Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 21

Project Titles and key words

- Generation of monoclonal and polyclonal antibodies

  Monoclonal, hybridoma, antibody, immunisation

- Enhancing the initiation of immune responses

  Vaccine, immunity, dendritic cells, antigen processing, demyelination, allergy

- Protection against skin cancer

  Skin cancer, transcription factors, chemoprevention, UV radiation

- Regulation of Stem and Progenitor cell fate

  Stem cell, cancer, genetics

- The role of altered metabolism in cancer formation

  Cancer, metabolism, mitochondria, microenvironment

- DNA repair in development and tissue homeostasis

  DNA repair, cancer, Fanconi anaemia

- Targeting Wnt signalling in colorectal cancer

  Wnt signalling, intestinal homeostasis, colorectal cancer

- Neural Bases of Action

  Motor control, Motor dysfunctions, Neuronal circuits

- Information Processing in Innate Aggressive Behaviour

  Behaviour; Computation; Neuron

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Generation of monoclonal and polyclonal antibodies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Monoclonal, hybridoma, antibody, immunisation</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>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[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>To generate monoclonal and polyclonal antibodies to improve the understanding of disease and develop biotherapeutic medicines for the treatment of disease.</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>Antibodies are natures own weapons to fight infection, that have been harnessed by science to work more effectively. They are used as tools for scientific research, enabling scientists to understand diseases and their underlying mechanisms.

At our establishment our company’s goal is to develop medicines for treating patients in all disease areas such as asthma, cardiovascular, cancer, and arthritis. In achieving this goal we use antibodies to better understand the cause, progression and mechanism of any specific disease we are researching. As our research progresses we often need to make new antibodies to help us further our understanding and exploit what we have learned to develop better medicines and treatments for patients.

Our benefits in the use of monoclonal and polyclonal antibodies are:
• Improved understanding of the cause of disease and mechanisms of disease progression
• Identify new targets that can be used to develop new medicines.
• Develop diagnostic kits for identifying patients with symptoms of disease</td></tr>
</table>

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

	• Making antibodies that treat the disease in the patient
What species and approximate numbers of animals do you expect to use over what period of time?	Over the next 5 years I expect to use up to: 500 mice 100 rats 250 rabbits
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?	Based on previous antibody generation work at our establishment and refinements in our immunisation technique I expect the adverse effects to be minimal (1% of procedures). When adverse effects do occur the majority of these will be transient mild discomfort that could be expected to occur post immunisation in mammals. At the end of each immunisation protocol, the 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	Our company has invested heavily to develop state of the art facilities and technology to make antibodies using several different methods, and we are committed to only use animals when it is necessary. Every request for new antibodies is thoroughly scrutinised to ensure that all possible alternatives that do not involve animal use have been considered or carried out, before the use of animals is endorsed. We will be using *in-vitro* antibody display technology first to identify antibodies that can be used to support our research projects. In the event that we are not successful or for targets that are not tractable with this *in-vitro* antibody display technology, only then animals will be used to generate monoclonal and polyclonal antibodies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Based on our extensive antibody generation experience and all the refinements in our immunisation techniques, we have minimised the number of animals that are needed for any single target.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Our antibody generation techniques that involve animals use mice as the primary source of monoclonal antibodies. We have selected a specific hyper-responsive mouse strain to use for immunisations. The advantages are that less antigen is needed to get an immune response and the immune response can be amplified when compared to what you would get in other mouse strains. Rats are used for

	those rare occasions where, due to the type of antigen, they will produce a better antibody than mice or where we failed to generate useful antibodies in mice. Rabbits are normally used for making antibodies when we require a large quantity of polyclonal antibody for our *in-vitro* assays. Typically these antibodies are used in diagnostic tests to monitor new medicines that have been given to patients in clinical trials. All our work is based around getting the best quality of antibody reagents for advancing our understanding of disease and developing high quality medicines for patients. We continually strive to do this as few animals as possible and with minimal suffering to the animals when they are used.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Enhancing the initiation of immune responses</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Vaccine, immunity, dendritic cells, antigen processing, demyelination, allergy</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)[3]</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[4]</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 objectives of this project are 3-fold. First, we wish to continue our basic research into how immune responses are initiated. Effective vaccines to several debilitating diseases such as malaria, HIV and hepatitis C are still not available and the vaccine to the Foot and Mouth virus that infects livestock is ineffective, requiring farmers to re-vaccinate every 6 months. There are likely to be many reasons for the failure of current vaccines and we do not claim to address all of these. However, all vaccines must undergo a series of processing steps before they can trigger an immune response. We aim to boost some of those steps and to increase our knowledge of others. Our second objective is to increase our understanding of an important type of white blood cell called the eosinphil. In the developing world these cells are thought to help control parasitic worm infections but in the developed world they are more commonly associated with allergic diseases such as asthma where they release toxic proteins that contribute to tissue damage. Our recent work has produced insights into how these toxic proteins are activated and we think further work might suggest new therapies. Finally, our work on an enzyme that contributes to vaccine processing (see above) has unexpectedly revealed that is also has an important role in normal kidney function. Mice that lack this enzyme develop kidney disease that resembles some human renal disease and renal cancer. We think that further study of the kidney disease induced by lack of this enzyme may provide new</td></tr>
</table>

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

	insights into human kidney disease.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We anticipate the benefits of the work will be as follows: (i) improved basic knowledge of the immune response, offering potential new ways to boost desirable immune responses and suppress undesirable ones. (ii) potential improvements to vaccines. Here we will test very specific ideas for improving the formulation of vaccines and will apply this to the foot and mouth vaccine as well as other vaccines including cancer vaccines. (iii) improved understanding of how the immune system can become primed to attack normal cells of the body in diseases such as multiple sclerosis (MS) (iv) improved knowledge of some types of kidney disease and kidney cancer (v) improved knowledge of eosinophil biology and potentially, identification of possible targets to modulate eosinophil function in allergic disease.
What species and approximate numbers of animals do you expect to use over what period of time?	We use mice, as they are readily genetically modified and yet mimic human physiology very well. We use experimental groups of 6-8 in most cases. More may be used when statistically necessary (for example in some of the models of human disease). We expect to have to breed up to 5000 mice (the great majority under a 'mild' severity limit), about 3000 of which will have informative genetic make-ups that they be used in a variety of 'read-out' protocols designed to test the effects of specific interventions (genetic or pharmacological) on the activity of the immune system. Some animals will be used for post-mortem tissue analyses, in which case no in-life experimental interventions will take place.
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 mice we will use will experience transient discomfort as a result of needle-based injections of substances that are expected to affect the immune system (challenging it direct, or modulating its activity against other experimental interventions). In addition some will experience local inflammation, swelling at the injection site and possibly transient fever that can accompany immunisations with agents that stimulate immune cells. Some animals will undergo irradiation, to knock-down the immune system, before it is "reconstituted" with defined cells. Successful reconstitution is not associated with significant long-term effects on welfare. A few animals will experience partial and in most cases transient paralysis as a result of inflammation or specific targeting of cells that produce myelin in the nervous system. We will use a scoring system to ensure that

	robust humane (and scientific) end-points will apply to these animals. Because demyelination (like that in human multiple sclerosis) can come and go, these end-points have to be set at a point where recovery is still possible. Animals will be monitored very carefully to make sure that they are killed humanely and promptly if this appears not to be occurring. In the asthma model lung function changes are expected but these are relatively mild and require sophisticated apparatus to be measured in living animals. We are interested in developing vaccines against a number of diseases, including cancer. We shall use a standard model of cancer metastasis to test our hypotheses, in which significant deviations from normal welfare will not be expected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We first test our hypotheses in vitro using cultured cells but to draw strong and unequivocal conclusions that are potentially informative about human health, animal studies are unavoidable. This is particularly true in the immune system where multiple cell types and multiple tissues are involved. The immune response can be broken down into stages and we can and will, where possible, study individual stages in vitro. If the results are encouraging we will then proceed to animal studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We can reduce numbers in 3 ways: (i) By only proceeding to animal experiments if the in vitro data are strong enough to justify it (ii) Where possible, by using multiple tissues from individual culled mice. Different lab members can usually make use of different organs/tissues, e.g. kidneys, spleen, bone marrow etc. (iii) Where possible by maintaining gene deficient (knockout) mice (-/-) by intercrossing the knockout mice rather than by intercrossing heterozygous mice (+/-) which produces 50% +/- progeny of limited value.
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 is the only species that can realistically be used due to the availability of strains lacking specific genes. Also analysis of cells and tissues in the mouse is made much easier by the availability of reagents for analysis and established models of disease/infection. We will minimise animal suffering by use of anaesthesia where possible and realistic and by defining and adhering to clear humane end-points beyond which no (or little) useful information would be gained. We will use a moderate model of demyelinating disease to screen for any significant differences that are caused by our interventions.

	Only then will we determine whether these changes are maintained in a more relevant model of human demyelinating diseases (e.g., MS). In this way we will reduce the numbers, and increase the value, of the animals undergoing this more intrusive procedure.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Protection against skin cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Skin cancer, transcription factors, chemoprevention, UV radiation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</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>This project will assess the importance of an endogenous defence mechanism to protect against the development of skin cancer induced by ultraviolet (UV) radiation.

UV radiation from the sun is the major environmental risk factor for skin cancer, the most common cancer worldwide. Skin cancer is a major cause of complications and even death in people who undergo treatment with immune-suppressive drugs such as organ transplant recipients and inflammatory bowel disease patients, and there is an urgent need to find an effective method of prevention and treatment. Public awareness campaigns to educate people to avoid UVR-exposure have not shown significant benefit in the UK.

We are using a mouse model of skin cancer to test the protective properties of small molecules that activate the natural defence systems. This knowledge will be essential for the development of new therapies.</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>The information generated from this research will elucidate the role of the defence pathway in protection against skin cancer. It may allow for the development of practical applications (topical and/ or dietary) for the prevention and treatment of skin cancers due to UV exposure, the most ubiquitous carcinogen that is relevant to humans. If successful, the benefits of this research will be of vital importance for recipients of organ transplants, whose risk of skin cancer is especially high (~100-</td></tr>
</table>

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

	fold higher than the general population) and for whom skin cancer is a cause of serious health problems and even death.
What species and approximate numbers of animals do you expect to use over what period of time?	The mouse was chosen because of the availability of strains that differ in the activity of the particular defence mechanism. They have been crossed into a hairless line, so as to avoid any immediate discomfort or longer-term irritation from shaving the fur. From our previous experience, we have been using 30 mice per experimental group. This number of animals gives statistically meaningful results. We expect to carry out approximately 15 experiments (with two groups in each experiment), resulting in a total number of animals used for this purpose of approximately 1000. We have to breed the necessary genetically altered lines (and cross them with the hairless mouse strain) and we estimate that we will need to use 5000 animals for this, from which the experimental groups will be drawn.
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 have generated specific mouse strains which either lack a potentially important defence mechanism or possess it in super-abundance. We will test their susceptibility to UV-induced skin lesions, as compared with normal control animals. We will test some non-toxic but potentially protective compounds in this system. It will be important to establish not only that such compounds are protective, but also whether they exert their effects through this defence mechanism or by some hitherto unknown process. The various mouse strains do not have any apparent welfare problems. The UV irradiations are of short duration and low dose, so they do not cause the redness and irritation that are associated with “sun-burn”. The skin tumours that may form are small and are not expected to cause significant problems to the mice over the course of the interventions.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal models would not allow for the evaluation of potentially protective agents to be delivered directly to the skin (in a cream, for example) or in the diet, nor for the modelling of the increased risk of skin cancer in immune-suppressed patients. Skin is a very complex and dynamic organ composed of many different cell types that communicate with each other; ultimately the effects of UV radiation on this organ have to be studied using animals. Other components of our research programme do of course use non-animal alternatives. For

	example, our basic studies on the body's defence mechanisms and the dose-dependent effects of the protective agents are carried out in suitable cell culture systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	At all times, experimental design will be kept as simple as practically possible, in order to maximise the information obtained from the minimum number of animals. If there is any doubt about the design of a particular experiment, professional statisticians will be consulted concerning the minimum number of animals required to allow a sufficiently powerful 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.	Individually ventilated cages or isolators will be used to house the immune-suppressed mice, as otherwise they might be prone to infection. The UV exposures involve low doses, so are not expected to cause obvious "sun-burn" and the temperature will also be carefully controlled to avoid any heating. Some mice will develop skin tumours, but it will not be necessary to allow these to develop to sizes at which they would impact on normal welfare or behaviour. Animals will be killed humanely well before this end-point might be reached.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Regulation of Stem and Progenitor cell fate</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Stem cell, cancer, genetics</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>-</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[8]</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>The purpose of this work is to establish how stem cells sustain and repair tissues, and how changes to DNA implicated in human cancer alter stem cell behaviour.</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 project will give new insights into the behaviour of normal adult tissue stem cells in maintaining and repairing tissues and how mutations alter stem cell behaviour, which will improve cancer prevention strategies in humans. In addition, measuring the effect of ultraviolet light, on skin stem cells, will provide a rational basis for cancer prevention advice, and defining the effects of low dose X radiation on stem cells will inform policy makers in setting safe limits for radiation exposure.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Up to 15,200 mice will be used over five years</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>This project will apply new genetic technologies to characterise the changes in behaviour of stem cells in mice.

To define normal stem cell behaviour, animals will typically be given a single injection of drugs to induce a genetic marker in stem cells, and then allowed to age, until they are humanely killed to collect their tissues. Experiments to study the effects of gene alterations linked to cancer will have a similar design. Animals may develop tumours,</td></tr>
</table>

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

	but if they do so they will be humanely killed if the tumours approach 1cm in diameter or if they develop signs of cancer such as weight loss approaching 20%. In some experiments animals may be treated with drugs given by injection. Doses used, determined from the scientific literature or pilot experiments will be at a level which results in clinical signs such as weight loss approaching 20% . To study the effects of ultraviolet light, animals will be exposed to light doses below the level which causes a sunburn type reaction, and causes no signs of distress to the mice. X rays will also be administered at low doses, below the levels that cause acute toxicity. In wound healing studies, small wounds will be created under anaesthetic and animals given post operative pain control. Any animals which develop adverse clinical signs of infection or bleeding at the wound site, or systemic signs such as fever or weight loss approaching 20% after the procedure or will be humanely killed. The drug treatment, ultraviolet light, X ray and wound healing experiments may be combined with genetic marking. We anticipate that the majority of animals will experience mild side effects due to the nature of the experiments. A small proportion of animals will be required to undergo procedures that may cause a moderate level of side effects such as weight loss approaching 20% or altered behaviour. When necessary, anaesthetics and post procedure pain control will be given to alleviate any adverse effects or the animals will be humanely killed. All animals will be humanely killed on completion of all procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project is investigating stem cells in adult mammals with the goal of discovering findings relevant to humans. Mice are the only species in which the required genetically engineered animals are available. Non-animal alternatives are not able to reproduce the complex behaviour of stem cells within a tissue environment, so animals are essential for these studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have developed and refined a new method of labelling cells. This new method will be used in experiments to label cells and to track their subsequent behaviour. Because thousands of cells can be labelled and analysed in each mouse, the use of this new method means that far fewer mice are required for these experiments compared with conventional approaches.

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 to be used, as the only species for which the required genetically modified animals are available. Most experiments will alter genes in only a small percentage of cells in a tissue, which are then tracked to reveal the effect of the alteration. The majority of unaltered cells function normally during the experiment. This means that the animal does not suffer ill effects that occur when all the cells in a tissue are altered. In addition we will always use the minimum required doses and durations of treatment, when giving animals drugs, X rays or ultraviolet light determined from published literature or small scale pilot experiments.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The role of altered metabolism in cancer formation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, metabolism, mitochondria, microenvironment</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>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[10]</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>Cancer is currently viewed as a genetic disease whereby well-characterised gene mutations are sufficient to drive unrestrained growth and proliferation. In order to support proliferation, cancer cells utilise a specific set of nutrients, among which glucose and glutamine are the most important. It has been shown that inherent dysfunctions of glucose and glutamine metabolism in some circumstances predispose to cancer formation. However, how altered metabolism drives tumorigenesis in not fully understood.
In this project, we intend to investigate the role of genes involved in cell metabolism in cancer formation. We will start by studying the role of these genes in altering cell metabolism using cell culture models. Then, we will move into animal models to define how each candidate gene triggers tumorigenesis in vivo.
Our goal is to find metabolic pathways required for the survival of these mutant tumours in order to determine potential anti-cancer drug targets. Furthermore, we aim to detect early signs of these metabolic changes in body fluids such as urine and blood, in order to discover novel biomarkers for the early detection of 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>The work will provide novel information about how altered metabolism affects tumorigenesis and will lead both to the discovery of novel cancer biomarkers and cancer therapy.
The main benefits from this study relate to new mechanisms of how tumours control their fate by</td></tr>
</table>

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

	manipulating cell metabolism. The information will likely be of importance to preclinical scientists interested in cancer metabolism. Secondary potential benefits translate to the value of the results beyond the laboratory, and to the possibility of a) the identification of new molecular targets and b) improved strategies for drug delivery/effectiveness, by virtue of a greater understanding of how aberrant metabolism impacts a tumour.
What species and approximate numbers of animals do you expect to use over what period of time?	Our work focuses exclusively on mouse models. We expect to use about 200 animals per year
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals may exhibit moderate clinical signs. Where subcutaneous tumours will be induced, the majority (~85%) are expected to be small epidermal tumours which will have no significant impact on the animals' general well-being. For mice where inducing/deleting agents are required to induce tumours, transient discomfort immediately after administration is anticipated (recognised by hunched posture and inactivity) but animals are expected to make a rapid recovery. Animals will be observed closely for any evidence of tumour growth by careful palpation and/or inspection of the injection site. For the other procedures such as metabolomics, imaging and pharmacological treatments at least 75% of animals will show no more than mild clinical signs, less than 25% will show moderate signs and of these less than 1% will require culling. Animals will be killed before they show sustained signs of ill health. All animals will be killed at the end of experiments, this will be undertaken using schedule 1 methods, perfusion fixation and removal or organs and tissues the method used will be dictated by the data output being measured.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	A large proportion of this study employs cell culture models, which will be used to investigate our hypothesis. However, these models cannot fully recreate the dynamic events that occur in a living system and recapitulate cell-to-cell interactions. We will move into animal models to validate our findings in a more physiological milieu and in the context of preclinical studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the smallest number of animals that will ensure robust results. We will calculate those numbers collaborating with experienced statisticians. Once experimental end points are

	reached cancer from affected animals will be harvested to facilitate continuing, complementary ex vivo/in vitro studies. Performing pilot studies of drug toxicity and metabolomic labelling on small cohort of animals will reduce the number of animals to be used. Finally, thanks to imaging techniques such as ultrasound scanning and magnetic resonance to track disease progression will avoid unnecessary animal usage.
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.	Mouse models have been chosen as they represent the least sentient species able to generate meaningful data i.e. that is likely to be directly applicable to the human disease. Animal suffering is minimised by the use of appropriate anaesthetic and analgesia. Where clinical signs are seen animals will be culled as soon as possible and before they are likely to develop signs of pain or distress that would exceed a moderate severity limit.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>DNA repair in development and tissue homeostasis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>DNA repair, cancer, Fanconi anaemia.</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)[11]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This PPL application aims to understand the role of a specific group of DNA repair pathways in mice. Over the last 12 years we have been identifying new components of a DNA repair pathway the loss of which leads to an inherited human illness called Fanconi Anaemia (FA). Using cell lines and biochemistry of purified FA proteins we have gained an improved understanding of how the FA proteins interact with other DNA repair proteins and how they fix damaged DNA. Our most recent work has shown that this DNA repair pathway helps protect and preserve DNA from chemical attack by a class of highly reactive molecules called aldehydes – these chemicals are by-products of metabolism and also common in our food and environment.

This discovery has led to several key areas requiring future exploration. We want to uncover the exact role of the Fanconi pathway in physiology and precisely what this DNA repair pathway does to prevent this human disease. The key unknowns are: why stem cells are affected in this disease? Where do these aldehydes, which damage DNA, come from? What kind of DNA damage do these aldehydes cause?</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>The importance of the FA proteins is underscored by the catastrophic phenotype of children with Fanconi anaemia. These children get bone marrow failure and developmental abnormalities coupled with a 1000-fold risk of developing cancer. A better understanding of what the FA proteins do in a broad physiological context is likely to provide important insights into certain human diseases such</td></tr>
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[11] Delete Yes or No as appropriate.
[12] At least one additional purpose must be selected with this option.

	as bone marrow failure, developmental defects and cancer as well as patients with this rare genetic disease. The identification of a common environmental mutagen that precipitates the FA defect may have consequences for public health.
What species and approximate numbers of animals do you expect to use over what period of time?	We use mice and genetically modified mice in our research. We anticipate using around 60,000 mice over a period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We anticipate that our mice may suffer developmental defects, predisposition to bone marrow failure (anaemia) and cancer. Over the period of the last project licence we have developed accurate methods to measure the clinical signs of mice and from these to predict outcome. This allows us to cull mice before they reach the severity limit, allowing us to gain maximum information from every mutant mouse we generate. Mice with developmental abnormalities are typically generated through our teratogenesis protocol in which embryos are exposed to aldehydes. These pups are usually culled before term and the developmental abnormalities are assessed. Occasionally these pups will be weaned and carefully assessed as soon after birth as possible. In general these mice have eye defects (absence of one or both eyes) or 'kinked' tails which in themselves do not pose a significant welfare problem. However, if future pups display unexpected harmful defects these mice will be killed or advice sought from the Home Office Inspector if the animals are of particular scientific interest. Mice generated during this project may develop bone marrow failure or cancer (which is usually leukaemia). Both of these diseases have a similar spectrum of clinical signs and severity which we have come to accurately assess during the previous project licence. The best indicator of either of these diseases is weight loss – therefore mice with a genotype that predisposes to these diseases are identified early. The mice are then weighed weekly. Weight loss can then be easily monitored and mice can be culled before they reach the severity limit. In addition the mice develop piloerection, a hunched posture and inactivity. These are signs of profound anaemia that can also be assessed through the paleness of the paws. If these clinical signs develop it is an indication that the mice must be culled. Undertaking the steps

	above maintains mice within the severity limit but also allows every mouse to be analysed reducing waste within the colony. Finally, we have one protocol that is severe in its severity. In this experiment mutant mice, which cannot effectively break down aldehydes, are exposed to an exogenous source of such aldehydes. Mice of a particular genotype (Aldh2-/-) are severely affected. Following exposure to the aldehyde these mice enter a collapsed state. This prostration is prolonged in mutant mice lasting more than 1 hour but less than 6 hours. These mice also show signs of ataxia, piloerection, and rapid breathing which will last less than 12 hours. Mice that have not recovered from prostration in 6 hours or have not fully recovered in 12 hours will be killed. Mice of all other genotypes undergoing this procedure have transient prostration (<20 minutes). This is an important experimental model to use as it gives us insight into the role of alcohol (the exogenous source of aldehyde) in individuals deficient in Aldh2. This is of particular importance to health as over ¼ billion people on earth have defective Aldh2. Therefore it is important that we understand the role of alcohol in models of Aldh2 deficiency.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The main reason for the use of this animal model is that we intend to investigate how such DNA repair pathways enable normal development, help mammals to deal with common environmental and dietary genotoxins, preserve stem cells and finally protect against DNA changes that lead to cancer. It is really only possible to study development in the context of a whole animal. Furthermore, to study both stem cell biology and cancer in a physiological setting necessitates the use of animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our experimental design will ensure we use the minimal number of mice to achieve statistical significance. When embarking on work with many variables we shall carry out small pilot studies so that we can refine our experiments. All of our Fanconi mice are sterile and born at low ratios compared to what is expected. This necessitates large breeding programmes with many mice of the wrong genotype being generated. Over the past 3 years we have generated conditional knockout strategies. This will therefore allow us to greatly reduce the number of mice that we have to breed to get useful genotypes.

	Finally, we will cryopreserve our strains. This prevents us needing to keep all of our mouse strains alive just to perpetuate the strain.
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 mice due to their genetic tractability and similarity to human development and stem cell function. The models that we have are the best available models to study the effects of aldehyde-mediated DNA damage. Furthermore, these are the only mouse models that recapitulate the key features of Fanconi anaemia. For the first time these models enable us to study the physiological role of the Fanconi DNA repair pathway. As mentioned above we have invested in the generation of conditional mouse models. These will allow us to further refine our models. These mouse models include tissue-specific models e.g. allowing us to study the role of aldehyde damage in T-cells without the mice developing bone marrow failure. They also allow us to switch off the DNA repair pathway in response to an inducing agent (e.g. tamoxifen). This allows us to generate knockout mice when we need them to, further reducing the possibility that the mice will develop disease when not in an experiment. As outlined above all of our mutant mice that may develop limiting clinical signs will be prospectively identified at genotyping (14 – 21 days old). These mice will be monitored carefully by day daily inspection for clinical signs and also through weekly weighing. Mice that are beginning to develop clinical signs will be culled and analysed before they reach the moderate severity limit whenever possible. In our one severe protocol mice will be monitored carefully for ill health. If the mice do not recover from prostration after 6 hours, or do not return to having clinical signs of a mild limit within 12 hours the mice will be killed to prevent any additional suffering. (1276 words)

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Targeting Wnt signalling in colorectal cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Wnt signalling, intestinal homeostasis, colorectal cancer</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>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Colorectal cancer is the third most common cause of cancer death in the UK. Most cases of this lethal disease are kick-started by the aberrant activation of an important cell signalling pathway, called the Wnt/□-catenin pathway, which mediates communication between normal cells in all animals, thereby controlling their development and tissue homeostasis. Notably, -catenin regulates the self-renewal of the intestinal stem cells: its activity is kept in check by the Adenomatous polyposis coli (APC) tumour suppressor, but if APC loses its function due to a mutation, -catenin is aberrantly activated outside stem cells and thus produces the cells-of-origin for colorectal cancer. APC mutations are found in >80% of sporadic human colorectal cancers, but also in heritable forms of this disease: for example, Familial adenomatous polyposis (FAP) patients carry germ-line mutations in APC, and therefore develop thousands of benign tumours during their teenage years, and full-blown cancer by middle age. Oncogenic □-catenin mutations have also been found in many other types of cancer. Yet, despite its importance in cancer, there are no validated small-molecule inhibitors of the Wnt/ -□ catenin pathway that could be developed into therapeutics.

The aim of our overall research programme is to understand at the molecular level how Wnt signalling activates -catenin and how this pathway is repressed by APC. We focus on newly discovered regulators of -catenin that have</td></tr>
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	potential in cancer therapy since their inhibition might block the aberrant activity of oncogenic -□ catenin. A small but important part of our research programme involves animals, in particular a genetic mouse model (called the *Apc-Min* model): mice bearing the *Apc-Min* mutation develop numerous intestinal tumours, similarly to human FAP patients. This model therefore provides a close approximation to the human disease. Our animal work has three specific objectives: (i) to determine the role of Wnt signalling molecules in intestinal tumorigenesis in the *Apc-Min* model; (ii) to define the functions of these molecules in the normal mouse intestine and during regenerative homeostasis; (iii) to test the therapeutic benefits of rationally-designed Wnt signalling inhibitors in the *Apc-Min* model.
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 overall aim of our animal project is to advance the scientific understanding of the physiological role of new Wnt/□-catenin regulators in the normal intestine and their role in promoting intestinal tumorigenesis. We aim to validate these regulators as novel drug targets, to develop inhibitors against them and to test their therapeutic benefits in the *Apc-Min* model. This could open up new avenues for the therapy and prevention of colorectal cancer in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	To reach our objectives, we require genetically altered mice, maximally 20'000 over 5 years, most of which (>80%) are needed for breeding purposes only and for constructing specific genetic strains.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of our mice (>80%) are not expected to experience any adverse effects. However, a small fraction of them (<20%) carry the *Apc-Min* mutation, and will thus develop intestinal tumours. They may therefore show moderate signs of this disease, including pale feet, ruffled fur, inactivity and lack of appetite. Mice will be humanely killed once they present with overt disease.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complexities of the normal and cancerous human intestine cannot be modelled adequately *in vitro* or *ex vivo*. It is therefore essential that we use an animal model that allows us to determine the physiological role of Wnt signalling molecules in the most appropriate tissue setting in a living organism, to validate these molecules as therapeutic targets and to test putative inhibitors of these molecules as

	anti-cancer drugs.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Wherever possible, we plan to use non-regulated procedures and approaches including biochemical, biophysical and structural analysis, cell-based assays in human cell lines and functional tests in the fruit fly *Drosophila*, an animal model which we have used extensively to discover new Wnt signalling genes. The experimental design follows well-established protocols, based on minimal cohorts that allow statistically significant results. We hold regular meetings to optimise our breeding strategies and the management of our mouse colonies. Our experiments are based on a well-established tumour model, allowing us to rely on extensive previous experience to ensure that we use the minimum number of mice. Pilot experiments with small cohorts and statistical analysis will be used wherever necessary to minimise animal numbers. Cryopreservation of strains will be used to avoid having to keep live stock unnecessarily. We will collect tissue samples from multiple body sites, and provide tissues and mouse strains to other scientists, to maximise the information from a single animal or strain.
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 become *the* leading vertebrate model, due to their relative ease of breeding and genetic manipulation, and because they are the lowest sentient mammal that is closely related to humans. Much is known about mouse development and tissue homeostasis, and sophisticated genetic technology is available that allows rigorous and highly refined experimentation. Our model of choice (the *Apc-Min* model) provides a close approximation to the human disease, and we expect the insight we gain from this model to be highly relevant for human therapy. Our experiments require only minimal invasive procedures, mostly intraperitoneal injections, which will be carried out by highly trained staff and will typically cause no more than transient discomfort. All mice will be housed and maintained to the highest international standards for welfare. By choosing a well-established model, we minimise unknown effects on mice and subsequently pain, distress and suffering. The signs of the neoplastic disease in the *Apc-Min*

	model are well known, and obvious on inspection. Mice carrying the *Apc-Min* mutation will be monitored daily for signs of the disease, and humanly killed following disease onset. We will only subject animals to neoplasia studies once we have sufficient evidence from non-regulated procedures that our genetic manipulations or drug treatments will alleviate their disease.

Neural Bases of Action
Motor control, Motor dysfunctions, Neuronal circuits

- Summarise your project (1-2 sentences)

We study the organization and function of neural circuits controlling movement.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

With our work we expect to gain new insights into how motor networks are assembled and how perception is integrated into motor action. Understanding the nature of these circuits, the genetics of them, how they are assembled and how they function is fundamental for the understanding of how we produce purposeful movements and why we fail to do so in a number of neurodegenerative disorders.

- Outline the general project plan.

With our work we will try to identify, at the electrophysiological and genetic level, the neuronal populations responsible for specific aspects of the motor programme.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

These studies require tracing and recording of specific neuronal circuits, which could not be done in humans. However, our tracing and recording methods are minimally invasive (see below) and cause minimal distress to the animal, which is in fact normally engaged in behavioural tasks that it performs at will and with interest.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

We believe that with this approach we will be able to understand novel aspects of the neuronal control of movements and to identify neuronal populations that are crucial for specific features of motor execution. There is an ever-increasing incidence of neurodegenerative disorders that affect motor function to various degrees. These have an enormous impact on the life of million of patients, with motor defects ranging from dyskinesia to complete loss of voluntary movements. We believe that our findings will be useful to identify and target more precisely those neuronal populations whose impairment leads to these severe motor defects.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

Mice are the experimental species of choice because it is possible to generate and acquire genetically modified strains, which allow the visualization and manipulation of selected neuronal populations; presently this can not be achieved with other species and it is crucial for the success of the proposed project. We expect to use 2000 mice for the tracing and electrophysiological recordings and to breed a total of 4000 transgenic mice over 5 years to maintain the stocks and provide animals for the experimental procedures.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

The role of neural networks activity in motor performance can only be studied in the intact, freely moving animal. Implantation of chronic indwelling electrodes in humans is only permissible in a very small number of clinical situations and thus is impractical for research purposes. We have collaborated (and will continue to do so) with colleagues who devise computational models of motor networks and have on occasion used these to design experiments and predict their outcomes, but the models are extraordinarily simple in comparison to the complexity of the brain, and cannot substitute for experiments themselves. We intend to use the minimum number of animals consistent with achieving our experimental aims. The animals are often tested for long periods and thus considerable information is obtained from each animal, minimising the total number used. With an appropriate use of statistical methods and the use of inbred strains we keep the use of the animal at a minimal required level. Whenever possible we make use ex vivo recordings. This will reduce the instances in which we have to perform in vivo acute or chronic recordings which greatly decreases the number of animals used under these protocols.

- Explain why the protocols and the way they are carried out should involve the least suffering.

In order to trace neuronal circuits and record neuronal activity we implant microelectrodes chronically or acutely. The implantation of electrodes in defined brain regions might seem intrusive at first but the presence of the implants is completely painless. The surgical approaches used are the least severe available, involving the smallest amount of tissue damage. Animals are given extensive post operative care including antibiotics and analgesics. Animals are closely monitored throughout the experiments and any signs of problems with implants or other aspects of surgery are immediately dealt with, or, if this is not possible, the animal will be killed. Similarly, animals are closely observed and monitored during the recording experiments and during interactions with other animals.

Information Processing in Innate Aggressive Behaviour
Behaviour; Computation; Neuron

- Summarise your project (1-2 sentences)

It is not known how neurons in the brain control instinctive behaviours essential for survival. The goal of this project is to identify the key mechanisms used to implement the computations underlying innate behaviours in the mouse.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

Neurons in the brain receive information via so-called dendrites - long extensions resembling tree branches - which have properties that allow them to transform information before it reaches the output end of the neuron. It is not known whether these dendrite properties are used in the functioning brain, but if they were then each neuron could behave like a mini-network of high computational power. This would allow neural circuits to carry out tasks more complex than those considered in current models of brain computation. It is therefore essential that we understand how dendrites compute and whether their computational properties control the input-output relationship of neural circuits.

- Outline the general project plan.

We will work on innate aggressive behaviour, because aggression is controlled by evolutionarily primitive brain areas, which are conserved across all vertebrate species, including humans, and because this behaviour can be triggered rapidly and reliably, which is essential for linking it to cellular and molecular events. We will start by identifying the populations of neurons activated during aggression using behavioural assays and high-resolution imaging. Using physiological recordings we will determine the properties of the selected neurons and of their dendrites, identifying the relevant inputs and how they are processed. Once key molecular mechanisms of input integration are identified, genetic modifications together with physiological recordings and behavioural assays will be used to establish causal links between specific computational mechanisms and the behaviour.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

The main procedures used in this work will be physiological recordings, imaging and injection of substances, which require a surgical procedure to gain access to the brain. Adverse effects are expected to be minor, and will mostly result from post-operative complications following surgery. If mice show signs of ill health, distress or suffering, they will be humanely killed.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

This work will enhance and advance our knowledge on how the brain processes information from the outside world and converts it into behaviour, in particular, aggressive behaviour. This information could lead to the design of new highly selective drugs for treating aggression in medical conditions such as schizophrenia and autism, which could be used with minimal side effects to manage aggression levels. The programme of work will also generate a number of technical advances in cell-specific genetic manipulations and microscopy, including the development of software. These will have an impact across all fields of Neuroscience and will also be of use to researchers in other areas of science, such a cellular biology.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

This work will use less than 3000 mice over 5 years. Mice will be used as they are an appropriate model for neuronal physiology studies and reliable transgene technologies are established for this species. We will use the same animal for performing experiments and controls, which reduces the number of animals and increases statistical sensitivity. Statistical power will be further increased by using different methods simultaneously, and to maximize the data generated from a single animal, different procedures will be done sequentially and contribute to multiple steps of the project.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

The key goal of this project is to link behaviours, such aggression, with cellular and molecular mechanisms, and therefore it requires experiments performed in behaving animals with intact neuronal networks. While we have considered other techniques such as primary neuronal cultures, these are unfortunately inappropriate, since the culturing procedure alters the organisation of the network and crucially, precludes behavioural assessments. Throughout the project, data-based computer models will be used replace the use of animals when possible, and to guide experimental design.

- Explain why the protocols and the way they are carried out should involve the least suffering.

To minimize harmful effects we will use non-invasive imaging and well-established physiological techniques, and whenever possible, physiological recordings will be carried out on anaesthetised animals. When using pharmacological agents, dose-response curves will be generated *in vitro* to guide *in vivo* application and minimize side effects. We will use genetic models that allow regulation of the activity of the gene under study using well-established agents to induce or delete the candidate gene, thereby reducing the likelihood of generating severe brain function perturbations.