Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 12

Projects with a primary purpose of: Translational and applied research - Human Sensory Organ Disorders (skin, eyes and ears)

Project Titles and keywords

1. **Repairing the injured retina after blunt ocular trauma**
    - Eyes, Nerves, Blunt Trauma, Neuron, Death

2. **Circadian and photic regulation of physiology and behaviour**
    - Circadian, Light, Sleep, Retina, Aging

3. **Mechanisms and treatments of eye disease**
    - Blindness, eye, vision, gene therapy

4. **Biocompatibility of human decellularised matrices**
    - Cornea, Decellularised corneas, Biocompatibility, Wound Healing

5. **Novel Vitrectomy Cutter: Anim Surgery Study**
    - Ultrasonic Vitrectomy Technology

6. **Enhancing visual function in retinal disease**

Project 1	**Repairing the injured retina after blunt ocular trauma**
Key Words (max. 5 words)	Eyes, Nerves, Blunt Trauma, Neoron, Death
Expected duration of the project (yrs)	5 year(s) 0 month(s)

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

Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.
Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objectives of this project are to determine changes that occur after blunt trauma to the eye, which is connected via the optic nerve to the brain and relays information about vision. We are particularly interested in learning how neurons deal with the injury that makes them vulnerable to death,

	and the lack of axonal regrowth that follows traumatic eye injuries. This will allow for a better understanding of the mechanisms after blunt eye trauma and will help us to identify therapeutic drugs that will be used to protect nerve cells from death and encourage nerves to re-grow.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The project will provide important data that will improve our understanding of the changes that occur to the different neuronal cells in the eye after blunt trauma and provide an insight into what is required to promote photoreceptor (mixes light and colour) and retinal nerve cell survival, removal of scar tissue and promotion of nerve regeneration. This will underpin the discovery of novel therapeutic drugs that will be used to promote nerve cell survival, scar tissue removal and nerve regeneration. There are currently no treatments available. Therefore, advances in our research will benefit affected patients (civilian and military) who are increasingly being affected due to a variety of reasons including eye injury from car air bags, projectiles (e.g. tennis balls etc), closed fists and bomb blasts. In particular our therapeutics will be useful for soldiers coming back from war, of whom 15% suffer from such eye injuries and the disabilities that reduced vision causes
What types and approximate numbers of animals do you expect to use and over what period of time?	Rats: 1,500 Mice: 500 Over a period of 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?	Harm results from blunt trauma to the eye which will be created under general anaesthesia using a carefully calibrated apparatus that accurately fires a projectile at a pre-determined velocity at a specifically-defined location on the side of the eyeball. This causes a bruise that replicates the pathological features in humans suffering from blunt trauma. Importantly, our injury device will not cause the globe to rupture. In the vast majority of cases, there is no

	adverse response to the reduction in sight since rats/mice do not use vision as a primary sense. The maximum likely severity for all procedures is moderate, although some of the procedures will be mild. There are clear guidelines in place in our facility to ensure that suffering in animals is minimised by either administration of pain-killers or termination of experiments. Intravitreal injections/topical eye drops may cause irritation at the site of injection/application but in our extensive experience this has not been the case. As with the injury, we will remain vigilant for any adverse effects and will promptly provide pain relief or treatment if appropriate, or humanely kill the animal. Schedule 1 methods will be used to kill all animals or animals will be perfused with 4% paraformaldehyde under terminal anaesthesia
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-protected animal alternatives	There is no adequate substitute for using the *in vivo* models described in this application. Establishment of potential clinical relevance of regulatory molecules interacting in a dynamically changing central nervous system injury site can only be achieved in an animal model. Although it is not ethical to experiment on humans, our experiments are informed from human tissue samples from blunt trauma patients, including analysis of biomarkers and DNA from fluids from the eye and blood. The rat/mouse is our prototypic laboratory animal and has been rigorously characterised by ourselves for the blunt eye injury paradigm and shown to be representative of the human condition by us and others. The tools for the project have all been prepared in relation to the models described herein and continuity of the study in this species will be essential for significant progress to be made in a timely and efficient manner.

2. Reduction Explain how you will ensure the use of minimum numbers of animals	Our model is calibrated and is extremely reproducible, reducing the number of animals that we use. Some of the end-point measurements (e.g. axon regrowth, scar formation etc) may be essentially qualitative and for these we use 3-6 animals per treatment group. In most experiments with quantitative end-points, 6 animals are randomly assigned to each treatment group, a number calculated as the minimum required to provide statistically significant results. This has been determined on the basis of our previous experience with procedures, the methods of analysis and after consultation with statisticians to calculate power. Since blinded rats are not apparently debilitated, bilateral blunt eye injuries are used where possible to enable the size of treatment groups to be reduced by 50%.
3. Refinement Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The model selected closely resembles the features seen in humans after blunt trauma. Most therapeutic agents are evaluated and optimised *in vitro* prior to *in vivo* application. We keep our experimental time points in longitudinal studies to a minimum and use archival control results where possible. Multiple analyses are done on harvested tissues. We use the minimum number of interventions and minimal volumes for drug delivery during experiments and continually seek methods to reduce these by studying alternative drug delivery strategies. These refinement steps significantly reduce animal usage and severity. All injuries are performed under anaesthesia and animals are given pain relief for the first 24 hours after injury and thereafter are pain free. We closely monitor animals for signs of pain and treat any discomfort proactively. In general, animals do not show any adverse signs after blunt trauma.

<table>
<tr><td>Project 2</td><td colspan="2">Circadian and photic regulation of physiology and behaviour</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Circadian, Light, Sleep, Retina, Aging</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>X</td><td>Basic research</td></tr>
<tr><td>X</td><td>Translational and applied research</td></tr>
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<tr><td></td><td>Forensic enquiries</td></tr>
<tr><td>X</td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">This project aims to understand how the body clock drives rhythms in processes throughout our body, ranging from activity and sleep to learning and memory. These circadian rhythms are controlled by a master clock in the hypothalamus of the brain, which is set to the correct time by light detected by the eye.

Despite the enormous progress that has been made in this field over the last 20 years, the mechanisms by which the circadian system regulates these diverse responses is unclear. Moreover, this system can be disrupted by our environment, by disease and by aging. Why the circadian system is sensitive to such disturbances is also largely unknown.

Up to 30% of the UK population is involved in shiftwork, and due to jetlag as well as the use of artificial light and light-emitting electronic devices, circadian rhythm disturbances are increasingly common in modern society. Moreover, disturbances in circadian rhythms in disease and aging can</td></tr>
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	contribute to the symptoms of these conditions and therefore provide a tractable target for intervention.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	As a result of modern life, circadian disruption is increasingly common, occurring due to shift work, jet-lag and the widespread use of artificial lighting. In addition, it has become clear that circadian disruption in a common feature of many different diseases, as well as aging. Circadian disruption can result in impaired health and performance, as the internal body clock becomes out of phase with the external world. This also results in impaired sleep and quality of life. However, the underlying mechanisms by which circadian disruption affects health are poorly understood. As such, there are few treatments available. Understanding how the circadian system regulates our wider physiology and behaviour and how these processes go wrong will provide new therapeutic targets for circadian disruption.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will exclusively use mice. Over 5 years, we expect to use 13,500 animals.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of our work involves non-invasive measurements of physiology and behaviour. This is largely based upon monitoring the activity/sleep or performing behavioural tests in wildtype and genetically-altered mice to determine how they respond to different light/dark cycles and acute light exposure. This may involve singly housing mice or using mice with retinal or neuronal deficits. This work has few expected adverse effects. However, some behavioural tests require the use of food as a reward, which requires limiting the animal's normal food intake. However, this work also require measuring parameters such as the electrical activity of the brain. This involves surgically implanting devices that transmit this information, which can result in post-operative pain or discomfort, which will be treated

	with analgesics. Our work also involves administration of substances to modify circadian or light signalling pathways throughout the body, or more specifically at the level of the eye or brain. This involves injecting drugs or other substances to alter the expression of specific genes into the circulation or directly into the eye or specific areas of the brain. This may result in post-operative pain or discomfort, which will be treated with analgesics. In vivo studies are supported by ex vivo work on tissues collected 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	This work involves the study of complex physiology and behaviour in systems throughout the body. As such, no alternatives to animal models are available. However, we commonly employ cellular clock models to replace the use of animals where possible. Moreover, collaborations with groups studying human subjects and patient groups also replace the use of animals and inform our in vivo work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Breeding of genetically altered animals will be closely monitored to prevent over-production. Animal numbers will be reduced by careful experimental design (e.g. power calculation). In addition, we routinely archive our transgenic mouse lines to prevent over-breeding. Finally, we regularly employ randomisation and blinding to ensure our results are reproducible, as well as employing within-subjects experimental designs where each animal can act as its own control.
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	A primary aim of this project is to measure physiology and behaviour over multiple days without disturbing the animals. As such, we use a wide range of non-invasive methods, including measuring activity and sleep using movement sensors, video recordings of behaviour and measuring body temperature using thermal cameras (rather than implanted devices). We have also developed a range of assays of visual function in mice that do not require anaesthesia or

(harms) to the animals.	invasive procedures. Where used, all surgical procedures will be conducted with aseptic techniques with appropriate analgesia and post-operative monitoring. This will include home cage activity, sleep and/or body temperature measurements (as described above).

Project 3	**Mechanisms and treatments of eye disease**	
Key Words (max. 5 words)	Blindness eye vision gene therapy	
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 objective of the project is to develop and test treatments for human eye diseases. The project also aims to advance knowledge of disease mechanisms and degenerative processes in the eye. There are a number of ocular disorders (such as retinitis pigmentosa, glaucoma, macular degeneration , diabetic retinopathy) which result in blindness and for which there are no lasting or effective treatments. In the UK there are currently around 360,000 people registered with their local authority as blind or partially sighted and therefore there is a great public need for the investigation of mechanisms of eye disease and the development of new treatments for vision loss and blindness.	
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)?	It is expected that any positive results of this project will be used to apply for approval to perform human clinical trials. In the future this will lead to new or improved therapies for diseases and disorders of the eye and would therefore provide a direct benefit to human health and wellbeing.	

What species and approximate numbers of animals do you expect to use over what period of time?	The project will mainly use mice. Up to a maximum of 40,000 mice may be used over the entire 5 year duration of the project. A smaller number of rats (up to 500 at maximum) may also be used during the entire 5 year duration of the project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The project will use rodent (mainly mouse) models of human eye diseases and disorders. These are created through genetic alterations, experimentally induced methods, or a combination of both. In many situations, rats and mice rely on tactile whisker driven responses, rather than sight. Consequently, under standard husbandry conditions, visually impaired or blind rodents seem to be able to behave, grow and breed in a similar way to normally sighted animals. However, some of the methods needed to induce retinal degeneration have the potential to cause distress or welfare impairment to the animal. Rarely, rodent models will be used that have hearing impairment in combination with an eye disorder. This is because these problems are often associated in human conditions (such as Usher syndrome). Every effort will be made to reduce the welfare cost to these animals by the most refined husbandry methods. The project will also assess treatments for eye disease. These will include gene therapy, cellular replacement strategies, pharmacological intervention, dietary supplementation and environmental alteration. Most treatments used are expected to cause no adverse effects. However, some environmental alterations (for example, long-duration housing in constant darkness) have the potential to cause distress. Treatments will be assessed by physiological, behavioural, optical imaging and anatomical techniques. We will routinely perform visual electrophysiological testing and ophthalmological examination to assess visual function and eye health in the live animal. These are similar to techniques used in an ophthalmology clinic and are minimally invasive. Procedures for assessment of behavioural responses to light (for example, use of light tight environmental chambers) have the potential to cause distress. These will be

	conducted relatively infrequently and every effort will be made to ensure protocols are continuously refined to reduce animal suffering and distress. Control measures and humane endpoints are used so that any adverse effects experienced by animals are moderately severe at the maximum. However, most animals in the project will experience mild severity adverse effects or none at all. All animals will be humanely killed at the end of a specific set of procedures. They will not be kept alive and re-used for other experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals must be used for this project since the development of therapies requires assessment of efficacy and safety in live animals. The eyes of lower, non-mammal species are quite different to those of humans and so do not make good models to use to test potential clinical therapies in. Wherever possible, treatments will be initially investigated in the non-animal alternatives such as cells and tissue from human donors.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers will be kept to a minimum by using the most appropriate experimental design and statistical analysis. Wherever possible we will use technologies, such as imaging, to enable longitudinal studies in the same animal.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We want the treatments that are being developed to eventually be applicable for use in humans. For this we need an animal model with a physiology, immune system and eyes that are fundamentally similar to ours. Therefore a mammalian animal model must be used. Mice and rats will be used because they are well defined model organisms and well characterised disease model strains with inherited ophthalmological dystrophies already exist. To minimise potential harms to the animals as far as possible, we use the best possible equipment for experimental eye surgery and assessments. We often use clinical grade equipment that has been

	modified for animal use. We make sure our methods are conducted within the internationally recognised guidelines for animal eye research, laid down by the Association for Research in Vision and Ophthalmology (ARVO). We also make sure anyone conducting complicated procedures is highly skilled and adequately trained. Many people working on the licence will be clinical ophthalmologists, specialised in human eye surgery and transferring those skills for use in animals.

Project 4	**Biocompatibility of human decellularised matrices**	
Key Words (max. 5 words)	Cornea, Decellularised corneas, Biocompatibility, Wound Healing,	
Expected duration of the project (yrs)		
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)	Corneal blindness is a major cause of visual impairment globally. Traditionally, corneal blindness is treated using transplantation of a donor cornea, however, there is a shortage of donor corneas worldwide and many donated corneas are not considered suitable due to non-structural reasons. We have developed an alternative to donor corneas, derived from the corneas that are deemed unsuitable. These corneas have been treated to create a non-viable corneal replacement, known as Theagen. We plan to test the safety of Theagen by implanting it below the skin (a subcutaneous dorsal flank model) in adult rats, to see that it does not trigger an immune reaction within the rat.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or	Current treatments for corneal blindness require a large donor pool, which is not accessible in many parts of world. This project will prove that Theagen is a safe product that does not produce an	

animals could benefit from the project)?	immunological response and will be the first step in creating a commercial product. Theagen will be available as a dry product that can be easily shipped and stored on the shelf. This will allow surgeons and patients in this country and in many developing parts of world, access to treatments that were not previously available. The scientific benefit of this project will be realised through peer-reviewed publications and further grant applications developing the work.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use between 23 and 29 adult Wistar rats for 4 different experiments for this project over a period of 4 months.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The expected severity is moderate as we plan to use anaesthesia, analgesics and antibiotics in the initial stages of the study, until primary healing is achieved, to minimise the discomfort to the rats. We anticipate that the surgery will cause some discomfort but this will be controlled with appropriate pain relief. The animals will be euthanised at the end of each experiment and the implant and surrounding tissue collected for further analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In vitro assays that are designed to evaluate biocompatibility and immune sensitivity mimic only one process, not the entire foreign body response. This is not a good representation, as you cannot see the interplay of the entire physiological system. It is not feasible to produce an adequate model of this complex system *in vitro* therefore; the project requires a living animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This confidence in concept study will use the minimum number of animals to provide safety and efficacy data to demonstrate Theagen does not elicit a foreign body response, along with exploring the biocompatibility with host tissue. The study mirrors that designed by our collaborator in their recently published paper, which involved statistician advice.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The rats will be acclimatised for one week, then put under anaesthesia and have an implant surgically placed in a pocket under the skin in the flank. At research time points, rats will be euthanised, and the implant and immediate tissue will be removed and analysed. The size of Theagen means the implant will sit comfortably in vivo without causing excess irritation. Rats are also the accepted animal model for evaluating foreign body responses to implants. The animals will receive the appropriate pre- and post-operative analgesia. The surgery will be performed under full anaesthesia. Subcutaneous implantation is a standard procedure is known to cause the least amount of irritation and discomfort due to the least amount of surgical/implant induced damage. Animals are known to behave normally almost immediately after surgery. Any lasting discomfort can be managed through a simple pain relief regime. The minimum time point required to achieve data in this project is one week, which will allow for inflammation due to surgery to subside and any effect of the implant to be seen.

Project 5	**Novel Vitrectomy Cutter: Anim Surgery Study**	
Key Words (max. 5 words)	Ultrasonic Vitrectomy Technology	
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)	Pars plana vitrectomy (Vitrectomy) is a surgery to remove the vitreous humour, the transparent jelly, from inside the eye. The vitreous is located behind the iris and the lens, and it is in front of the retina. The general indications for a Vitrectomy are: retinal detachment, macular hole, diabetic vitreous haemorrhage, removal of intraocular foreign body, removal of membranes (scar tissue) from the retina, endophthalmitis or post-operative intraocular infection and complicated cataract surgery. All of these are serious and potentially blinding conditions, if left untreated. The retinal surgeon makes between two and four, depending on the case, small cuts through the sciera or white part of the wall of the eye. Instruments are passed through these cuts, including a tiny light source, a cutting and aspiration device, called vitrector or vitrectomy cutter, and an infusion cannula. The cutting device cuts and aspirates the vitreous gel while the infusion port replaces the gel removed with fluid so that the ocular architecture is maintained. The	

	procedure normally takes 1-2h in a non-complicated case. Possible complications during the procedure are, amongst others: Bleeding inside the eye, holes occurring in the retina due to tractional forces induced by the vitrector and Retinal detachment. Although commercially available vitrectors that rely on guillotine cutting are generally accepted as effective by ophthalmic surgeons, they have several limitations. Some of these limitations may not be broadly recognized by surgeons, as users frequently have difficulty perceiving defects without some defect free alternative to compare to. The current study aims to determine the effectiveness and safety of a novel vitrectomy device which we believe will reduce surgical time, damage and complications and to compare it to current vitrectomy devices.
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 study should provide important information to help us to decide whether this new technology is safe and effective to be tried in a pilot study in-vivo in human eyes. This novel technology could be revolutionary to ophthalmic surgery and become a game-changer in vitreo-retinal surgery. The liquefaction and excision of the vitreous body using low power ultrasound harmonics is a promising new alternative to guillotine pneumatic vitrectomy systems, which we hope will allow for the fabrication of lees invasive intraocular instruments (smaller gauge), reduce surgery time, reduce the intraoperative complications, reduce post-operative discomfort and speed up the postoperative recovery time. Shorter surgical times will enable more patients to be operated on per theatre session which would reduce the time patients are currently waiting for surgery with consequent (and crucial) cost savings clinically. Thus, this novel technology could potentially be of huge benefit to both patients and the medical profession.
What species and approximate numbers of animals do you expect to use over what period of time?	20 commercially available pigs will be used over 36 months.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All procedures will be conducted under balanced general non-recovery anaesthesia, meaning that the animals will be rendered insentient throughout the experimental procedure and will not experience any suffering beyond the induction of general anaesthesia. As such, no adverse effects to the animals' wellbeing are anticipated.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The determination of the anatomical changes associated with the use of this novel technology requires the presence of in vivo model as the natural occurrence of changes in the tissues following death. Hence the need for the evaluation of.this novel cutter in-vivo and in an animal model. Likewise, It is not ethical to conduct experiments on humans in vitrectorny surgery studies, especially where those experiments require the removal of the eyes for the histopathological investigations.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Previous use of in-vitro methods (flow tests, molecular assessments and histopathological studies) allows us to obtain valued information about this novel technology and it limits the numbers of animals required for the in-vivo investigation stage. The use of High Speed Video Script and surgical records allows a longitudinal assessment of this novel technology. This strategy also reduces the number of animals required. Furthermore, the storage of all of the unused tissue in the histopathological studies at the end of our investigations could allow us to perform further studies without recourse to perform new animal live experiments, being this, another additional way to reduce the number of animals required.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	Its morphology is similar to the human eye. Anatomical and structural differences with the other models. Size of the eyes similar to human ones. All animals, including controls, will be under GA to reduce or avoid any operative discomfort or painful experience during the vitrectomy procedures. The

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	pigs will be terminated at the end of the procedures with an overdose of anaesthesia, and the eye removal for the investigation studies will be done after death.

Project 6	**Enhancing visual function in retinal disease**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall objective of this project is to develop new and enhanced methods for enhancing vision in animal models of human retinal disease. This will be done using human-stem cell derived retinal cells and novel pharmacological approaches. There is a huge clinical need being addressed here, with dry age-related macular degeneration being the leading cause of irreversible visual impairment amongst elderly people in developed countries. There is currently no effective treatment for this disease. Also, as we still understand very little about the basic biological processes underlying retinal degeneration, this project also seeks to discover more about the neuronal changes that occur during retinal degeneration with reference to the normal retina.	
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 offers the prospect of developing new more effective differentiation methods for generating functional human retinal pigment epithelium (RPE) cells for use in the treatment of patients with macular degeneration. Because dry AMD is a disease with complex aetiology and no effective treatment, cellular therapy with fully functional stem cell derived RPE currently offers one of the best chances of an outright cure. In addition to the obvious clinical benefits of generating functional RPE cells for use in patients, this work will also extend our understanding of the	

	basic biology of human RPE cells and the degenerative processes underlying retinal degeneration. Our experiments examining retinal neurotransmission are also likely to identify new potential drug targets for improving vision in patients with retinal disease.
What species and approximate numbers of animals do you expect to use over what period of time?	During this project we will be using established rodent (rat and mouse) models of human retinal degeneration. We expect to use approximately 320 mice and 160 rats per year of this study.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The breeding of retinal degenerate rats and mice has no adverse effects on these rodents other than those anticipated for the visual system. The procedures that we intend to perform on the animals are well established and we anticipate few problems in terms of adverse effects. If adverse effects occur they are likely to be moderate but there is the possibility that animals could experience occasional severe adverse effects (i.e. unexplained death). In this instance, the protocol in question will be modified to stop the future occurrence of such effects. At the end of this study, all animals will be killed for histological analysis of the retina.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although we make extensive use of *in vitro* (laboratory-based, non-animal) methodology in this project, there is currently no effective way to model vision outside the living animal. As such, we have to use animals in this study in order to reliably determine if human stem cell-derived retinal cells can actually function to sustain vision. The cells must be capable of supporting retinal function *in vivo* (i.e. in living animals) to be successful in the clinic and the use of animals in this project is therefore essential to achieve this aim.
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 procedure

	only the minimum number of animals required to produce valid statistical comparisons between experimental groups. Also, our decision to transplant human cells will be guided by the performance of these cells during pre-transplantation *in vitro* functional assays. This methodology will reduce the likelihood of us transplanting cells, which are functionally ineffective. We will also monitor breeding of genetically altered animals closely in order to ensure that only the required number of mice and rats are produced for use in this project.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats and mice will be used in this project as the retina and visual system of these rodents has been widely studied and used to great effect to further our understanding of human vision and to develop viable treatments for ocular disease. The specific rodent models we will use to recapitulate retinal degeneration are well established and have proven ability to aid in the development of new therapeutic agents for the treatment of retinal disease. We will make every effort to minimise welfare costs throughout this project by conducting the minimum number of procedures on individual animals required to meet our scientific objectives. In order to minimise suffering, we will administer analgesia after every surgical intervention and monitor experimental animals carefully for signs of distress or abnormal behaviour following any regulated procedure. Any animal deemed to be suffering will be removed from the study using an accepted humane method of euthanasia.