Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 2

Project titles and keywords

- **Frontal lobe functions in control of complex behaviour**

  Frontal lobes, prefrontal cortex, electrophysiology, attentional and control systems, non-human primate

- **Humane killing of piglets, kids and lambs.**

  Percussive, non-penetrating, piglets, kids, lambs

- **Animal models of arthritis and bone loss**

  Arthritis, Rheumatoid arthritis, Osteoporosis, Bone

- **Molecular ecology of bats**

  Bats, genetics, ecology, conservation

- **Muscle development in zebrafish**

  Zebrafish, muscle, hedgehog, muscular dystrophy, myotube patterning

-
- **Regulation of transcription in normal tissues & during cancer evolution**

  Genomics, transcription, cancer

- **Pathophysiology and therapies of headaches**

  Headaches, brain, pathophysiology, trigeminovascular system

- **Mechanisms and targets for chronic pain**

  Pain, nerves, non-nerve cells, pain killers

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Frontal lobe functions in control of complex behaviour</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Frontal lobes, prefrontal cortex, electrophysiology, attentional and control systems, non-human primate</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)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td><s>Yes</s></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The critical functions of the primate frontal lobes are indicated in a wide range of neurological and psychiatric conditions, where impaired frontal lobe function creates severe disorganization of thought and action. The frontal lobes are thought to create the organized sequence of attentional episodes that make up goal-directed behaviour. Functional brain imaging (fMRI) of human frontal lobe activity has revolutionized our understanding of where in the frontal lobe cognitive operations are performed, in particular identifying a specific multiple-demand (MD) network active in the organization of diverse cognitive activities. The coarse spatial resolution of fMRI however, prohibits detailed analysis of network function at the level of single neurons. In this project we examine how frontal lobe neurons control the successive attentional episodes of complex, sequential and novel behaviour. Our objectives are to gain understanding of how the attentional and control functions of prefrontal cortex are mediated at a neuronal and mechanistic level. Furthermore, we aim to specify neuronal activity in prefrontal cortex impacts upon activity in selected regions of posterior cortex within a functionally connected network of cortical regions within and beyond prefrontal cortex.</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</td><td>From this work we expect to gain novel understanding of the way that populations of prefrontal neurons work to control brain activity, producing coherent, effective behaviour. Our work complements the large-scale information available</td></tr>
</table>

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

project)?	from human fMRI, but unlike fMRI delivers neuronal level measurements of function in this critical brain region. Specific short-term benefits include are advances in basic research: i) Determining the functional role of distinct frontal lobe systems in behavioural control: our scientific understanding of MD network activity will progress beyond the limited spatial and temporal resolution provided by fMRI; in particular our data will be the first to compare detailed physiological activity across these hubs which is essential in moving from coarse network definition to detailed understanding of neuronal level function in this critical brain system. ii) Determining critical frontal lobe processes in complex multi-step behaviour: At the level of neural activity we know little of how complex behaviour is controlled in a sequence of component episodes. Accordingly we have only the most limited understanding of deficits in sequential behaviour in a wide range of clinical conditions. To help resolve such uncertainty, our data will address a range of fundamental questions, including development through learning, how each episode is assembled, and how the nervous system manages transitions from one episode to the next. iii) Development of a suitable behavioural model for rapid task learning: The frontal cortex is most critical in rapid learning of solutions to novel behavioural problems. In neuroscience, however, the great majority of studies have focused on highly artificial tasks, requiring extremely long-term training. Through adaptation of a semi-naturalistic foraging scenario, we aim to produce a suitable task context for neurophysiological study of rapid task learning. Success in this objective would be of broad value to the community of neuroscientists working on frontal lobe functions. In the medium-term we also expect further benefits: iv) Improved understanding of frontal lobe conditions and input to clinical research and management: Impairment of prefrontal function is among the most disabling – but at the same time least understood – aspects of neurological and psychiatric disease. In the medium-term, we expect fundamental knowledge from neurophysiology to feed into detailed planning of future clinical research and practice. (v) Provision of essential data for formal network modelling: these physiological data will also provide input for detailed neural network models explaining

	how the brain represents and controls the events of integrated, goal-directed behaviour. Such models are critical to move beyond understanding of brain function based on single regions to analysis of integrated networks; and to link understanding of brain function from single neuron activity to integrated whole-system behaviour.
What species and approximate numbers of animals do you expect to use over what period of time?	Four rhesus macaque monkeys; we expect each animal to be on study for between 3-5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Adverse effects arise from the necessity for invasive recording from the brain, for daily recording sessions to measure neural activity, and for motivation by control of fluid intake: i) Repetitive restraint - animals may experience periods of stress related to behavioural testing because the nature of the procedures requires prolonged head-restraint during training and testing stages (several hours per day, several days per week, for 3-5 years, albeit with some days off per week and with some interpsaced 'vacation' periods with no testing) ii) Fluid control – some level of fluid control/restriction is likely to be required on most days to help motivate animals' training and performance. There is therefore a moderate risk some animals may experience transient periods of mild dehydration, particularly during the initial stages of training or when tasks suddenly increase in complexity; and transient weight loss may occur when starting on fluid control. This is mitigated by using only the minimum necessary amount of fluid control throughout, by giving some ad lib access to water at weekends, and by giving ad lib access to water on all days in the 'vacation' periods. iii) the potential for stressful motivational/training techniques such as the rare use of pole-and-collar. This will be mitigated by only using pole-and-collar only on rare occasions and only for short durations. iv) the necessity to use implants means that there is a moderate risk that their implants may become mildly infected; there is also a small risk that the implant may become damaged/fall off and require repair or replacement or become damaged. v) there is a very small risk of seizure, infection, and/or haemorrhage associated with neuronal recordings and surgical implantation of recording devices. At the end of the experiment the animals will all be

	humanely killed and perfused so that their brain tissue can be examined.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Achieving the scientific objectives of this research project requires that we measure neuronal activity in awake, behaving subjects, which involves invasive techniques that cannot be performed in humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reduction in this project is achieved by: a) reducing the number of animals necessary; b) reducing the duration of each daily experimental session; and c) reducing the number of experimental sessions (thereby reducing the time an individual animal spends on study). Our experiments are based on a "within-subject" design, such that research questions are based on comparisons in one experimental condition vs. another, which can be embedded within the same behavioural task. We design the behavioural tasks to be as simple as possible given the experimental objective. This reduces the duration of individual experimental sessions as much as possible and ensures that few (if any) animals need to be removed from study for failure to learn/perform the task. We also design the tasks to enhance differences between experimental conditions (wherever possible) to reduce the amount of data necessary to achieve statistical reliability. Finally, we design our experiments such that a single animal/experiment can contribute to multiple objectives (without increasing the cost to the animal), thereby reducing the total number of animals necessary.
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 need to use non-human primates in this project because rodents do not possess a granular prefrontal cortex like that of anthropoid primates; this is crucial for these projects because we are investigating the role of MD cortex which is part of a network of interconnecting and interacting regions that includes granular prefrontal cortex. We need to use macaques because structural and functional similarities between the macaque brain and the human brain are much closer than is the case in New World monkeys, and because the intellectual abilities of Old World monkeys are known to be markedly superior to those of New World monkeys (e.g. marmosets) who would be unable to learn all of the variants required of our flexible rule-guided decision-making tasks. All our work builds upon an extensive behavioural, anatomical, and physiological scientific literature that is unavailable to this extent in any other species and which avoid what would otherwise be the unnecessary replication of this work in a new laboratory species.

	We have taken every measure possible and established multiple intervention points to ensure that each individual animal will experience the minimum potential possible for any adverse effects (pain, suffering, distress, or lasting harm) and only when it is absolutely necessary to achieve the scientific objectives; and to ensure that that no more animals are used than is absolutely necessary. We have designed the scientific programme in order to maximise the amount of data that can be obtained with each experiment, with many experiments contributing data to more than one objective. The individual experiments are designed to build upon one another to ensure that they are conducted with the most up-to-date information possible and to achieve maximum scientific benefit from each animal. We have incorporated flexibility in terms of recording techniques to ensure that the best available recording technique will be used for each scientific objective. This will maximise the quality of data and minimise the amount of time the animal is required to be on protocol. We have established intervention points between each new procedure to ensure that no new procedure will be performed unless the NVS, NACWO, and researchers agree that the animal is not likely to experience an unreasonable increase in risk for adverse effects. This will not however, compromise the utility of the data obtained prior to these intervention points should it be deemed inappropriate for the animal to continue. We have developed a progressive method of instituting reinforcement training/fluid control (and monitoring its efficacy through behavioural monitoring) that will serve as the basis for developing individualised programmes for each animal (to be done with the NACWO). This will ensure that no animal will experience any more restriction than necessary to overcome specific hurdles in training. We will use the latest technology/methods to increase the quality of the data thereby minimising the duration of experiments and the time each animal is required to be on protocol. We have specifically designed (and continue to refine) our restraint systems (both headposts and restraint chairs) in order to minimise the discomfort experienced by the animal during prolonged periods of restraint. Finally, we continually liaise with the NVS/NACWO to refine our behavioural and health monitoring procedures.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Humane killing of piglets, kids and lambs.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Percussive, non-penetrating, piglets, kids, lambs</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)[3]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
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<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[4]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Occasionally stockmen will be faced with the problem of seriously sick young livestock that are beyond treatment. Usually, the choices that are available to them are either leaving the neonates with their mothers in the hope that they may recover, or casualty slaughter. Casualty or surplus slaughter of young animals on-farm is usually carried out by administering a blow to the head, which is generally performed by swinging the young animal against the floor or a wall. Although widely used as a means of casualty slaughter the effectiveness of this method is heavily dependent on the strength and skill of the person and consequently the probability of achieving an immediate kill in all cases is low. Furthermore a lack of proper training and human error can leads to pain and distress to the animal. It is also a method of killing that is aesthetically unpleasant for both the operator and any bystanders.

Survey results showed that the majority of young sick lambs are left to die whilst piglets are usually killed by a blow to the head. The majority of respondents were not satisfied with their current method of casualty or surplus slaughter and all of them expressed an interest in an alternative device. Unfortunately currently there is no alternative method available for killing young livestock.

Captive bolt devices are used widely for the humane stunning and killing of adult livestock. The device used in this study (the CPK2 gun) has been</td></tr>
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[3] Delete Yes or No as appropriate.
[4] At least one additional purpose must be selected with this option.

	specifically designed for the killing of large birds and is suitable for use with neonates. Previous research has demonstrated that the skull development of piglets is sufficient for a percussive blow to induce a fatal concussion. In addition, a pilot trial was conducted with kids, which identified the CPK2 device as the optimum tool when compares with 2 penetrating captive bolt guns. The work conducted under this licence will define the robustness, effectiveness and aesthetic acceptability of the device. The results from the proposed research would underpin legislative change to include the CPK2 gun for the humane killing of neonate piglets, lambs and kids
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 new EU Regulation 1099/2009 on the protection of animals at the time of killing permits the use of a percussive blow to the head as a killing method for neonatal pigs, sheep and goats up to 5 kg live weight. This study will contribute to animal welfare by providing an effective and reliable tool that will provide a more effective and reproducible method of killing neonate pigs, sheep and goats, thereby overcoming the limitations of the methods currently available.
What species and approximate numbers of animals do you expect to use over what period of time?	The experiments will be conducted on 200 lambs, piglets and kids over a period of 20 months which is dependent on the seasonal variation in reproduction in lambs and kids.
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 usual method that farmers adopt to kill neonates is to administer a blow to the head by swinging the young animal against a wall. This form of mechanical stunning is not reproducibly successful and the use of a percussive gun will reduce the risk of failure. Should the CPK fail to result in the death of all animals, a more powerful non-penetrating tool will be available.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The assessment of an effective killing method cannot be carried out without its application to dispatch animals. The percussive gun under trial has been demonstrated to humanely kill kids, turkeys, geese and ducks. The experimental animals are destined for casualty slaughter on the farm under uncontrolled conditions. Therefore, the use of a mechanical killing device will improve their welfare.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A statistically valid number of animals has been selected for this trial which will clearly demonstrate whether the method is effective of not. Based on a confidence level of 95% a sample size of 200 is recommended as a sensible balance between the

	cost of the study in animal terms and demonstrating the degree of efficacy.
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 choice of species is that specified in EU Regulation (1099/2009) when a percussive blow to the head is used to kill neonates. The farming industry do not currently have a proven device/tool to deliver such a blow. In the event of an animal surviving this treatment, it will be dispatched using a more powerful captive bolt gun and a higher-cartridge strength will be selected and a further 200 animals tested. Participating producers will be instructed that animals that are suffering any pain and distress must not be held back and kept alive for this project but must be humanely dispatched as soon as is practicable.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Animal models of arthritis and bone loss</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Arthritis, Rheumatoid arthritis, Osteoporosis, Bone</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)[5]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall objective of this project licence is to identify new medicines for the treatment of chronic inflammatory diseases such as rheumatoid arthritis (RA) and metabolic bone diseases such as osteoporosis (OP).

RA is a disease of the joints and the cause is unknown. Patients have inflamed painful joints, cartilage and bone destruction, fatigue and a reduced life expectancy. Current treatments are associated with side effects and do not completely stop disease progression and not all patients respond to treatment. There is still a clear unmet clinical need for better treatments which will give patients a better quality of life.

OP is a disease that affects the bone and causes a decrease in its density, which ultimately results in fractures. Patients have back pain, height loss, spinal deformity, a reduction in the ability to perform routine tasks and increased morbidity and mortality. Current treatments aim to decrease bone absorption resulting in a net increase in bone formation. However, these treatments have side effects and none have unequivocally demonstrated an ability to fully prevent the occurrence of new fractures. Thus there is still a need to develop new medicines for the treatment of OP.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or</td><td>This project is expected to identify novel medicines and processes involved in chronic inflammatory diseases such as RA and metabolic bone diseases such as OP which will lead to new treatments and</td></tr>
</table>

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

animals could benefit from the project)?	improved quality of life for patients.
What species and approximate numbers of animals do you expect to use over what period of time?	Rodents (mice, rats) and rabbits will be used in this project. It is estimated that an average of 8150 animals could be used annually in this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals will undergo procedures that may involve injections and they may experience moderate discomfort as they develop symptoms of disease, such as swollen paws. Overall the level of severity for procedures in this project is moderate. At the end of studies 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	RA and OP are complex diseases that involve interactions between tissues and various cell types. This complexity cannot be mimicked *in vitro* hence the need to assess the effect of new medicines in animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Due to the nature of disease models incidence of disease may not be 100%. Our experience with the experimental protocols will be applied to ensure appropriate group sizes are used to identify statistically significant differences between groups, whilst minimising the numbers of animals undergoing the protocol. Group sizes are constantly reviewed and experts in statistics are consulted to ensure the minimum number of animals are used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodents are the mammalian species of lowest neurophysiological sensitivity on which these chronic inflammatory diseases and metabolic bone diseases have been modelled. The majority of animal models in this project are well established both in-house and within the literature and have been shown to model different aspects of human disease. However, no single model accurately reflects human disease and it is therefore necessary to study different models that model different components of human disease. All procedures have been ethically reviewed and all animals undergoing procedures are monitored closely by trained staff that work closely with a veterinary surgeon. In addition, distress scoring sheets are used to monitor disease severity and these are under constant review to ensure the correct level of disease scoring is achieved with

	minimum stress to animals. Humane endpoints are employed to limit suffering and disease burden.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Molecular ecology of bats</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Bats, genetics, ecology, conservation</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)[7]</td><td>
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<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>
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<tr><td>Preservation of species</td><td>Yes</td><td></td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>I will explore the causes and consequences of genetic variation within and among bat populations. Within this broad aim, my research has two major objectives. First, I will continue long-term research on the genetic composition of the greater horseshoe bat population at Woodchester Mansion to better understand the genetic constitution of successful individuals (i.e. those that survive and leave many offspring) in comparison with unsuccessful ones. The study is one of the longest-running population studies on mammals – the bats there have been marked for > 50 years, and we have obtained DNA profiles all animals in the population since 1993. We therefore have in-depth knowledge of relatedness, maternity and paternity within the colony.
Second, I routinely conduct studies on the population genetics of bats to determine whether populations are fragmented or inbred (and hence of conservation concern), and to reconstruct patterns of how bats recolonized northern Europe after being forced into southern Europe during the last Ice Age. I also aim to use this knowledge to predict how patterns of existing genetic variation may be affected by future climate change. For example, most genetic diversity is still present in southern Europe as animals were forced into ‘glacial refugia’ there during the last Ice Age. Models predict that these refugial areas may be especially susceptible to climate warming however.</td></tr>
<tr><td>What are the potential benefits</td><td>We will understand whether bat populations are</td></tr>
</table>

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

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	suffering detrimental effects of reduced genetic diversity of inbreeding and this knowledge will determine whether mitigation such as increasing landscape connectivity (and hence migration and gene flow) among colonies is needed. We will understand if climate change is likely to have severe impacts on reducing genetic diversity in populations of European bats, and highlight populations that should be given the highest priority for conservation.
What species and approximate numbers of animals do you expect to use over what period of time?	We will sample a range of European bat species and anticipate taking biopsy samples from fewer than 5000 bats during the 5 years 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?	We take 3mm biopsy punches from the wing membranes of bats. The small holes produced heal rapidly, often within two weeks in the summer. I have used this method for over 20 years and have never witnessed any obvious adverse effects on the animals. The procedure is mild in severity, and the animals are released back to the wild after the punch is taken. The animals are captured frequently for many years after the procedure is completed, and show no adverse effects from the biopsy.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no realistic alternative to using living wild animals in our studies. We need high quality DNA for our studies, and this cannot be obtained reliably from droppings.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We sample the minimum number of individuals necessary to achieve our aims. Bats are typically ringed so resampling the same individual is avoided. We already have DNA samples from many bats in our main study population, so will sample all newly born bats at the colony, all immigrants, and occasionally bats that were sampled many years ago whose DNA may have degraded, and if we develop novel genetic markers. In population genetic studies, peer-reviewed scientific papers recommend that at least 20 -30 individuals are sampled to assess genetic diversity when working with a population that has an unknown level of diversity, and we use this as our yardstick.
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	**The methods we use are used throughout the world, provide reliable sources of DNA and are the least severe methods known that can provide such high-quality data. We will manage the capture, sampling and release of the bats carefully based on 27 years of field experience.**

measures you will take to minimise welfare costs (harms) to the animals.	**Capture methods such as hand-netting and mist-netting are licensed by Statutory Nature Conservation Organisations.**

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Muscle development in zebrafish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Zebrafish, muscle, hedgehog, muscular dystrophy, myotube patterning</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)[9]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</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[10]</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 are studying how muscle is patterned and differentiates in the vertebrate embryo. We will study how certain signalling and structural proteins impact on muscle pattering and regeneration after injury. Related to this, we are studying models of human Duchenne muscular dystrophy (DMD), a severe muscle wasting disease.

The precise way in which different muscle types are formed in the embryo are unknown, and it is not well known which genes are involved in this. In addition we do not know how certain structural proteins influence the formation/functioning of myofibers, and how these proteins influence another cell type important for muscle maintenance; the muscle stem cells (satellite cells). DMD is an incurable disease, and only a limited number of drugs that can alleviate symptoms are known.</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>We will produce data that will clarify how different muscle types are induced in fish and this may be a paradigm for muscle patterning in higher vertebrates, including human and mouse. A better understanding of the regeneration and muscle degeneration process may allow us one day to exploit this knowledge to the benefit of human patients with muscle diseases. For instance if we find chemicals that reduce the muscle degeneration in our fish model of duchenne muscular dystrophy, this might lead in the future to the development of a</td></tr>
</table>

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

	treatment for this disease in human
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate that we will use 12625 adult zebrafish, these are generally only used for matings. These numbers are determined by stock keeping requirements, a minimal number is require per stock to ensure maintenance or generation of the a required transgenic line, unwanted lines will usually be maintained as a frozen as sperm sample. Maximally 2500 of these will be used to generate 5 transgenic lines per year, the rest are required to maintain existing stocks. We will use maximally 210 young larvae for muscle injury and muscle regeneration studies, and maximally 1000 8 day old larvae for gene and protein expression studies, to study processes and muscle patterning events that do not occur in embryos
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In most cases we will work on embryos, therefore adult fish are only required in natural matings to generate these embryos. Occasionally to identify the correct genetic type of animal we will take a small part of the fin, this will regenerate and does not significantly impair their well being. In a small number of cases we will apply a small muscle injury to fish embryos or larvae. Finally we may raise embryos to adulthood that have been injected with DNA or RNA. In some cases fish may be anaesthetised required for eg. fin clips, observation, expression of oocytes or muscle injury. Anaesthesia may cause light transient bleeding from their gills. Rarely fish may have difficulty waking up or very rarely they may fail to recover at all. There is a small chance that injuries can become infected. Injection of DNA or RNA constructs should not cause harm but unanticipated dominant effects might occur.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are studying patterning and regeneration of vertebrate muscle using zebrafish as our model organism, this needs to be studied in a living embryo or larva because these processes occur as a consequence of a precise interplay between different tissues that send and receive patterning signals. For instance, apposition of the midline notochord to the muscle precursors is important for correct formation of muscle fibre types. Such conditions are impossible to recreate using cell culture systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reduction is achieved by using embryos instead of protected adults, adults are mainly used to generate embryos and the numbers are the minimal numbers required to ensure safe stock keeping. Where protected larvae are used we will use minimal numbers to achieve significant results (5

	larvae per group)
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.	Fish are the simplest model vertebrate in which these studies can be performed, and our experiments are on embryos or larvae which have a lower level of awareness relative to adults

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Regulation of transcription in normal tissues & during cancer evolution</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">genomics, transcription, cancer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">five years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[11]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</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 colspan="3">The human body is composed of over two hundred unique types of cells with specialized roles and yet every cell contains the same genetic information called the genome. The differences between types of cells are largely determined by master regulators of transcription which direct how the genome is ‘read’ in each cell. The overall goal of this project is to define how the mammalian genome is deployed transcriptionally to create specific cells, tissues and species. We aim to use this knowledge to identify malfunctions in these regulatory mechanisms which are associated with the development of diseases such as cancer.</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">This project will provide novel information about the mechanisms controlling tissue-specific transcriptional regulation and about how these mechanisms may guide cancer evolution. Such information is expected to be of potential clinical interest. Understanding how specific tissues are ‘wired’ is essential to therapies involving cell replacement. Furthermore, identifying the transcriptional malfunctions associated with diseases such as cancer may uncover new treatment strategies.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">This project will use the mouse and rat as the experimental animals. We expect to use up to 20,200 mice and 1,125 rats in regulated procedures during this five year project.</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</td><td colspan="3">Many of our studies use tissue samples collected from animals which have no expected adverse phenotypes and which have been killed by a humane, non-regulated procedure.</td></tr>
</table>

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

level of severity? What will happen to the animals at the end?	Some studies use rodents carrying mutations which may affect the development and function of their liver or mice treated with carcinogens to induce liver cancer. These adverse phenotypes may result in mild to moderate levels of severity. Experimental animals are 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	Post-mortem tissue samples from animals are essential for our studies to understand normal tissue-specific transcriptional regulatory networks. Non-animal approaches such as cell culture are of limited use for our research since the transcriptional regulatory systems found in cultured cells have invariably drifted substantially from those in the original tissue from which they were derived. **For our experiments investigating transcriptional regulatory systems during tumour evolution, mammalian tumour samples are crucial for similar reasons.** Tumour biology is profoundly influenced by its environment within the body and tissue culture methods are unable to mimic this complex interplay.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers are minimised by designing breeding strategies to produce experimental animals as efficiently as possible; by collecting multiple samples from individual animals to create a tissue repository for future experiments; and by the use of statistical analyses to determine the minimum number of animals required to achieve a significant result.
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 mouse and rat are the experimental animals of choice for this project. They are well-studied experimental species; many inbred strains exists which are crucial for our studies; and there is reliable technology to genetically manipulate mice.** Appropriate health monitoring protocols are implemented for mice expected to have an adverse phenotype. When appropriate, suitable analgesia & anaesthetic regimes are used to minimize suffering or the animal is killed by a humane method.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Pathophysiology and therapies of headaches</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>headaches, brain, pathophysiology, trigeminovascular system</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 yrs</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>Yes</td><td></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>A. Mapping the brain pathways that are involved in abnormal neuronal excitability related to headaches
B. Characterise their electrophysiological properties and receptors involved
C. Investigate the actions of potential therapeutic approaches.</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>Lack of understanding of the causes of primary headaches, has been a major limitation to the development of effective treatments. The primary potential benefit relates to new knowledge on the changes in brain nuclei and neuronal excitability that occur in headache disorders. The published information is likely to be of interest to biologists/physiologists and clinicians with an interest in the mechanics of headache. The secondary benefit relates to headache sufferers, in so far as the results may at some later stage be of such a great value.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>~ 520 Adult rats, ~220 adult mice; 5 yrs</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</td><td>Non-terminal procedures will be of mild severity. Adverse effects may include:
- Deaths resulting from anaesthetic or surgical complications are most uncommon (<1%)
- Mild pain related to the surgical procedures</td></tr>
</table>

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

happen to the animals at the end?	- Post-surgical infections may occur after laparotomies (< 1%) - Rare re-opening of wounds or wound infection - It is possible that some novel uses of substances may unexpectedly cause irritant effects. - In the terminal phase, the procedure is conducted under non-recovery general anaesthesia (unknown severity), which is sufficiently deep and stable to ensure that the animal is insentient throughout. All animals will be euthanized.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The determination of neuronal changes associated with primary headaches not only requires the presence of neurons at a state of nociceptive condition, but also it requires intact brain pathways that influence each other. The investigation of such pathways cannot be ethically conducted in humans as it involves major invasive approaches and removal of neural tissue for ex-vivo investigations. Therefore, there is no feasible alternative that would entirely replace the use of living animals that would allow the objectives to be met.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The proposed experimental designs and methods of analysis of the results have been discussed with the Statistical Unit, to give effect to the principle of reduction. The method of single neuron electrophysiological recordings allow us to perform multiple recordings in the same animal, which reduces the number of animals and furthermore allows direct comparison of treatments in the same animal, thus improving the power of the statistics performed. In anatomical studies, when appropriate, we perform unilateral or one-sided observations in the brain, allowing us to use the contralateral or opposite side as an internal control. The ARRIVE guidelines will be followed when reporting in vivo experiments in animal research to contribute in reducing the number of animals in similar studies in the field in the long term.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use the rat because we can model trigeminovascular nociceptive activation and its pathways in a species with a rich and well-studied neurobiology. The rat has become a reliable model system for predicting the anti-migraine efficacy of test compounds, and by using the same species we can compare our data to previous studies that have been done. We also propose the use of mice models for research. The discovery of genetic mutations in certain migraine conditions has opened up the opportunity for developing pure

	transgenic models in mice of these conditions, and thus use whole animal systems that directly resemble the human genotype and observe and compare phenotypes with the wild-type animal. The recommendations by of the Good Laboratory Practice for reduction of experimental bias which results in a major refinement will be followed. In both species we use mainly non-recovery studies.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Mechanisms and targets for chronic pain</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Pain, nerves, non-nerve cells, pain killers</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[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>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[16]</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">Chronic pain associated with diseases such as diabetes and rheumatoid arthritis reduces patient’s quality of life and can be resistant to the available pain killers.
Our research aims to identify new mechanisms underlying chronic pain in order to find new targets for pain therapy.</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">This project will identify new mechanisms which are responsible for chronic pain in animals and humans. The identification of key players in such mechanisms will provide new therapeutic targets for the relief of chronic pain in diseases like diabetes and arthritis.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">We will use mice and rats not exceeding 2000/year.</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 colspan="3">The use of animal models is crucial to our understanding of pain pathophysiology and the development of novel analgesics. We expect that our animals will walk less and lose some of their explorative behaviour. At the end of the experiments the animals will be killed humanely and tissue may be collected for analysis.</td></tr>
<tr><td>Application of the 3Rs</td><td colspan="3"></td></tr>
<tr><td>1. Replacement
State why you need to use</td><td colspan="3">The use of animal models is crucial to our understanding of pain pathophysiology and the</td></tr>
</table>

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

animals and why you cannot use non-animal alternatives	development of novel pain killers. The translational animal models which are going to be used in this project provide unique systems responding to drugs used in the clinic.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible each animal will be used as its own control. Where this is not possible groups of animals will be utilised. In these cases the numbers in each group will be the minimum required to allow valid statistical analysis.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodents will be employed in these studies as they are the lowest vertebrate group on which these types of experiment can be conducted and their extensive use in biological research has already provided much information on pain processes. The severity of the models will be limited as far as possible by limiting the time for which animals are kept following induction of the pain model.