Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 18

Project Titles and key words

- Metabolic alterations of pregnancy

  Pregnancy, metabolic disease, offspring, therapies

- African Trypanosomiasis

  microscopic parasites

- 5-HT circuits regulating energy/glucose balance

  5-HT, appetite, brain, obesity, type 2 diabetes

- Investigating long-range synaptic pathways in cortical regions

  Hippocampus, Synapse, electrophysiology, cannabinoid, neuroanatomy

- Molecular Neuroscience of Ligand-gated and G-protein coupled receptors

  GABA, Nervous system, Neurological disease, Ion channels, neuro-transmitter receptors

- Information Processing in Innate Aggressive Behaviour

  Behaviour; Computation; Neuron

- Metabolic alterations of pregnancy

  Pregnancy, metabolic disease, offspring, therapies

- Factors contributing to liver failure

  Liver Disease, Hepatic encephalopathy, acute liver failure (ALF), acute on chronic liver failure (ACLF), Cirrhosis

- Oocyte functionality post cryopreservation

  Fertility preservation, xenografting, nude mouse, human ovarian tissue, oocyte functionality.

- The role of RING finger proteins in malignancy

  PML, BRCA1, RING finger, Malignancy, DNA repair

- Collection of Blood and Arthropod Feeding

  Arthropod maintenance, blood, primary cells

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Metabolic alterations of pregnancy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Pregnancy, metabolic disease, offspring, therapies</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)[1]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</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">Pregnancy is associated with a series of metabolic changes in the mother that are necessary to support the nutritional needs of the developing baby. These can have consequences for the health of the pregnant woman and her baby during pregnancy and in later life. In normal pregnancy, these changes include raised cholesterol levels as well as increased insulin resistance, a condition that usually leads to diabetes, and high blood levels of bile acids (chemicals made by the liver as a way to remove cholesterol from the body).
In high-risk women, these changes cause metabolic disease of pregnancy. Metabolic disease of pregnancy can cause increased rates of sickness and death of the pregnant woman and her baby. They also have implications for the subsequent health of the children of affected pregnancies. Moreover, metabolic changes in pregnancy may have important health consequences for women who do not have diseases of pregnancy e.g. women who have had a large number of pregnancies have an increased risk of developing heart disease in later life, and this is thought to be due to continuous exposure to raised levels of cholesterol.
This work aims to elucidate the factors that drive gestational metabolic changes and how these factors can lead to metabolic disease of pregnancy. The impact on the embryo and children will be also determined. Additional experiments will enable evaluation of therapies that can be applied to prevent metabolic disease in pregnancy.</td></tr>
</table>

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

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The proposed research will have an impact on human health for a wide spectrum of individuals. The results will be of relevance to women with metabolic pregnancy disorders, *e.g*. gestational diabetes, cholestasis and obesity. Children of affected pregnancies who are more susceptible to metabolic syndrome may benefit from this work. There will also be economic benefits to the NHS if this research identifies effective treatments to reduce metabolic disease of pregnancy and susceptibility of children and young adults to metabolic syndrome. This work will inform affected women of ways they can improve the subsequent health of their children. Pharmaceutical companies that invest in strategies to prevent obesity, diabetes and fatty liver will benefit from our proposed research. This research is investigating factors that are involved in the aetiology of these diseases, and will provide insights into strategies that could be tackled by drugs or other therapeutic interventions in young adults that are susceptible to metabolic syndrome. The work will also have an impact in the field of the developmental origins of health and disease, as we have developed new experiments to investigate factors of pregnancy that cause subsequent susceptibility of the children of affected pregnancies to metabolic syndrome.
What species and approximate numbers of animals do you expect to use over what period of time?	The species we expect to use are mice. The estimate number for the duration of the project is 8000.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The proposed research plan involves mating of animals and characterisation of the metabolic profile of the offspring through collection of organs after killing the animals in a humane way. In the cases of more invasive methods, such as surgical procedures *e.g.* to remove reproductive organs or supply a compound or imaging, general anaesthetics will be used in combination with anaelgesics, painkillers and proper post-operative care to keep pain and suffering in the absolute minimum. Surgery will be carried out using the same kind of aseptic techniques that are used to avoid infection in human operating theatres. Special diets and other non-invasive methods such as routine tests to assess glucose and insulin function that will be used in this research are not expected to cause any pain and animals will be treated in a

	humane way in every occasion. No animal is expected to experience more than moderate severity and many will experience no more than mild.
Application of the 3Rs	
3. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will employ non-animal experimental tools as alternatives to the use of live animals wherever possible, For studies of metabolic alterations, we have an active human research programme to collect samples from pregnant women and the fetus where possible from cholestatic cases and non-pregnant controls. This includes blood, urine, faeces, placenta, intestine, liver and uterine biopsies, fetal samples and amniotic fluid.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Based on the animal data, we always aim to reflect findings at the clinical level by collection of human samples (*e.g*. blood, urine, faeces and placenta where feasible) or by performing population studies or by developing non-animal tools with human resources where appropriate. Moreover, the proposed experimental designs and methods of analysis are always discussed with statisticians so that we can maximise the information obtained from the minimum resource. Also, more than one researchers share the same animals to address their questions. In this way, we aim to minimise the numbers of animals used for our studies.
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.	It is necessary to use mice with pregnancy disease to investigate the aetiology of metabolic disorders of pregnancy as it is not possible to obtain liver, pancreas and fat from pregnant women and their children. Moreover, use of animals is a useful method to determine causes of disease as genetic and lifestyle influence, often referred to in population studies, can be eliminated. This will allow better evaluation of data and more solid conclusions to be drawn. Also, based on studies performed by the applicant and others, there is already a considerable amount of background information on the hormonal and metabolic parameters of mice that will facilitate experimental planning and validation of the results. Our research plan involves mating of animals and screen of metabolic profile through collection of samples after killing the animals in a humane way. In the cases of more invasive methods general anaesthetics will be used in combination with anaelgesics, painkillers and proper post-operative care to keep pain and suffering to the absolute minimum. Special diets and other non-invasive procedures such as routine glucose and insulin tolerance tests that will be used in this research are

	not expected to cause any pain.

African Trypanosomiasis

- Summarise your project (1-2 sentences)

We want to understand the response of our immune system to infections with African trypanosomes, microscopic parasites which cause serious human and livestock diseases in sub-Saharan Africa and are listed by the WHO as priority Neglected Tropical Diseases

- 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.

This work is part of an integrated programme of studies involving direct study of the disease in the field, the use of laboratory animal model infections, and the use of in vitro cultures. There are three goals.

First we want to understand virulence – that is why most individuals get very sick with sleeping sickness and will die without treatment but some apparently only get a mild disease. We think this is connected to barriers preventing invasion of the brain by the parasite, and that these are related to particular types of immune reaction in different individuals. Understanding this may allow us to target drugs that prevent invasion of the brain, and in the case of livestock, selectively breed animals that have some degree of resistance to infection.

Second, we want to identify new diagnostic markers for infection, and in particular the advanced stage of disease known as the late stage where parasites enter the brain. Our initial clues in this work come from clinical studies in Uganda and Malawi but we now need to employ model infections in mice to understand the process by which the levels of candidate markers vary in the bloodstream. This in turn will be used to inform clinical validation studies in Uganda. The potential of this work is to provide a dipstick type test (similar to home pregnancy test kits) for disease staging that as well as being quick and easy to read, also will not require the current practice of taking a lumbar puncture (spinal tap).

Finally, we are interested in alterations in food intake and body weight in infection. We believe that these are also potentially new markers of disease progression, as well as being of potential profound importance to the nutrition of patients. We need to understand the mechanisms of these alterations.

- Outline the general project plan.

Our project work is informed directly by clinical studies with trypanosomiasis patients. From these studies we identify candidate molecules that are involved in the immune response and which are potential diagnostic markers or mediators of immunological disease effects and develop hypotheses relating to their role in disease or applicability as clinical diagnostics. As part of these studies, it is necessary to infect mice with trypanosomes. While we use *in vitro* culture extensively for whole animal effects of infection there are currently no adequate in vitro simulations. Also while we work also with human subjects, our studies are for ethical reasons of an observational nature; we cannot carry out experimental interventions.

In a typical experiment, mice would be infected with trypanosomes. The infection in mice

follows a similar pattern to that in humans, except it progresses more rapidly. Almost all our studies will use each infection to study more than one of the above research questions. As an example, if we want to study the basis of weight loss during infection, we will during the infection take very small blood samples from which we can also determine how the immune response develops thus enabling us to address questions on virulence, and also in the same experiment these blood samples will be used to measure the levels of diagnostic markers.
We have a lot of experience with the mouse as a model of trypanosomiasis, in most studies animals will be euthanized before overt symptoms develop, animal numbers are kept to a minimum using statistical models to ensure maximum power, and we collaborate and liaise regularly with all other groups working on this disease around the world so as to ensure no duplication of experiments take place.

- 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.

Infections of mice may cause transient stress and discomfort at the time of inoculation, and also as the infection develops. We control for the latter with a clinical grading score system developed over many years that ensures that any animal demonstrating symptoms above a mild (protocol 1) or moderate (protocol 2) level are euthanased. The monitoring procedures to measure the developing infection and immune responses involve taking tiny blood samples and we expect these to cause minimal discomfort and stress. Some animals will require surgery to implant transmitters, and this may cause post-operative stress and discomfort.

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

As noted above, African trypanosomiasis is a serious tropical disease. There is a desperate need for new diagnostics and drugs. Current diagnostics for disease staging are highly invasive requiring lumbar puncture, and development of a less invasive blood test would be of high value, encouraging early diagnosis. Understanding the immunology of the infection will help us understand why up to 10% of people treated with the currently available drugs either die or develop permanent neurological damage, and provide insights into more rational chemotherapeutic approaches.

- 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.

We only use mice, a species in which very well defined models of trypanosome infection have been developed and described over 3 decades. We know from our clinical studies that mice show the same pattern of infection and host-response to humans but with a more rapid tempo.
We will use altogether up to 150 mice per year for this work. We have a lot of experience with the mouse as a model of trypanosomiasis, in most studies animals will be euthanized before overt symptoms develop, animal numbers are kept to a minimum using statistical models to ensure maximum power, and we collaborate and liase regularly with all other groups working on this disease around the world so as to ensure no duplication of experiments take place.

- 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.

While *in vitro* methods for the growth of African trypanosomes are now available (and our used in this lab), this project is primarily interested in the response of the host to infection, and this requires either clinical or animal model studies. All our work is grounded on our

own work in the field on host-response in human trypanosomiasis, where we measure immune response and diagnostic candidates in blood and CSF samples, in relation to detailed clinical case history. These results (submitted to peer review through publication and grant application) then are used to develop hypotheses that can only be tested in animal model systems.
• Explain why the protocols and the way they are carried out should involve the least suffering. While it is impossible to avoid some element of suffering when one experimentally causes an infection with a parasitic disease, suffering is minimised by letting the infections progress for the minimum duration commensurate with the experimental aims, and the use humane end points based on a clear clinical grading score.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>5-HT circuits regulating energy/glucose balance</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>5-HT, appetite, brain, obesity, type 2 diabetes</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)[3]</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>
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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>Yes</td><td></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>Obesity and type 2 diabetes represent major medical and economic challenges for the 21st century. However, strategies for obesity treatment are limited, reflecting a profound worldwide unmet clinical need. For the past 15 years, compounds influencing a particular brain chemical called 5-HT have been at the forefront of obesity treatment, but these drugs have been withdrawn from clinical use due to off-target effects. Our strategy is to pursue the therapeutic mechanism underlying these compounds because we already have global clinical evidence that they are effective for human treatment. Therefore, the main focus of the research programme is to unravel the way these obesity drugs work.</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 work is expected to provide novel and therapeutically relevant information about the molecules and pathways that regulate energy and glucose homeostasis. It will advance our knowledge of how molecular processes in the normal state are mis-regulated in obesity and type 2 diabetes. Pathways and factors involved in obesity and type 2 diabetes may be identified that could lead to the discovery of new strategies for treating these common global conditions.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>The mouse has been selected for most of this work as it is the lowest model organism in which energy balance has been extensively characterised and is the species in which reliable transgene technology is best established. When designing the experiments, we perform statistical analysis to ensure that we use the minimum</td></tr>
</table>

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

	number of mice per group that will be informative using power analysis. In order to reduce the number of breeding pairs, the mice will be kept as homozygous mice, where possible, provided that they do not have a harmful phenotype and wild type littermates are not required for analysis. Up to 4,500 mice and 500 rats will be used over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We do not expect animals to experience adverse effects and thereby the expected severity for most animals is mild or unclassified. The maximum severity under this protocol is moderate, which will be experienced by a subset of the animals. In particular, some animals will undergo surgery, including brain surgery, and we do not anticipate that adverse effects will be observed. At the end of the protocol, all animals will be humanely killed.
Application of the 3Rs	
3. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At present, there is no alternative to animals for studying the complex behavioral, physiological, and neuroanatomical processes of appetite and the regulation of metabolism. The mouse has been selected for most of this work as it is the lowest model organism in which energy balance has been extensively characterised and is the species in which reliable transgene technology is best established. Where rats are a better model for a specific component of the neurocircuitry of human metabolic disease, they will be used. Humans are not suitable for this work because technology is not sufficient to identify specific neurons regulating energy balance. For example, fMRI can only identify gross brain regions that are activated in response to meals, but cannot provide any further information. We are already aware of the general brain regions regulating energy balance. What is now required is an identification of specific neurons within these broad regions so that we can identify specific targets for obesity and type 2 diabetes treatment.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When designing the experiments, we perform statistical analysis to ensure that we use the minimum number of mice or rats per group that will be informative using power analysis and consultation with a statistician, where necessary. Mice or rats will be assigned to treatment groups using a Latin Square design to simulate random assignment. In order to reduce the number of breeding pairs, the mice will be kept as homozygous mice, where possible, provided that they do not have a harmful phenotype greater than moderate severity and wild

	type littermates are not required for 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.	We will utilise an extensive knowledgebase for refinement. The mouse has been selected for most of the experimental work as it is the lowest model organism in which energy balance has been extensively characterised and is the species in which reliable transgene technology is best established. Where rats are a better model for a specific component of the neurocircuitry of human metabolic disease, they will be used. To generate transgenic mice, inducible constructs (including inducible viral techniques) will be used whenever possible. The mice should not display a phenotype until candidate gene expression, deletion, activation, or inhibition is induced. Experimentation used will be rigorously peer-reviewed (e.g. funding applications, publications, literature) and carefully planned to ensure against unjustified duplication of procedures. All staff will demonstrate and have documented competence prior to independent experimentation. We will only use well-established reagents and protocols to induce expression, deletion, activation or inhibition of the candidate gene/neurons and assess health/behaviour. Where the target modulation produces a metabolic phenotype, we will apply strategies and compounds that improve the phenotype. Where possible, we will physiologically influence endogenous □ndocannabino/neurotransmitter /peptide levels in wild type mice or rats with food restriction or feeding. We will manipulate the duration of food restriction to influence the cascade of events modulating energy balance. Different types of animal housing (single, pair, group) will be considered in advance of each experiment, on a case by case basis, depending on the scientific outcome required. Unless experimentally required, animals will be group housed in recommended husbandry and care conditions. The work in this project will be undertaken in accordance with the surgical procedures will be undertaken adhering to the guidelines described in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2010).

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Investigating long-range synaptic pathways in cortical regions</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Hippocampus, Synapse, electrophysiology, cannabinoid, neuroanatomy</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)[5]</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>
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<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[6]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project aims to determine how different sub-regions of the brain communicate with each other. In particular investigate the micro-circuitry of neurons that play a role in memory and learning and how these circuits are disrupted during disease such as dementia or epilepsy. We will determine the receptors are involved in these processes, which will aid us designing new therapies to target various neurological disorders.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This work is expected to provide novel information about the operation of cortical networks. It will advance our knowledge of how information can be stored as changes in synaptic weights in a network.

The new tools developed in this project will be valuable to other scientists interested in neural development and synaptic plasticity. The information/data will be available for data sharing and computational modellers.
This work also has the potential to develop a novel memory enhancer.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We expect to use Rats and mice, both wild-type and genetically modified. We expect to use 1350 rodents over 5 years.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse</td><td>Most animals produced under the 5 different protocols are not expected to exhibit any harmful side effect (severity limit moderate to mild). The</td></tr>
</table>

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

effects and the likely/expected level of severity? What will happen to the animals at the end?	endpoint will be to use the animals to produce acute brain slices to allow in vitro electrophysiology to be performed.
Application of the 3Rs	
3. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We aim to build artificial computational networks, but the results obtained from this approach would still require verification in naturally developing brain tissue from a mammalian species. No computer model is currently available that can replace the use of animal tissue for this objective, as there is insufficient information on the network connectivity and circuit activity involved. Nevertheless, computer models will be used to assist the interpretation of the data obtained in experiments from animal tissue.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will ensure that the minimum number of animals will be used by maximising the information obtained from each animal. For this, experimental design will be optimised to obtain answers to the questions addressed, and statistical power analysis will be employed ahead of commencement of experiments. However, physiological experiments are special in that the number of animals will largely depend on the success rate of recording. Therefore, we will ensure that research personnel receive extensive training.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are sufficiently close to humans to reveal principles of synaptic communication and are species that are much used in behavioural and cellular studies of the synaptic circuitry and the □ndocannabinoids system, which enables us to build upon a large body of research already carried out, and to relate our findings to previous results. Our primary model is stimulation and recording from a slice preparation in vitro. This is the most refined model that can be used for the study of synaptic communication of relevant architecture. We will employ state-of-the-art stimulation and recording techniques to maximise the information yield from each experiment.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Molecular Neuroscience of Ligand-gated and G-protein coupled receptors</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>GABA, Nervous system, Neurological disease, Ion channels, neurotransmitter receptors</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)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
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</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Neurotransmitter receptors are important for cell to cell communication in the brain and for controlling neural circuit activity. Their dysfunction is associated with many neurological diseases, makingthem important targets for the development of new and existing therapeutic agents. We are investigating how these receptors work at the molecular level, where and how drugs and modulators bind to these proteins, and how this affects their function during normal physiology and disease states.</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 aim to achieve a greater understanding of how neurotransmitter receptors function during normal physiology and how mutations cause dysfunction during disease. We aim to uncover new drug binding sites and therefore new therapeutic opportunities for treating neurological diseases.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We use rodents: the numbers required, including those for breeding colonies, will be approximately 2000-3000 per 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>The severity for our procedures is classed as ‘mild’. Animals will provide nervous system tissue for acute in vitro experimentation and behaviour, as well as being used to create animal lines with altered genetic constitution. Animals will ultimately be culled.</td></tr>
</table>

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

Application of the 3Rs	
3. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals are used as there are no alternatives to studying the function of neurotransmitter receptors in the intact nervous system. The complexities of the nervous system and its proteins have not, so far, been accurately replicated in other cell types that do not involve the use of animals. We do use immortal human cell lines for characterising our receptors and drugs, but these provide limited information, being constrained by the extent to which they emulate native neurons. We also use cell lines for structure-function studies, where we alter the structure of the receptor protein and assess the impact on its function in the presence of drugs. However, sometimes, the expression of certain proteins requires the use of animal oocytes (eggs). To completely understand how drugs and modulators affect receptor function in the nervous system, it is necessary to create animals with altered genetic constitution. This powerful approach enables the functional and behavioural assessment of drug action which is only made possible with animal tissue.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We constantly reduce the number of animals used for tissue preparation by sharing material between various lab members, and other licensed labs, through coordination. All experiments using tissue for neuronal cultures, brain slicing or behaviour, are planned very carefully to use the minimum number of animals that will provide clearly discernible results that withstand statistical analysis. For tissue culture, to reduce animal usage, we often use early neonatal tissue. Oocytes are extracted from a single animal and this tissue pool is shared between 7 labs.
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 use rodents for preparing tissues, and for genetic and behavioural studies. These are considered worldwide laboratory standards for such work and there is a wealth of data already published validating the use of such species. We perform transfections on cultured neurons to direct them to express mutated forms of receptors, thus reducing the need for large numbers of

	transgenic animals. Equally, developing viral transfection technology further reduces the need for transgenic colonies. Animal welfare is paramount. Lab members maintain their own transgenic colonies and handle them on a regular basis to minimize stress. Approved training (modules 1-4) is obligatory. All behavioural tests are based on extensive experience of animal behaviourists within UCL. Animals are not re-used and we are vigilant to spot any aberrant behaviour resulting from new transgenic lines.

Information Processing in Innate Aggressive Behaviour
Keywords: 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 start by focusing on aggressive behaviour, and identify 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 behavior.

- 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 behavior. 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.

- 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.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Metabolic alterations of pregnancy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Pregnancy, metabolic disease, offspring, therapies</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</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 colspan="3">Pregnancy is associated with a series of metabolic changes in the mother that are necessary to support the nutritional needs of the developing baby. These can have consequences for the health of the pregnant woman and her baby during pregnancy and in later life. In normal pregnancy, these changes include raised cholesterol levels as well as increased insulin resistance, a condition that usually leads to diabetes, and high blood levels of bile acids (chemicals made by the liver as a way to remove cholesterol from the body).
In high-risk women, these changes cause metabolic disease of pregnancy. Metabolic disease of pregnancy can cause increased rates of sickness and death of the pregnant woman and her baby. They also have implications for the subsequent health of the children of affected pregnancies. Moreover, metabolic changes in pregnancy may have important health consequences for women who do not have diseases of pregnancy e.g. women who have had a large number of pregnancies have an increased risk of developing heart disease in later life, and this is thought to be due to continuous exposure to raised levels of cholesterol.
This work aims to elucidate the factors that drive gestational metabolic changes and how these factors can lead to metabolic disease of pregnancy. The impact on the embryo and children will be also determined. Additional experiments will enable evaluation of therapies that can be applied to prevent metabolic disease in pregnancy.</td></tr>
</table>

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

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The proposed research will have an impact on human health for a wide spectrum of individuals. The results will be of relevance to women with metabolic pregnancy disorders, *e.g*. gestational diabetes, cholestasis and obesity. Children of affected pregnancies who are more susceptible to metabolic syndrome may benefit from this work. There will also be economic benefits to the NHS if this research identifies effective treatments to reduce metabolic disease of pregnancy and susceptibility of children and young adults to metabolic syndrome. This work will inform affected women of ways they can improve the subsequent health of their children. Pharmaceutical companies that invest in strategies to prevent obesity, diabetes and fatty liver will benefit from our proposed research. This research is investigating factors that are involved in the aetiology of these diseases, and will provide insights into strategies that could be tackled by drugs or other therapeutic interventions in young adults that are susceptible to metabolic syndrome. The work will also have an impact in the field of the developmental origins of health and disease, as we have developed new experiments to investigate factors of pregnancy that cause subsequent susceptibility of the children of affected pregnancies to metabolic syndrome.
What species and approximate numbers of animals do you expect to use over what period of time?	The species we expect to use are mice. The estimate number for the duration of the project is 8000.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The proposed research plan involves mating of animals and characterisation of the metabolic profile of the offspring through collection of organs after killing the animals in a humane way. In the cases of more invasive methods, such as surgical procedures *e.g.* to remove reproductive organs or supply a compound or imaging, general anaesthetics will be used in combination with anaelgesics, painkillers and proper post-operative care to keep pain and suffering in the absolute minimum. Surgery will be carried out using the same kind of aseptic techniques that are used to avoid infection in human operating theatres. Special diets and other non-invasive methods such as routine tests to assess glucose and insulin function that will be used in this research are not expected to cause any pain and animals will be treated in a

	humane way in every occasion. No animal is expected to experience more than moderate severity and many will experience no more than mild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will employ non-animal experimental tools as alternatives to the use of live animals wherever possible, For studies of metabolic alterations, we have an active human research programme to collect samples from pregnant women and the fetus where possible from cholestatic cases and non-pregnant controls. This includes blood, urine, faeces, placenta, intestine, liver and uterine biopsies, fetal samples and amniotic fluid.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Based on the animal data, we always aim to reflect findings at the clinical level by collection of human samples (*e.g*. blood, urine, faeces and placenta where feasible) or by performing population studies or by developing non-animal tools with human resources where appropriate. Moreover, the proposed experimental designs and methods of analysis are always discussed with statisticians so that we can maximise the information obtained from the minimum resource. Also, more than one researchers share the same animals to address their questions. In this way, we aim to minimise the numbers of animals used for our studies.
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.	It is necessary to use mice with pregnancy disease to investigate the aetiology of metabolic disorders of pregnancy as it is not possible to obtain liver, pancreas and fat from pregnant women and their children. Moreover, use of animals is a useful method to determine causes of disease as genetic and lifestyle influence, often referred to in population studies, can be eliminated. This will allow better evaluation of data and more solid conclusions to be drawn. Also, based on studies performed by the applicant and others, there is already a considerable amount of background information on the hormonal and metabolic parameters of mice that will facilitate experimental planning and validation of the results. Our research plan involves mating of animals and screen of metabolic profile through collection of samples after killing the animals in a humane way. In the cases of more invasive methods general anaesthetics will be used in combination with anaelgesics, painkillers and proper post-operative care to keep pain and suffering to the absolute minimum. Special diets and other non-invasive procedures such as routine glucose and insulin tolerance tests that will be used in this research are

	not expected to cause any pain.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Factors contributing to liver failure</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Liver Disease
Hepatic encephalopathy
Acute liver failure (ALF)
Acute on chronic liver failure (ACLF)
Cirrhosis</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)[11]</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[12]</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>The progression from liver injury to liver failure varies considerably between individuals which may be dependent on the inflammatory processes that develop. Our objectives are to examine the role of inflammation in liver disease and develop interventions to these processes.</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 potential benefits are the development of novel therapies to treat liver disease and the complications that occur as a result of it. We also aim to develop a fundamental understanding of the processes to improve the scientific knowledge of the causes of disease.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We will mainly use mice and rats for the studies, with the possibility that we may require rabbits for some investigations. We estimate that we may use up to 7500 mice; 4500 rats and 100 rabbits over a five year period.</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>Some animals may experience discomfort or complications (such as infection) as a result of surgical procedures, though any animal exhibiting signs of distress will be humanely killed at the earliest opportunity.
Animals will be humanely killed at the end of the studies.</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
<tr><td>1. Replacement</td><td>Due to the nature of liver disease and it’s</td></tr>
</table>

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

State why you need to use animals and why you cannot use non-animal alternatives	complications, typically multiple organ systems and the circulation are involved. It is currently not possible to mimic the use of multiple systems and cell types in artificial models and requires the use of whole animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies will be coordinated to obtain the most information possible from individual experiments. The research team works with statisticians to ensure that the minimum number of animals are used to obtain statistically valid results.
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 will be used for the majority of studies, making use of genetically modified animals to investigate the role of specific proteins in the development of liver disease. Rats (and occasionally rabbits) will provide larger models for use with apparatus that cannot be used in the small models. Rats will also provide larger sample sizes to reduce the overall number of animals required. Careful monitoring will be conducted in all studies to minimise suffering and remove any animals exhibiting signs of distress.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Oocyte functionality post cryopreservation.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fertility preservation, xenografting, nude mouse, human ovarian tissue, oocyte functionality.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>One Year</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>~~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[14]</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>The aim of this project is to derive new information about whether the cryopreservation (freezing) process has any lasting effects on eggs produced from previously-frozen pieces of human ovarian tissue. The new technique of ovarian tissue cryopreservation and re-implantation gives women who have been made infertile by cancer treatment the chance to have a baby, but many questions remain unanswered and this research aims to answer some of them. Although cancer survival rates amongst young people are improving, the treatment often causes infertility as it effectively poisons the ovaries. At present, the only option a woman has to preserve her fertility before cancer treatment is to undergo an emergency cycle of IVF and freeze her eggs or embryos. However this is not appropriate for all patients or types of cancer, and invariably delays treatment, sometimes by up to six weeks. The process of ovarian tissue cryopreservation eliminates most of these problems, as the woman has part of her ovary removed using keyhole surgery, frozen and re-implanted after successful treatment. Even small pieces of ovary contain many hundreds of immature eggs which could be matured and ovulated naturally. However, at present it is unknown whether eggs produced from this tissue are of good quality or are more likely to have problems with their chromosomes. This project aims to answer these questions.</td></tr>
<tr><td>What are the potential benefits likely to derive from this</td><td>The aim of this project is to derive new information about whether the cryopreservation process has</td></tr>
</table>

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

project (how science could be advanced or humans or animals could benefit from the project)?	any lasting effects on eggs produced from this tissue. This will be of great importance to young women diagnosed with cancer before they have had a chance to have a family, as it will help to improve the options they have for fertility preservation.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate using 250 nude mice in total for this project, over the course of the five year duration of the licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Each animal used in this project will have a small operation under general anaesthetic, where two small pieces of human ovarian tissue will be transplanted onto the inner lining of the abdomen. We do not anticipate anything more than some minor bleeding from the skin and abdominal wall which will be stopped immediately during the operation. The animals may experience some post-operative pain which will be controlled by the use of pain-killing agents. After 5 months, the animals will receive a total of six injections of human hormones on alternate days. The injection sites will be alternated, and we expect that these injections will only cause momentary needle-stick pain. The likely severity level of these interventions is mild. All animals will be killed humanely 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	It is difficult to use a non-animal model for the production of mature human eggs as the methods available are expensive, time-consuming and have lower success rates. The eggs produced by using these methods are also of poorer quality compared to those produced in animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are performing preliminary studies in the laboratory (without using animals) to investigate whether the thawing process affects how much of the ovarian tissue survives freezing, and will use the best thawing protocol to provide tissue for the animal studies to minimise the number of animals used in the project. We have involved a statistician in our experimental design to ensure that the maximum amount of useful results can be obtained from using the minimum number of animals throughout the project.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to	The nude mouse provides a very good mammalian environment for the growth and maintenance of human ovarian tissue, and does not reject it as foreign due to having an incomplete immune system. This makes them the most appropriate animal to use in this project. The surgical procedures will be performed by experienced

minimise welfare costs (harms) to the animals.	surgeons with the animals under general anaesthetic, and the transplanted tissue will be kept small to minimise the effects on the animal. Pain-killing agents will be used to ensure the animals are comfortable post-operatively.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The role of RING finger proteins in malignancy.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>PML, BRCA1, RING finger, Malignancy, DNA repair</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)[15]</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[16]</td><td>-</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Our laboratory has had a longstanding interest in understanding the function of proteins carrying a particular zinc binding region (called the “RING finger domain”). We have focused on two such proteins, namely PML (for ProMyelocytic Leukaemia) and BRCA1 which are both involved in repairing damage to DNA in the cell and play an important role in human cancer. The PML gene is disrupted by a chromosomal rearrangement in a type of leukaemia (Acute Promyelocytic Leukaemia, APL). In addition to being involved in leukaemia, altered expression of PML has been associated with a number of common tumours including those involving the brain, lung and prostate. Alterations in the BRCA1 gene also represent an important healthcare issue, with carriers of mutations having a very high risk of development of early onset breast and ovarian cancer, which are associated with a relatively poor prognosis. Our major objective is to understand the function of PML and BRCA1, considering how loss or alteration of the proteins contributes to altered cell biology and the development of cancer.

With respect to our work on leukaemia, we are using mouse models to establish the role played by PML in normal blood development. We are also using mice to gain more information about the type of bone marrow cells in which the disease arises and establish the relationship between cell of origin and the pattern of mutations found in different populations of leukaemia cells, determining how they affect clinical outcome and response to</td></tr>
</table>

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

	therapy. An important further objective is to decipher the role played by RING finger proteins such as PML and BRCA1 in DNA repair; this will not only help us understand how tumours develop, but may also identify vulnerabilities of particular tumours which can be exploited to improve treatment outcomes.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Understanding the molecular genetic basis of these cancers should lead to measures for early diagnosis, help refine disease diagnosis, improve outcome prediction and development of better treatment approaches.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse 7000 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	For breast cancer studies the adverse effect would be breast cancer development. For the analyses concerning Pml and other genes involved in leukaemia the adverse effect is expected to be onset of blood cancer in some animals. For this project mice are monitored very carefully for signs of illness, with strict criteria adopted when mice are euthanized to ensure that they do not suffer.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have already undertaken extensive studies, based on study of patient samples. While this has been informative, it does not allow us to understand the mechanisms underlying the stepwise progression to leukaemia or other malignant diseases. We need to use animal models to achieve our objectives, which provide primary cells for experimental analysis and allow testing of anti-tumour agents. It is not possible to reliably determine whether particular mutations will induce tumours by use of *in vitro* assays alone, since these do not reliably model the *in vivo* situation in terms of the cellular environment, nor do they take into account the latency period required for tumour development. Moreover, *in vivo* efficacy of therapeutic agents cannot be reliably extrapolated from results of *in vitro* assays.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical analysis will predict the minimal number of animals needed to achieve meaningful results *i.e*. to be sure that any differences are likely to be real rather than due to chance, as well as ensuring that biologically important differences are not

	missed. These statistical estimates take into account different possible outcomes of the experiments performed.
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 is the most appropriate model for these studies, given the large body of work published by investigators in the field concerning normal blood development in this species, forming a firm basis for comparison with alterations resulting from targeted mutations or expression of leukaemia-associated fusion proteins. Similarly, there is an extensive body of work on mammary gland and breast cancer development in this species, which makes it the most appropriate species for this aspect of the project.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Collection of Blood and Arthropod Feeding</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Arthropod maintenance, blood, primary cells</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)[17]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>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[18]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The licence will cover the following 2 objectives:
1) To provide blood to scientists at The Pirbright Institute in support of research and diagnosis
Blood from pigs and ruminants is required to isolate live primary cells to be maintained as cell cultures in the laboratory.
Within the laboratory primary cells will then be utilised to study the replication and immune response of highly important viral pathogens e.g. African Swine fever virus, Bluetongue virus, Peste des Petits Ruminants Virus, Foot and Mouth Disease virus, bovine respiratory syncytial virus as well as vaccine candidate antigens.

Primary pig blood cells in large numbers are especially required for isolation and replication of African swine fever virus (ASFV). The number of viable primary cells needed for ASFV diagnosis exceeds the blood volume obtainable from a live individual pig. However for certain applications all cells need to be obtained from the same individual as immune competent cells from different individuals can not be cultured together.

Therefore this objective has 2 different protocols:
1. Obtaining large volume of bloods under terminal anaesthesia (pigs only)
2. Obtaining normal blood volumes from healthy non-infected individuals (pigs, cattle, sheep and goats)
2) To maintain arthropod colonies</td></tr>
</table>

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

	The Pirbright Institute maintains colonies of arthropods including midges (*Culicoides*) and soft ticks (*Ornithodoros*) which are biological vectors for a wide range of economically important diseases of livestock (e.g. Bluetongue virus, African swine fever virus) and humans. These arthropod colonies need regular blood-feeding for egg development and production of the next generation. *Culicoides* and mosquito colonies are maintained via blood-feeding on an artificial feeding device using commercial blood. Occasionally a generation of adult insects may refuse to feed on the artificial blood feeding system, resulting in very low egg production. Hence live mice will only be used to feed midges or *mosquitoes* in the extreme emergency of feared colony collapse. Currently soft ticks do not feed efficiently on the artificial feeders and colony maintenance therefore requires that they blood-feed on live mice.
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 Pirbright Institute is the principal UK centre for research on exotic virus diseases of farm animals. The Institute also houses National, EU, OIE and FAO Reference laboratories for more than 10 viral diseases. The diseases studied cause major social and economic impacts in affected countries and if introduced to the UK result in movement of animal bans and loss of international trade in addition to causing animal suffering and welfare issues. Primary cells obtained from blood of pigs and ruminant are a vital resource to carry out research and diagnoses for numerous important livestock and zoonotic pathogens. Additionally the immune response of primary blood immune cells towards vaccine candidates against viral pathogens will be analysed. Overall the research and diagnosis utilising these primary cells is vital to keep the UK free from important virus diseases of farm animals and zoonotic diseases. The arthropods maintained under this licence are all biological vectors for important viral livestock and/or zoonotic pathogens such as Bluetongue virus and ASFV. Research is carried out to determine the efficiency of infection, replication and transmission of different virus strains within arthropods and to identify both virus and host factors that are critical in this process. Such information is vital for the

	development of models to estimate the likely spread of disease and to develop control strategies for arthropod-borne viruses
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 1 pig/ week is currently used for the supply of large blood volumes obtained under terminal anaesthesia Throughout a normal year it will be expected to utilise about 20 mice for the blood-feeding of soft ticks as these ticks only blood feed every 6 months. Additional mice might be used to prevent colony collapse should a generation of insects refuse to feed on the artificial membrane blood feeding system. About 200 individuals of pigs, sheep, goats and cattle may be used as regular blood donors throughout a single year, these animals will be released to the herd after taking the sample and health check by a veterinarian.
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 collection of large blood volumes from pigs and the feeding of arthropods on mice are both being carried out under terminal anaesthesia. The only expected adverse effect is an insufficient anaesthetic effect either in duration or depth. The level of anaesthesia of the animals will be monitored and an extra dosage of anaesthesia will be administered if necessary. Following the procedure the animals will be euthanized. Blood collections from healthy ruminant and pig donors are classified as mild. Rare side effect might be a hematoma or an excessive stress response to the handling. Such animals will be rested and not used as blood donors until fully recovered. These animals will be released form the licence back to the national herd
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Many of the viral pathogens in question do not replicate within established cell lines or primary blood cells from model hosts, hence primary cells established from the blood of the natural hosts are a requirement. Such primary cells will have to be isolated within 12 hours from obtaining the bloods sample thereby making blood from commercial suppliers unsuitable. Additionally freezing primary cells reduces their viability significantly and may alter cellular subsets.

	Soft ticks currently refuse to blood-feed using artificial membrane feeding systems. These arthropod species can only be reared by feeding them directly on live animals. Occasionally a generation of adult insects usually maintained through blood-feeding via artificial membrane system may refuse to take up a blood meal. These individuals may be allowed to blood feed once on live animals to prevent colony collapse.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Attempts are in progress to establish cultures of a pig macrophage cell line in sufficient quantities to reduce the requirement for primary pig cell cultures for replication of ASFV. The number of pigs used has been reduced by 50% from 500 to 250 in the last two years already. The *Culicoides* and mosquito colonies are to date fully maintained through blood feeding on artificial membrane systems and feeding on live mice will only have to be considered in the imminent threat of colony collapse. Overall this achievement has reduced the requirement for mice dramatically from 10000/ 5 years on the last licence down to 500/ 5years in this licence Attempts will continue to develop a method allowing reliable feeding of tick colonies on an artificial blood- membrane system.
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.	Primary cells utilised need to be from the natural host species as many viral pathogens do not replicate in established cell lines or blood cells obtained from model hosts. The Pirbright Institute investigates highly important livestock viruses resulting in the need for pig and ruminant primary cells. For pig cells large numbers of cells can only be obtained from blood volumes not obtainable by normal blood sampling procedures, therefore larger blood volumes from pigs will be obtained under terminal anaesthesia. Mice have been shown to be a suitable model system for blood-feeding arthropods as many arthropod species willingly feed on anaesthetised individuals and mice anaesthesia protocols are well established. Thereby the feeding of arthropods can be carried out under terminal anaesthesia resulting in reduced pain and discomfort to the animal