand how they contribute to the processing of information within the nervous system, because we have not been able to monitor their operation within intact circuits. The zebrafish is an excellent model system used world-wide for in vitro and in vivo experiments because it allows the performance of imaging and electrophysiological experiments in intact animals. This is possible because the zebrafish is small, transparent during early stages of development and it is easier than rodents to genetically manipulate.

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

The use of animals is absolutely essential for this project since at present it is the only means of obtaining fundamental information about the physiological properties of cells in the intact circuit. The number of animals used (maximum 9K) will be kept to a minimum by making sure that all experiments are meticulously designed and by ensuring a high level of training for the staff involved in the various procedures. All animal work will be closely monitored to make sure that the procedures adhere to the principles of Replacement, Reduction and Refinement. The studies performed under this programme of work will be published in high- impact journals and presented at national and international conferences.

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

The adverse effects might include some discomfort and a potential increased stress level. However, these potential adverse effects will be monitored during procedures (e.g. heartbeat) and minimized using the best practise. For the reasons, the level of severity should be mild or no more than moderate. Fish in distress will be immediately culled by schedule 1. At the end of the procedure, the fish will be culled by Schedule 1.

Application of the 3Rs

Replacement

The research questions proposed in this project aim to define the critical physiological steps required for the maturation and function of hair cells. Currently we do not properly understand how hair cell synapses contribute to the processing of information within the nervous system, because we have not been able to monitor their operation within intact circuits in living animals. This is now possible with zebrafish because several transgenic lines expressing fluorescent proteins are available and, more importantly, zebrafish are small and transparent. This will allow

us to investigate how a large number of synapses cooperate to carry out an information-processing task. Unfortunately, there is no substitute for the integral sensory systems or brain in a living animal in order to understand how they work. Although animal models are still the only means to understand the intricate mechanisms implicated in the development and synaptic transmission in hair cells, we are currently working with colleagues to build a computational model of auditory function using the data collected by our experiments.

Reduction

All personnel working in the laboratory are trained to carry out all procedures involving the use of animals to a high level of competence, especially on the complex skills required to dissect the auditory tissue. This will keep the number of animals required to a minimum. We also have weekly lab meetings where we discuss any technical issues and evaluate (and revise if necessary) the experimental design.

We will consult our biostatistician regarding statistical analysis of our data to ensure that the optimum approach is being used to minimise the numbers of animals required to answer the questions posed. Where applicable we will adhere to the ARRIVE guidelines (https://www.nc3rs.org.uk/arrive-guidelines). The measurements obtained will be electrophysiological or imaging data from hair cells of neurons, mainly in the lateral line sensory pathway. Our aquarium staff has a wealth of expertise working with a variety of GA lines. This wealth of expertise will ensure that all GA lines bred will be kept to a minimum to maintain the line and to supply experimental stock for analysis.

As part of good laboratory practice, we will write a protocol for each experiment including: a statement of the objective(s); a description of the experiment, covering such matters as the experimental treatments, details of the experimental material, and the size of the experiment (number of groups, numbers of animals/group); and an outline of the method of analysis of the results (which may include a sketch of the analysis of variance, an indication of the tabular form in which the results will be shown, and some account of the tests of significance to be made and with the treatment differences that are to be estimated).

Refinement

The use of the zebrafish as a model demonstrates the use of a species of a lower neurological sensitivity compared with mammals. Moreover, zebrafish are potentially an excellent model system for hearing in mammals because there is a great deal of similarity in the two auditory systems at the anatomical, molecular and physiological

levels.

For embryos of fish that are at motile stages of development before they are protected by the Act, we still use best practise anaesthesia for all operations and embryonic surgery.

All personnel involved in the project will attend at least PIL A and PIL B of the Home Office course (as requested by the University) and will also undergo further schedule 1 training. This will ensure that all animals are handled competently. Personal Licence holders are thoroughly trained and carefully supervised until they achieve high competence in the regulated techniques they will apply.

Project 19	Regulation of mesendoderm formation in vertebrates
Key Words	Gene regulation, zebrafish
Expected duration of the project	5 year(s) 0 months

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

Purpose

Yes	(a) basic research;

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

This project aims to understand how tissues such as blood, muscle and bone (known as ‘mesoderm’) and gut, pancreas and lung (known as ‘endoderm’) form in an embryo. With a better knowledge of the genetic programs needed to create these tissues and organs we can apply this to making tissues and organs outside the body, by coaxing stem cells or other cells to use the same mechanisms. Such ‘in vitro’ generated tissues and cells can then be used to replace damaged tissue in humans or animals after injury or disease. Alternatively this understanding may allow us to reactivate embryonic programs to repair tissue in the body without the need to generate tissues outside the body for replacement.

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 ability to make tissues, or even organs, outside the body for transplant is a goal of modern medical science. However, currently scientists cannot precisely recreate tissues with 100% efficiency. For instance, during the process some cells may not form the wanted cell type but form another cell type altogether. In other cases the 3D structure of the tissue cannot be recreated and the cells do not work efficiently without that structure. In order to overcome these difficulties, it is necessary to study first the embryonic process of making tissues, so as to better recapitulate it in vitro. The formation of tissues and organs is controlled by genetic programs which are switched on and off by regulators in the cell, known as Transcription Factors (TFs). However, our knowledge of these genetic progams is incomplete, as is our knowledge of how TFs are themselves regulated. In this project we aim to use genomics technologies to identify components of these genetic programs and discover other regulators that modulate the activity of TFs. In doing so we will be able to characterise more precisely the genetic mechanisms that generate organs and tissues. This will aid in our ability in the future to efficiently recapitulate these mechanisms to make tissues outside the body. Refining these processes will help to

develop better therapies for replacing damaged tissue, which is expected to have enormous benefit to human health in the future

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

We will use Danio rerio (zebrafish) in this research. We expect to raise around 7000 genetically altered zebrafish over the course of this five 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?

We analyse the embryos produced when genetically modified adult zebrafish are allowed to mate. These adult zebrafish may carry a gene for a fluorescent protein, or they may have a genetic mutation. However since the fluorescent genes and mutations that these zebrafish have do not have any effect in the adults, they behave in the same way and produce fertilized eggs as normal zebrafish do, so there are no adverse effects. The expected level of severity for all our experiments is mild. When the zebrafish reach the end of their breeding life they are killed humanely in accordance with Schedule 1 methods.

Application of the 3Rs

Replacement

In vitro or in silico systems are not currently capable of fully replicating the complex nature of early embryonic development, specifically the morphogenetic movements of cells and precise spatial interactions between different cell types that bring about normal embryonic development.

Reduction

The latest technologies for introducing genetic changes in zebrafish cells will be used to ensure the procedure is as efficient as possible, thereby reducing the number of animals that need to be used.

Refinement

Zebrafish were chosen for this work for several reasons. Research over many years has shown that the basic mechanisms of tissue formation are the same in zebrafish as in frogs, chickens, mice and other vertebrates, and thus what we learn from a zebrafish embryo is applicable to other animals, including humans, and means we do not need to use a mammalian system such as mice to study this process. Zebrafish eggs are also laid into the water and fertilized immediately, which means the embryos are accessible from the earliest stages when the cells being studied begin to form making them the best model to efficiently study these early processes. In order to minimise harm, we will use the latest technologies to ensure we use the minimum number of zebrafish possible, we keep our zebrafish in a state-of-the-art

aquarium that has health monitoring systems to ensure optimal water chemistry. The zebrafish are monitored n a daily basis to ensure they are healthy and any that are not are treated, or if treatment is not possible they are humanely killed.