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Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 28

Project Titles and key words

- Gait Analysis in Mice

  Gait, kinematics, mice

- Salmonella Virulence

  Bacterial, pathogen, virulence, immunity

- Immunogenicity and vaccinology of bacterial pathogens of man

  bacteria, vaccines, Salmonella, Chlamydia

- Mechanisms underlying brain dynamics

  Sensory processing, sleep, EEG, neurons, synapses

- Molecular pathways in neuroregeneration

  Spinal cord injury, stroke, neuroregeneration

- Rodent Brain Activity Map

  Brain electrical activity, perception, behaviour

- Understanding how synapses work in health and disease

  Neuroscience; Brain; Synapses; neuropsychiatric diseases

- Development of vaccines for infectious diseases and cancer

  Vaccines, cancer, influenza, hepatitis B, peptides

- Immunology, pathogenesis & transmission of orbiviruses

  BTV, Immunology, Reassortment, Pathogenesis, transmission

- Characterization of a KY dependent hypertrophic pathway

  Muscle, hypertrophy, atrophy, z-disc

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Gait Analysis in Mice</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Gait, kinematics, mice</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>2 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This is a preliminary study in a programme that aims to examine the effects of osteoarthritis on the shape of bones in affected joints. It is already known that osteoarthritis (OA) is associated with a progressive degeneration of articular cartilage and bone destruction. However, these changes in bone shape have not been quantified. Within this project gait analysis data will be obtained and then combined with computer modelling, to provide information about 3D bone shape in normal mice and provide a better understanding of the relationship between limb function and bone anatomy in healthy animals. The same approach will subsequently be applied to animals with OA.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This study will to relate bone shape to function and lead to the use of gait analysis and computer modelling to study and predict joint disease progress and effects. Other musculoskeletal conditions might be also explained by studies using the techniques developed in this project.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>For this project 15 adult wild type mice will be used.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the</td><td>The only possible adverse effects are nicking of the skin during shaving, or perhaps slight skin tearing on removal of the markers (attached by skin adhesive). These are both highly unlikely; skilled personnel with experience of both techniques will be used and all attention will be taken to avoid</td></tr>
</table>

[1] Delete Yes or No as appropriate.
[2] At least one additional purpose must be selected with this option.

end?	these complications.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The long term aim of this project is to measure bone changes due to OA. Initial data of bone shape and limb function in normal, healthy mice are therefore essential. The data from this work can also form the baseline for studies of other musculoskeletal conditions; this study complements and quantifies the work of others without adding to the animal burden.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Since the animals are unaffected and unchanged by the gait analysis, and no surgical procedures are required to be performed, the minimal amount of harm or effect is induced and the minimal number of animals is required. Forty normal mice will provide sufficient data for meaningful statistical analysis of all parameters measured. These data can be compared with the known variability of gait studies in other species or other techniques, and will then serve to support decisions about the number of animals required for specific studies of OA.
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 smallest rodents for which accurate gait analysis measures can be made. Genetic strains of mice are available which exhibit spontaneous joint diseases, which can then serve as models of the diseases to be studied. The animals will be anaesthetised by inhalation for marker placement (which is painless) and removal will be achieved painlessly; there are no aspects of the procedure which cause pain or suffering.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Salmonella Virulence</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Bacterial, pathogen, virulence, immunity</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td>
<table>
<tr><td>Basic research</td><td>Yes√</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>In this project we will study mechanisms by which Salmonella causes disease and how our immune system works to resist this. Infection of humans with Salmonella can lead to gastroenteritis and typhoid fever, depending on the strain type. It is estimated that over 90 million cases of Salmonella gastroenteritis and 13 million cases of typhoid fever (with approximately 130,000 deaths) occur globally each year. There is no vaccine against non-typhoidal Salmonella and current typhoid vaccines provide only partial protective efficacy.
Infection of humans with Salmonella Typhimurium usually results in self-limiting gastroenteritis but this strain causes a systemic disease in mice that is similar to human typhoid fever. This very useful model system has been exploited intensively over the years and has provided a great deal of information of the pathophysiology of the infection process, the basis of host defence and immunity, and bacterial virulence factors involved. Much of this information is known to be relevant to human disease and has been exploited in the design of a novel vaccine against typhoid fever, which has been shown to be safe and immunogenic in clinical trials in humans.
A large part of Salmonella pathogenesis is associated with its ability to grow inside host cells, and we make extensive use of cell lines of human and mouse origin to study the biochemistry and cell biology of infection. However, these systems can never fully represent the complex environment and host response to infection that occurs in vivo. For example, an essential aspect of the host immune</td></tr>
</table>

[3] Delete Yes or No as appropriate.
[4] At least one additional purpose must be selected with this option.

	response involves communication between different cell types and their activation. These interactions and responses are highly coordinated in time and space, involve cellular migration and occur both within and between different tissues. Therefore, some work on living mice is unavoidable to properly assess potential virulence defects, bacterial population dynamics and spread throughout organs as well as immune and other cellular responses. We will study the effects of deleting genes of *Salmonella* with respect to its ability to multiply and spread in mouse tissues. We will study the immune responses of mice to these strains, taking advantage of mouse strains that are already available and which have known immune defects. We will also exploit state of the art imaging techniques to be able to follow the infectious process over time in a non-invasive manner. Animal suffering will be minimised by regular checking of animals for relevant symptoms that constitute the end point of the experiment, and the use of mixed infections to eliminate mouse-to mouse variability and hence the number of animals required to achieve statistical significance.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Through this research we are likely to discover new processes of pathogen and host cell biology, which could have implications for other important pathogens that propagate within our cells. Our work is also likely to provide valuable information for designing vaccines, which are still needed to provide effective long-term protection against *Salmonella* and other bacterial pathogens.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 6000 mice will be used over the 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?	Depending on the dose, the inoculation route and the strain of mouse, between 2 and 180 days after inoculation the majority of animals inoculated with *Salmonella* will develop mild to moderate symptoms of systemic infection consisting of reduced activity, hunched posture and ruffled fur. We will assess animals for these symptoms and those that display all three symptoms will constitute the end point of the experiment and will be killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our aim is to understand the pathogenesis of *Salmonella* (a bacterial pathogen which causes a wide variety of diseases in humans and other animals) and to learn more about host processes that influence the outcome of infection. An essential

	aspect of this work involves testing the pathogenic potential of different bacterial strains in the well-established murine model of infection. This helps us to establish the importance of genes that play critical roles in the pathogenic process and provides information about their interactions and possible use in vaccine design. Much of our work involves experiments in which *Salmonella* grows in immortalized host cell lines. However, these systems can never fully represent the complex environment and host response to infection that occurs *in vivo.* For example, an essential aspect of the host immune response involves communication between different cell types and their activation. These interactions and responses are highly coordinated in time and space, involve cellular migration and occur both within and between different tissues. Therefore, some work on living mice is unavoidable to properly assess potential virulence defects, and immune and other cellular responses.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All experiments will be designed so that the minimum number of animals necessary will be used. Breeding strategies are performed by qualified personnel and aimed to avoid unnecessary animal generation (animal surplus). A competitive index assay has been designed to evaluate the virulence of mutants in which a 1 to 1 mixture of wild type to mutant bacteria is inoculated into each animal. Because of the lack of inter-animal variation, significant differences in the virulence of strains can be obtained using fewer animals. Bioimaging will reduce the numbers of animals used due to serial imaging.
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 appropriate species in which to model systemic infection by Salmonella. The mouse model is especially appropriate as it enables (1) measurement of bacterial virulence by enumeration of colony forming units of bacteria at different time points and from different organs following inoculation by the oral, intraperitoneal or intravenous routes, (2) population dynamics of the pathogen to be analysed by new methodologies involving mathematical modelling and (3) whole body imaging of the process of disease progression using live animals. This form of imaging will reduce the numbers of animals used due to serial imaging, and refine the procedure by allowing infection to be monitored more closely. Animal suffering will be minimised by regular checking of animals for relevant symptoms that constitute the end point of the experiment, and the use of mixed infections to

	eliminate mouse-to mouse variability and hence the number of animals required to achieve statistical significance.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Immunogenicity and vaccinology of bacterial pathogens of man.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>bacteria, vaccines, Salmonella, Chlamydia</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Bacterial infections represent a major challenge to global health. In the developed world, the rise of anti-microbial resistance poses new threats to healthcare in hospitals and the community. In the developing world, the lack of vaccines and preventative measures contributes to bacterial infections being ranked as leading causes of mortality and morbidity. In this project, we will be studying i) how selected molecules derived from globally important pathogens contribute to the process of bacterial invasion and the development of disease and ii) whether these molecules can be used to develop new vaccines to prevent bacterial infection. We are focusing on two bacterial pathogens: Salmonella causes disease ranging from gastroenteritis through to typhoid fever; different types of Chlamydia cause either sexually transmitted disease or blindness. We first select a small number of molecules on the basis that they have good candidacy as virulence factors or as vaccine candidates. This information comes from the scientific literature or from the results of our own research using chemical analysis, cell culture or computer modelling. The molecules are then isolated from the bacteria or produced by genetic engineering in other bacteria, so that we can test how the body responds when it is exposed to them. If the results suggest the immune response can target these molecules, then we will use them to vaccinate mice and see whether the immune response that is induced protects from infection. The results we obtain from these studies will determine whether there is sufficient evidence to</td></tr>
</table>

[5] Delete Yes or No as appropriate.
[6] At least one additional purpose must be selected with this option.

	take these molecules forward as potential vaccines for use in man.
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 bacterial pathogens that we are studying all cause serious diseases in humans, and some also affect the health of domestic and farm animals. The major benefits from our research will come from the translation of new knowledge on how these bacteria cause disease into new vaccines to prevent infection or delay the development of disease.
What species and approximate numbers of animals do you expect to use over what period of time?	This research will use approximately 300 mice per year for five years. About one third of these will only be injected with molecules derived from bacteria to see how the body responds. Two thirds of the mice will be used to test whether these molecules can be used as vaccines against bacterial infection.
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?	Mice injected with bacterial molecules are not expected to show any ill health beyond a slight local response to the injection (as we might get upon vaccination). To test vaccines in mice, we will first immunize them and then they are challenged with live bacteria. Bacterial infections may make the animals sick, for example displaying lethargy and or fever, but humane endpoints are used to minimize suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although we have made advances in studying how the body responds to infection using test tube approaches, and in making computational models of how the immune system behaves, it is not possible to reproduce the response of an intact animal to vaccination in this way, or to see how bacterial molecules affect the body to cause disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We do not randomly screen for bacterial molecules but evaluate only those that pass strict criteria that make them likely to work. These experiments are often done in the test tube or on a computer. When we test vaccines in mice, we design experiments to maximize the information from the minimum number of animals e.g. running experiments in parallel to reduce the number of control mice required. We have developed imaging tools that let us see bacteria non-invasively in live mice. This reduces the number of animals needed in each experiment, as we can now measure bacterial numbers in the same individual over time. **Experiments are designed with due reference to randomisation and the avoidance of bias, and we report the experimental design and results in a way that is easily verifiable and reproducible.**

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 immune systems comparable to man and the resources available to study mice allow much more information to be collected than if other species of animal were used. We use bacterial infection models validated worldwide for drug and vaccine studies and that induce the minimum of ill health in the mice compatible with the goals of the study.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Mechanisms underlying brain dynamics</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Sensory processing, sleep, EEG, neurons, synapses</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td>~~Yes~~</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project aims to understand ways in which coordinated brain electrical activity corresponds to the objects we sense, how this is consolidated during sleep and how this can be deranged in diseases associated with learning disability.

Our current understanding of how the brain works is based on observations that show changes in electrical activity produced by sensory inputs. Patterns of changes occur over time and in different parts of the brain and can produce a response – for example a motor output (movement of arms and legs etc.) or emotional output (feelings of happiness or excitement). How these responses are generated and how they are modified by sleep remain unknown.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Data arising from this project will then be used to construct biologically accurate computer models of large-scale brain function. The output from these models reduces the number of animals needed for such studies and can be used predictively to interpret the types of activity seen in conventional EEG recordings in healthy subjects and patients with cognitive dysfunction to aid diagnosis and treatment.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Over the 5 year time period of the project we expect to use 1950 rats and 400 mice.</td></tr>
<tr><td>In the context of what you</td><td>The majority of procedures to be performed are of</td></tr>
</table>

[7] Delete Yes or No as appropriate.
[8] At least one additional purpose must be selected with this option.

propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	an 'unclassified' level of severity – the procedure will be akin to terminal anaesthesia used to humanely kill animals in veterinary practice. A small number of procedures are classified as moderate and may lead to temporary stress in affected animals. At the end of ALL procedures animals will be killed by terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals in order to gain access and manipulate small areas of brain tissue in a way that is not ethical in humans. However, we are expanding our capability to use post-surgery tissue from human patients to replace animal use as much as possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have been very successful in generating and using detailed computational models of brain activity over the last 20 years. Refining models reduces the number of animals needed to reach experimental and clinical end-points.
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 provide the ideal balance between level of cognitive sophistication and availability of scientific precedents. Mice are necessary owing to the ease of making transgenic constructs. Any potential harm to the animal will be minimised by strict adherence to approved procedure methods and overall high quality of animal husbandry.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">MOLECULAR PATHWAYS IN NEUROREGENERATION</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Spinal cord injury, stroke, neuroregeneration</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes
X</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</td><td>Yes</td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Stroke, multiple sclerosis, brain and spinal trauma are diseases with high incidence and prevalence that lead to severe long-term neurological disability. To date, there is still a lack of effective treatment that may limit long-term neurological dysfunction and disability, mainly due to a lack of understanding of the pathophysiology of these disorders. It is therefore an urgent scientific priority that novel therapeutic approaches are developed. The success of this will depend upon both a better understanding of the biology of the nervous system and upon the development of novel technologies. However, our understanding of how neurons respond to brain and spinal injury is still incomplete and this limits the design of effective therapeutic schemes.
Here we would like to investigate the molecular reasons for the failed ability of the nervous system to regenerate and recover after injury in animal models that allow studying the mechanisms of human stroke, spinal cord injury and multiple sclerosis. These animal models include experimental stroke and nerve injury, with emphasis on spinal cord injury. These diseases are still orphan of a therapy that may benefit neurological recovery. Our studies aim to clarify the reasons for failed regeneration and recovery and to test new regenerative therapies to promote recovery of function. The final goal is to transfer this knowledge into the clinic, limiting human suffering and social burdens.</td></tr>
</table>

[9] Delete Yes or No as appropriate.
[10] At least one additional purpose must be selected with this option.

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)?	These studies may benefit human diseases of high importance such as stroke, spinal cord injury and multiple sclerosis. Currently, neuroprotective strategies available for these disorders do not have a visible clinical impact on long-term disability and patient suffering. Our work in animal models may provide understanding of how neuroregeneration, essential for neurological recovery, is regulated at the molecular level. This will allow the design of novel therapies to enhance regeneration that would drive functional recovery in these human diseases, limiting disability and suffering.
What species and approximate numbers of animals do you expect to use over what period of time?	About 4500 mice
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The expected adverse effects will be impaired neurological function and pain of moderate level. In the first week mice will have impairment in hind limb function and in bladder voiding. However, in all the injury models used mice recover spontaneously (visibly after 1 week) to an extent sufficient for them to eat and drink independently. In fact, the spinal lesion we will inflict allows for some level of physiological recovery, being the most refined lesion model that has clinical utility for spinal cord injury. At the end of the experiments, animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The goal to develop novel therapies for human neurological disorders must be tested in animals, as in vitro cell culture based systems do not provide the sufficient biological complexity proper of human organs and diseases.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In vitro testing in cell culture will be extensively used to verify initial hypothesis before investigation in animals. In addition, only the necessary number of animals needed to reach statistical significance for each given experimental question will be employed. A common sense approach will also be adopted whereby if our treatment strategies were clearly having no effect those particular experiments would cease and our strategy revised or revoked.
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	Mice are used extensively in neurodegenerative disease research, including for neuroregeneration in models of nervous system injury. They allow reliable data for comparison in humans. These rodents are relatively low order sentient animals (i.e., compared with non-human primates, cats, dogs, etc.,) however these species are accepted by

minimise welfare costs (harms) to the animals.	the scientific community as useful animal models for the kind of basic and translational research we intend to carry out. In addition, based upon the need for data cross comparison and the availability of a large number of transgenic lines for mice, mice are our species of choice. Our experiments will include moderate but not substantial severity protocols. In fact, we strived to choose the most representative animal models that resemble these human diseases and are good predictors of effectiveness of treatment under investigation without resorting in the substantial severity. Additionally, suffering will be principally minimised by optimal operating technique and providing adequate analgesia and good post-operative care. Whenever there will be a choice of route of administration of drugs or virus the least invasive will be adopted. Pain and distress will be carefully monitored after lesion and appropriate measures will be taken if signs of pain/distress will be observed.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Rodent Brain Activity Map</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Brain electrical activity, perception, behaviour</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[11]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>(Yes)</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To arrive at a fundamental understanding of how the brain works, we need to observe the electrical activities of very large numbers of identified neurons in a brain during its normal operation. Current electrophysiological approaches towards this goal provide a low spatial resolution (electroencephalogram, local field potentials, etc) or focus on local circuits with single cell resolution (single cell microelectrode techniques, two photon cellular resolution imaging) but miss the “bigger picture”.
The basic vision of our research is use innovative optical methods based on genetically encoded indicator and actuator proteins to monitor and control electrical activities of neurons, respectively, to overcome these limitations and to analyze the spatio-temporal dynamics of neuronal communication in the upper layers of cerebral and cerebellar cortical during different brain states and behavior.
We expect that further development and generalization of this approach will have a broad impact in cognitive sciences and the understanding of diseases that have been associated with dysfunctions in large-scale co-ordination of neuronal networks such as schizophrenia and autism spectrum disorders.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Many of the devastating brain-based pathologies known in medical practice have neither cures nor effective treatments, in large part because it is difficult to provide a treatment for a dysfunctional organ when one does not know how it works.</td></tr>
</table>

[11] Delete Yes or No as appropriate.
[12] At least one additional purpose must be selected with this option.

	Understanding of our brain's circuits will facilitate development of advanced cures and effective treatments of diseases that have been associated with dysfunctions of neuronal networks. Moreover, the fundamental importance of generating a fruitful theory of brain function lies in the fact that as humans, more than any other species, we are defined by the higher cognitive abilities generated by our brains. Thus, scientific understanding of our brains will enable deeper knowledge of ourselves and of our minds, all of which would be highly beneficial.
What species and approximate numbers of animals do you expect to use over what period of time?	The current project will last 5 years. For the experimental work, we will need to use rodents (mice or rats).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We like to treat animals in a way that they don't feel unnecessary pain or distress. However, surgery under general anaesthesia will be necessary to observe brain activity. Since recovery from surgery is likely an adverse effect to the animal, we rate the level of severity as up to moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All experiments that can be done in cultured cells will be done in those, using methods to minimize the number of animals. We also use computer simulation to explore possible biophysical mechanism. Currently, we can reliably study higher brain functions such as perception, motor control and learned behaviour only in living animals. Rodents are likely the simplest models in which brain processes that underlie human cognition can be studied with confidence.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Although scientifically driven, our approach that combines several methods (e.g. imaging, anatomy, and behaviour) in single animals enables far more data to be obtained from single animals than would be obtained from a much larger number. The use of minimal number of animals will be further dictated by the high cost involved in animal care and welfare.
3. Refinement Explain the choice of species and why the animal model(s)	Rodents are among the simplest mammals in which to study functions that depend on the cerebral cortex.

you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice (and to a lesser extent rats) are currently the species of choice in most areas of biomedical research, where genetic manipulations such as production of transgenic animals have already been well refined. All surgical procedures will be conducted with state-of-the art techniques for analgesia and anaesthesia. All procedures are based on protocol steps that have been refined over many years. However, we will strive to find additional refinement, if possible at all.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Immunology, pathogenesis & transmission of orbiviruses</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>BTV, Immunology, Reassortment, Pathogenesis, transmission</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Bluetongue virus (BTV) is the most commonly known orbivirus having recently emerged in Northern Europe including n incursion the UK in 2007-2008. Orbiviruses are mainly transmitted between their mammalian hosts by blood-feeding arthropods – in the case of BTV these are Culicoides biting midges.
For BTV many different serotypes exist – 26 have been discovered so far however neutralising antibodies only protect against the homologous serotype.
Additionally these viruses have a segmented genome- allowing for genome segment exchange once two different viruses infect the same animal and/or the same midge (which occurs frequently in the field). This may entirely change their characteristics including their serotype
The protocols outlined in this licence will contribute key knowledge to the following currently unknown and essential questions:
1. What are the cellular immune responses such as T-cell responses in ruminants infected with BTV or EHDV ?
2. Can these cellular immune responses offer some protection of disease in infected ruminants even between different BTV serotypes?
3. What is the local immune response of the skin to the virus as well as to arthropod saliva?
4. Is the local immune response of the skin essential for virus dissemination and</td></tr>
</table>

[13] Delete Yes or No as appropriate.
[14] At least one additional purpose must be selected with this option.

	systemic immune responses? 5. Do immune responses play a role in the pathogenesis of these viruses (rather than being protective) and are they different between sheep and cattle? 6. How virulent are newly emerging strain for the UK sheep population? 7. How do these viruses exchange their genome in the mammalian host and how does this effect virulence, the immune response and the uptake by blood-feeding arthropods? 8. Do other important alternative transmission pathways exist which do not rely on biological arthropod vectors and what is their significance?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By advancing our understanding on the above summaries essential knowledge gaps the proposed works is envisaged to create the following benefits: - Contribute to better BTV/ EHDV vaccine development for ruminants which may cross-protect between serotypes - Understand the local immune response of the skin towards arthropod transmitted viruses (arboviruses) and its role in pathogenesis, persistence and onwards transmission – such knowledge would additionally be transferrable to human arboviruses - Elucidate the immune response to arthropod saliva and the effect on the incoming virus to inform vaccine development - Design more appropriate ruminant population control measures as virus transmission pathways are better understood - Identify factors that contribute to the severity of disease in sheep in comparison to mostly asymptomatically effected cattle and develop potential individual treatment options - Understand the mechanism of genome reassortment in ruminant hosts and identify genome segments responsible for virulence and transmissibility. Again this could lead to more specific vaccine development as well as better population control especially in those areas where several serotypes of BTV co-exist (co-emerge)
What species and approximate numbers of animals do you expect to use over what period of time?	Mostly ruminant species will be used in these projects as they are the natural hosts of the viruses investigated, hence all obtained result are directly significant.

	The exact numbers of animals used will partly depend on newly emerging strains of BTV and/or EHDV within Europe. Currently we are envisaging of using between 20-40 sheep and between 10-20 cattle year with occasional goat experiments across all protocols. However in response to new BTV outbreaks this number may have to increase. Most animals will be kept under the protocol for 28-35 days with the exception for the lymph-vessel cannulated animals and vaccinated animals, however if later infected with BTV or EHDV these animals will also most likely be kept between 28-35 days post infection. Occasionally if indication of viral persistence has been detected infected animals might be kept beyond 35 days post infection to monitor such viral persistence. Mice, guinea pigs and rabbits will also be used to raise essential monoclonal and polyclonal antibodies. The numbers will not exceed 20 mice/ year , 15 rabbits/ year and/or 15 guinea pigs/ year
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The proposed work is centred around BTV/ EHDV virus infection studies (by different routes) which will then analyse the immune response, virus replication, and duration of transmissibility to blood feeding arthropods. This analysis will be based on regular sampling of infected animals mostly blood but in certain cases also for lymph through insertion of a cannula into lymph vessels or skin biopsies. The expected adverse effects are therefore associated with the development of clinical disease following virus infection. Cattle and goats are mostly asymptomatically affected by BTV and EHDV. However depending on specific virus strains sheep can become severely diseased following infection with bluetongue virus. The protocols have humane endpoints in place which will not allow any sheep to develop clinical disease beyond the moderate severity banding. Wherever possible scientific endpoint have been implemented which do not require the development of clinical disease. All animals will be euthanized by a schedule one method either at the scientific endpoint or when approaching the humane endpoint
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The immune system is highly complex and inter-related and understanding of the immune response in infectious disease requires the use of living animals. Additionally our current knowledge of the immune system in any vertebrate species does not

	allow protection afforded by different vaccination strategies to be predicted. We perceive it as a specific strength that the studies into the immune response and pathogenesis of BTV and EHDV proposed under this licence will be carried out in ruminants, the natural host species, hence making any results directly relevant to the natural situation. Furthermore the *in vivo* work is strongly linked to the attempts to establish *ex vivo* and *in vivo* studies using organ, tissue and primary cell cultures to further understand the pathogenesis and specific cellular responses to arboviruses and arthropod saliva.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Initial proof-of-principle studies will be carried out on a smaller number of animals (4-6 per group) and follow on studies will only be initiated if preliminary results demonstrate occurrence and feasibility. Specialist statistical advice is always sought when designing new experimental studies. The numbers of animals used in experiments will be the minimum possible to achieve statistically robust data. The use of cannulated animal to investigate the local immune response of the skin towards BTV/EHDV will result in less animals being utilised as the normal migratory cell population and induced changes upon infection and arthropod feeding can be investigated in the same individual, thereby reducing animal-to-animal variation.
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.	Any animal developing > 40.5 C body temperature or express any signs of physical discomfort will be treated with the appropriate pain killer and anti-inflammatory medication upon veterinary advice. Through the experience gained under the previous licence specific scientific endpoints have been designed for certain protocols which will significantly reduce the impact on the animal in these procedures. Refinement of the surgical lymphatic vessel cannulation techniques, the use of harnesses to allow freedom of movement and the ability to maintain catheters for many days has resulted in higher yields of leucocytes from each animal, hence reducing the number of animals used.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Understanding how synapses work in health and disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Neuroscience; Brain; Synapses; neuropsychiatric diseases</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[15]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[16]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Understanding the brain is one of the ultimate frontiers in science and medicine. No other organ carries a higher disease burden than the brain and this is bound to increase due to ageing demographics. One of the main reasons why there are limited treatments for brain diseases is that we don’t fully understand the brain’s basic functioning. We are focusing on how nerve connections, also called synapses, work. Synapses are affected in numerous diseases including autism, schizophrenia, Alzheimer’s and Parkinson’s. Many of the devastating mental deficits found in these diseases correlate well with the start and degree of synaptic dysfunction and less well with neuronal death, which occurs only at very late stages. At present synapses cannot be studied in the living human brain as they are too small to be detected. We will study nerve connections in animal models of ageing and neuropsychiatric disorders by using an advanced type of microscopy technique. Our ultimate goal is to use this knowledge to guide the development of novel therapies for a variety of brain diseases.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">The overall aim of the work is to deepen our understanding of how nerve connections work, form and degenerate in order to find novel therapeutic targets and/or strategies, which may be beneficial for the treatment of nervous system pathology.</td></tr>
<tr><td>What species and approximate numbers of</td><td colspan="3">Up to 5000 mice and rats over 5 years</td></tr>
</table>

[15] Delete Yes or No as appropriate.
[16] At least one additional purpose must be selected with this option.

animals do you expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will implant a tiny glass window into the rodent's skull under general and local anaesthesia/analgesia. The wound heals within a few days, and there are no signs that the animals notice what is happened to them. The brain itself is not disturbed. Adverse effects are those typical of surgical procedures and include infections and anaesthesia side effects. Genetic models of neurological disease may experience altered mobility. The expected level of severity is moderate. Animals will be humanely killed at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At present synapses cannot be studied in the living human brain as they are too small to be detected with current imaging technologies such as MRI scans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	At every stage in the experiments, consideration will be given to ways in which we can reduce the number of animals. Several of the protocols that we use are designed in such a way to obtain the maximum possible data from a single animal. Furthermore, as far as possible, we will use cultures of neurons in a dish.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	In our experience sterile conditions, precision and care during surgery are the most effective strategies to minimise adverse effects. Any animal showing signs of infection or distress will be killed by a schedule 1 method in a designated establishment. All animals are maintained in IVCs (individually ventilated cages).

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Characterization of a KY dependent hypertrophic pathway</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Muscle, hypertrophy, atrophy, z-disc</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in Article 5)[17]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[18]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>We address the following questions: how do muscles grow in response to chronic demands? How do physical forces applied on muscles are translated into molecular mechanisms that ultimately result in a bigger/stronger muscle? We aim at identifying the molecular players and elucidating their specific function in that process.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Preventing muscle atrophy in bedridden conditions is a long-standing challenge. The identification of the sensors, mediators or downstream targets of the muscle hypertrophy mechanisms is co-substantial to the development of pharmacological strategies aimed at preventing muscle wasting due to paralysis, age or disease.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Over a 5-year period, we estimate that around 750 mice will be used for this purpose.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>Mice are subjected to exercise challenges. No adverse effect is anticipated, as these tests are derived from forms of exercise that mice would do spontaneously. Genetically altered mice may display some defects, for example, gate or size defects. We use specially designed welfare score sheets to continuously monitor these animals. In some cases, genetically modified mice may fall under the moderate level of severity. All animals will be killed at the end of the study.</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
<tr><td>1. Replacement</td><td>Muscle hypertrophy in response to mechanical</td></tr>
</table>

[17] Delete Yes or No as appropriate.
[18] At least one additional purpose must be selected with this option.

State why you need to use animals and why you cannot use non-animal alternatives	loads is not a physiological response reported in flies or worms. Unfortunately, no *in vitro* system described to date can effectively model neuromuscular disease or respond to mechanical loads and therefore we must use an animal model that, like the mouse, is similar in as many aspects as possible to the human anatomy, physiology and disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Standard breeding calculations and comprehensive project planning will be used to minimise the number of mutant mice bred. We will constantly analyse our data using statistical methods to ensure that the lowest possible number of animals per group will be used. We use power analysis to investigate statistical relevance of phenotypic data. Since we focus on skeletal muscle, we have developed a system to study the phenotype of specific muscles that have been electroporated to alter protein expression. The implementation of the in vivo electroporation platform would significantly reduce the use of animals in specific cases because: 1) Will allow the study of the effects on the muscle fibre of expression changes of specific target genes without the need of generating KO or transgenic lines; 2) Biochemical studies using recombinant proteins can be undertaken also circumventing the need of generating transgenic lines.
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.	**Why mice:** Unfortunately, no *in vitro* system described to date can effectively model neuromuscular disease or physiological muscle hypertrophy. We must use an animal model that, like the mouse, is similar in as many aspects as possible to the human anatomy, physiology and disease. Our models serve two basic scientific purposes. Firstly, it allows us to explore mechanisms of muscle hypertrophy and neuromuscular disease by revealing new molecular players that when mutated or overexpressed can cause muscle changes. And secondly, they represent unique resources to elucidate these pathways. Thus, although many aspects of the functional characterization of a protein do not require the use of animals, it is crucial to assess the impact that the loss of a member of a protein complex may have on other interacting partners *in vivo*. This gives us insights into the molecular mechanisms and provides markers that can be used to diagnose human disease. The mouse is the lowest vertebrate that can be used for this purpose with enough aspects of its genetics, anatomy and physiology shared with

	humans to generate biologically relevant data that ultimately can be extended to the generation of new therapeutic targets. In the case of existing mutant models such as Ky, these are not yet available in the fish. In addition, although many aspects of muscle function are indeed conserved in flies, worm and mice, crucial aspects of pathological analysis such as muscle regeneration, inflamation or ectopic accumulation of intracellular protein aggregates which are very relevant to human disease are not well recognized in flies and worms. Experimental refinement. Electroporation has been refined and made more efficient by an appropriate selection of the target muscle (Extensor digitorius longus), improvement of the injection technique (ensuring intramuscular delivery of the small muscle by maintaining the needle within the perimysium of the muscle) and improving absorption by a pretreatment with Hyaluronidase, a mucolytic enzyme that facilitates the spread of fluids through tissues. This new protocol will allow us to reduce the number of animals needed significantly.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Development of vaccines for infectious diseases and cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Vaccines, cancer, influenza, hepatitis B, peptides</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[19]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[20]</td><td>Yes</td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The work described in this project license is aimed at developing novel vaccines for mutating viruses, in particular influenza and hepatitis B, and cancer. The experiments will test the immunogenicity and therapeutic potential of candidate vaccines in murine models.

Influenza is a major cause of morbidity and mortality. Seasonal influenza kills as many as 250,000 to 500,000 people around the world every year. Vaccination remains the most important means of preventing and controlling influenza. Traditional influenza vaccines are designed to prompt an immune response to H and N proteins on the outer shell of the virus. However, these proteins have a tendency to mutate and vaccines need to be re-formulated every year based on the influenza strains likely to be most predominant.

In Europe there are an estimated 14 million citizens living with chronic hepatitis B virus (HBV) infection, nearly a quarter of those will die of liver failure or liver cancer as a direct result of the viral infection, accounting for >36,000 deaths per annum. As a consequence HBV is a significant burden to healthcare systems across Europe through the direct costs of treatment, as well as indirect costs linked to lost productivity and premature death.

One in three of us will be affected by cancer during our lifetimes. Given the ever increasing burden of disease and an ageing population, the costs of providing optimal care in Britain alone will rise by a</td></tr>
</table>

[19] Delete Yes or No as appropriate.
[20] At least one additional purpose must be selected with this option.

	staggering 62% over the next decade. Current healthcare models and practices are unsustainable. We are developing novel stratified therapeutic oncology vaccine initially for non-small cell lung carcinoma.
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 developing a synthetic universal influenza A vaccine designed to induce protective anti-viral cellular immune responses. Potentially our vaccine will not require annual reformulation, can induce long-lived immunity and will be effective in all populations including children and at-risk populations such as the elderly. For our novel hepatitis B vaccine we aim to induce immune control over the disease, increasing the likelihood of clearing infected liver cells and reducing the need for prolonged costly drug therapy. Our oncolgy vacccine, whist in early development, can potentaily offer a better, safer treatment option for improved long term prognosis for multiple tumour types.
What species and approximate numbers of animals do you expect to use over what period of time?	We wish to use both mice and rats in our research. Rodent models, whilst not directly predictive of immunogenicity or efficacy in humans, offer a valuable means of immunologically validating vaccines, providing essential data to assess immunological characteristics, dose frequency & interval selection, memory response, as well as evaluating different routes of administration. Different rodent strains may be selected to provide a more diverse MHC background to improve the potential scope of immunogenicity responses. Inbred or transgenic mice may be used to assess epitope specific CD4+ and CD8+ T-cell responses. We wish to carry out studies on inbred strains of mice which yield better reproducible experimental results thus allowing us to use smaller numbers of animals to gain statistically significant data. We estimate that we will use approximately 6,000 mice and 1,000 rats for this study. In addition, we may also use a small number (<500) of transgenic mice which express the human HLA molecule.
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 anticipate that only mild adverse effects will be observed for testing the immunogenicity of our vaccines in mice or rats. Animals are humanely killed at the end of the procedure. For testing the effectiveness of our vaccines to reduce the development of solid tumours in mice we may experience a maximum moderate level of adverse effects. These are mainly attributed to the formation of local and systemic tumours. For tumour generation of tumours underneath the skin we will

	humanely kill the animals when the tumour reaches the size of a pea. All animals will be humanely killed at the end of the testing period.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Rodent models, whilst not directly predictive of immunogenicity or efficacy in humans, are essential for immunologically validating vaccines, providing valuable data to assess immunological characteristics, dose frequency & interval selection, memory response, as well as evaluating different routes of administration. Rodent models provide invaluable information to establish ability of a vaccine to reduce tumour burden in cancer models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Careful statistical tests will be applied so that the minimum number of animals are used in each experiment. We aim to conduct a variety of immunological tests in each experiment to ensure that maximum output is achieved. Where possible we will use cells derived from donated human blood to achieve our goals. Other data will be obtained from biochemical testing or mathematical modelling.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will only use mice or rats for testing of vaccine candidates. The most suitable stain of rodent will be selected to support the type of immune response we require testing. The laboratory methods we use to assess the immune responses are state-of –the-art and ensures that maximal value can be gained from each experiment. Most animals will receive either intramuscular or subcutaneous injections of test vaccines which do not cause the animals pain or distress. Appropriate pain relief will be administered only when required. For tumour models, animals will be killed if tumour sizes are greater than the size of a pea or if the animals are distressed or loose weight.