RESEARCH REVIEW
2011

National Centre for the Replacement, Refinement
and Reduction of Animals in Research

The NC3Rs

The NC3Rs is a scientific organisation which leads the discovery, development and promotion of new ways to replace, reduce and refine the use of animals in research and testing (the 3Rs). It is primarily supported by Government, but also receives funding from the charitable and industrial sectors. The Centre has an annual budget of approximately £5.5 million and is the UK's major funder of 3Rs research. Further information about the NC3Rs can be found at **www.nc3rs.org.uk**

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Foreword

Funding research to discover new ways of replacing, reducing and refining the use of animals for scientific purposes (the 3Rs) is at the heart of the NC3Rs mission. Over the last seven years we have committed more than £20 million in grants to scientists in universities and other institutions, making us the main sponsor of 3Rs research in the UK.

During this time we have expanded the range of schemes we have available, providing access to funding for scientists at all stages in their careers. This includes the introduction in 2009 of a PhD studentship scheme and this year the launch of the David Sainsbury fellowships, which will support exceptional early career scientists during the transition into independent researchers.

We have also recognised the importance of innovation in our research funding model with the launch in 2011 of CRACK-IT, an exciting initiative connecting scientists from academia and industry. An important part of CRACK-IT is a new research competition, where scientists from universities and the SME sector have the opportunity to pitch for funding to solve business challenges with a 3Rs theme that we have identified jointly with the pharmaceutical, chemical, agrochemical and consumer product industries. This model provides a three-way partnership between the NC3Rs, industry and academia, where we will be providing research funding and the sponsoring companies in kind contributions, including access to data, equipment, samples and compounds for example.

CRACK-IT complements our strategic award scheme which supports research in priority areas that we have identified with the wider scientific community and where there are significant opportunities to advance the 3Rs. For the strategic awards, we ring fence funding and define the specific questions to be addressed. This has allowed us to drive new research ideas and approaches in areas such as the 3Rs in asthma research and the contentious topic of the euthanasia of rodents with carbon dioxide.

Our annual call for proposals to our project grant scheme continues to be our main route for funding research. This, like our other funding schemes, focuses on supporting high quality science with 3Rs potential. To achieve this, we use the same well established processes – employed by the major bioscience funders – of expert peer review, using national and international referees, and assessment by an independent panel. This rigour, and the involvement of some of the UK's most eminent scientists, such as Dame Nancy Rothwell and Sir Andrew McMichael as chairs of our grant panel, have bolstered the status of 3Rs research.

We have funded research across a diversity of scientific disciplines, supporting cutting edge technologies through to simple, elegant ideas. Importantly, our grants have been awarded to mainstream researchers who are leaders in their fields. For the first time, 3Rs research has been positioned at the centre of the bioscience sector rather than an activity restricted to a dedicated few or funded by a small number of organisations operating on the periphery of the scientific community.

The aim of this review is to showcase the impact of some of the research we have funded to date. We have selected grants from a range of disciplines, award years and institutions to highlight the breadth of our portfolio. As you will see, our research is not just delivering 3Rs benefits. It has much wider value, providing more relevant, robust and predictable models and tools for scientists to address some of the major human and animal health issues facing society.

Vicky Robinson, Chief Executive
Ian Kimber, Chairman

“We now have a model that avoids paralysis in our mice and has also led to the development of a new clinical trial design.”

Principal Investigator:
David Baker,
Professor of Neuroimmunology

Organisation:
Queen Mary, University of London

Award:
£368,512, in 2010,
over 24 months

A refined animal model of multiple sclerosis

Multiple sclerosis is a highly debilitating autoimmune disease

Multiple sclerosis is an autoimmune neurodegenerative disease which affects the central nervous system. It is characterised by loss of the myelin sheath which surrounds the axons of neuronal cells in the brain and spinal cord and is essential for the rapid transmission of nerve impulses.

Multiple sclerosis is the most common neurological condition in young adults with approximately 100,000 people in the UK affected. Disease onset is usually between the ages of 20 to 40, with women twice as likely to develop it as men. The disease typically involves episodes of paralysis followed by remission, however, it is chronic and progressively worsens. Symptoms range from pain, tremor and incontinence through to visual disturbances, difficulties with movement and coordination, and cognitive problems.

Traditional animal models of multiple sclerosis involve substantial suffering

Experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating disease that is widely used as a model of multiple sclerosis. It can be induced in a range of species from rodents to non-human primates by immunisation with myelin antigens. Studies of EAE in mice involve paralysis of the hindlimbs and sometimes forelimbs and, as such, are classified as causing substantial suffering under the Animals (Scientific Procedures) Act 1986. In a typical study, animals are killed at various time points to examine disease progression. This necessitates the use of large numbers of mice; eight to 25 per group depending on the experiment. Analysis requires detailed histology which is time consuming.

New treatments are required for the chronic phase of the disease

The EAE model shows several features of multiple sclerosis and has therefore been used extensively in research. It has also contributed to the development of new treatments. EAE models are, however, limited in that they represent acute central nervous system inflammation, whereas progression of multiple sclerosis has been shown to involve other mechanisms of neurodegeneration which are independent of the immune system. The latter phase of the disease is not well treated and there are few models available to study neuroprotection and repair.

A novel model to study nerve cell loss in multiple sclerosis

With NC3Rs funding, Professor David Baker, the Blizard Institute at Queen Mary, University of London, has developed a new mouse model of multiple sclerosis which uses fewer animals and avoids paralysis.

The model is based on optic neuritis which is typically an early symptom of multiple sclerosis in humans. Optic neuritis results in damage to the optic nerve and loss of retinal ganglion cells, which continues as the disease progresses. Two lines of transgenic mice have been crossed: one which has T cells which target a protein called myelin oligodendrocyte glycoprotein (leading to demyelination) and the other which expresses cyan fluorescent protein in the retinal ganglion cells. Levels of myelin oligodendrocyte glycoprotein are higher in the optic nerve than in the spinal cord and as a result the mice develop optic neuritis without EAE and the associated paralysis. Optic nerve damage can be tracked by following the expression of cyan fluorescent protein in the retinal ganglion cells. This provides a novel model which allows autoimmunity, neurodegeneration and neuroprotection to be studied and can be used to replace some use of the EAE model.

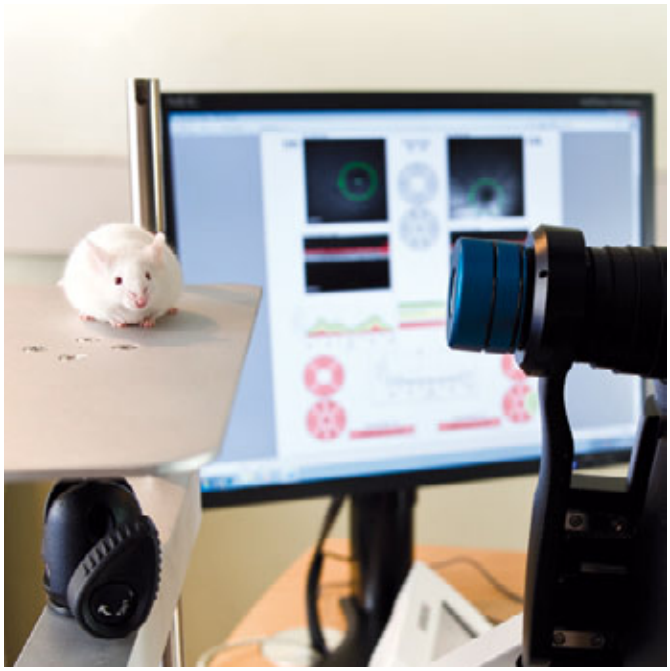
Optical damage can be monitored in the same animals for the duration of the study without the need to cull mice at different time points for histology.

Paralysis is avoided and animal suffering is minimised

The mouse optic neuritis model, combined with the fluorescence tracking of nerve cell loss, has a number of advantages over the EAE approach. Paralysis is avoided and therefore there is less animal suffering. This procedure is classified as causing moderate suffering under the Animals (Scientific Procedures) Act 1986, rather than the substantial suffering associated with the EAE model. Although optic neuritis can impair vision, mice do not use sight as their primary sense and are able to cope well. Furthermore, the whole experiment can be performed in one to two weeks rather than the typical three to four weeks with the EAE model and therefore any suffering is further minimised.

Nerve loss in mice is monitored using the same technology as in patients

A further advantage is that optical damage can be monitored non-invasively using optical coherence tomography, a technique which is also used for measuring nerve loss in multiple sclerosis patients. Optic damage can be monitored in the same animals for the duration of the study without the need to cull mice at different time points for histology. This means that the number of animals used can potentially be reduced compared to standard models. The imaging studies are currently being further optimised in order to maximise the benefits of the new mouse model.



A new clinical trial design for the treatment of multiple sclerosis

Based on the development of this refined model, Professor Baker has already secured funding from the Multiple Sclerosis Society of Great Britain and Northern Ireland and the UK Stem Cell Foundation to examine methods of neuronal repair. The model has also helped the development of a new clinical trial design for the treatment of multiple sclerosis in humans, with the first trial to start in 2011/12.

There has been one publication to date arising from this grant.

"Our *in vitro* model challenges the traditional dogma that animal studies of spinal cord injury can never be replaced."

Principal Investigator:
Sue Barnett,
Professor of Cellular Neuroscience

Organisation:
University of Glasgow

Award:
£294,404, in 2009,
over 36 months

An *in vitro* model of spinal cord injury to replace the use of rodents

Spinal cord injuries cause permanent disabilities such as paralysis

Damage to the spinal cord is a major cause of permanent disability, causing a loss of sensation and paralysis below the point of injury which arises due to breakdown in communication between the brain and the spinal cord. The central nervous system (CNS) has very little capacity for repair and there are currently no proven treatments for spinal cord injury. Strategies used to promote CNS repair include cell transplantation and drug and antibody therapies. However, it is now accepted within the scientific and clinical communities that repair is ultimately likely to require a combination of these treatment strategies.

Animal models of spinal cord injury involve severing nerve fibres

The most commonly used model of spinal cord injury involves axotomy – severing of the nerve fibres – in the spinal cord, of rats and mice. As in humans,

where this occurs in the spinal cord determines the degree of paralysis and disability. The procedure is typically classified as causing moderate or substantial suffering under the Animals (Scientific Procedures) Act 1986. Animals may require long term post-operative care; for example, having their bladders emptied manually. The surgery required for axotomy is technically demanding and studies can be difficult to reproduce because of variation in the level of injury. Ten rats or mice are used per treatment or time point in a typical study, plus control animals.

One pregnant rat can provide enough embryos for an experiment *in vitro* which if carried out *in vivo* would use 30 animals. This represents a 97% reduction in animal use.

A new *in vitro* model of spinal cord injury

With NC3Rs funding, Professor Sue Barnett, University of Glasgow, has developed an *in vitro* model of the CNS which is able to mimic certain key aspects of intact spinal cord injury. This provides a new model to study CNS cell/cell interactions and to test potential therapeutics, replacing the use of animals for some experiments.

Cells are obtained from rat or mouse embryonic spinal cords and used to establish mixed neural cell cultures. Over a period of weeks, a carpet of spinal cord axons develops. The axons are ensheathed by myelin, interspersed with nodes of Ranvier, produced by the surrounding oligodendrocytes. Many of the key axonal, glial and nodal proteins found *in vivo* are expressed.

Depending on the substrate the axons become arranged in long tracts which can be cut using a scalpel. A cell-free area develops and the lesioned nerve fibres respond, as *in vivo*, with myelin loss, changes in the expression of specific neuronal proteins and cell death. No neurite growth occurs across the cell-free area. Importantly, however, neurite outgrowth and myelination can be stimulated by reagents which have been shown to promote nerve growth in animal models.

Animal use and suffering is significantly reduced

Although the new model does not entirely replace the use of animals, one pregnant rat can provide enough embryos for an experiment *in vitro* which if carried out *in vivo* would use 30 animals. This represents a 97% reduction in animal use. In addition, the *in vitro* assay avoids the animal welfare issues associated with surgery and caring for rats with spinal cord injury.

The *in vitro* model is currently being optimised as a screen to test the combination therapies which are likely to be required for progress in the treatment of spinal cord injury. Such screening would be difficult in animals because of practical issues including cost.

“We have combined three cutting edge technologies to use tissue from patients as an alternative to animals for studying asthma.”

A tissue engineered model of the human asthmatic airway

The UK’s annual healthcare cost for asthma is estimated to be £2.5 billion

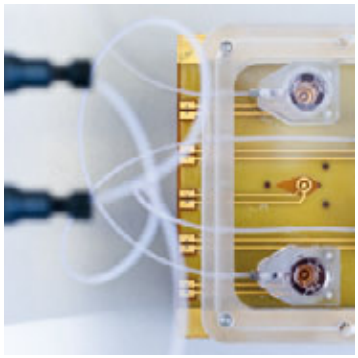
Asthma is an inflammatory disease of the airways that results in recurring episodes of breathing problems such as coughing, wheezing, chest tightness, and shortness of breath. It affects 5.4 million people in the UK, and is responsible each year for 1,500 avoidable deaths, as well as 20 million lost working days. The annual UK healthcare cost for asthma is estimated to be £2.5 billion.

There is currently no cure for asthma. Other than one class of drug which inhibits a specific inflammatory pathway, and a therapy that targets specific allergic antibodies (providing some symptomatic relief for 1 to 2% of the asthma population), there have been no effective new treatments for asthma since the introduction of beta2 receptor antagonists and corticosteroids in the 1960s.

Principal Investigator:
Donna Davies,
Professor of Respiratory Cell and Molecular Biology

Organisation:
University of Southampton

Award:
£299,875, in 2007,
over 29 months



There has been poor clinical translation of drugs developed in animal models

A range of animal species from rodents to monkeys are used in asthma research to model the three key hallmarks of the disease: airway obstruction, airway inflammation, and airway hyper-responsiveness. The mouse is the most commonly used species although the structure of the mouse lung and mechanics of breathing differ markedly from that in humans. Mice also lack a cough reflex. Most models involve sensitisation of the mouse with an allergen such as ovalbumin, with a subsequent allergen challenge. This is classified as moderate severity under the Animals (Scientific Procedures) Act 1986 because of the respiratory distress caused.

Mouse models have provided important insights into the cellular, immunological and molecular changes that occur in asthma. Nevertheless, most drugs based on preclinical studies have shown little clinical benefit in human asthmatics and the utility of animal models is under increasing scrutiny by asthma researchers. *In vitro* and *ex vivo* approaches have been used but their utility is limited by the poor availability of tissue; the paucity of technologies to non-invasively monitor cell behaviour; and the lack of immune cells which are a critical component of the disease. Importantly, none of the currently available animal models or *in vitro* or *ex vivo* approaches are amenable to studying the complex interplay between genetic and environmental factors which are believed to cause asthma.

Most drugs based on preclinical studies have shown little clinical benefit in human asthmatics and the utility of animal models is under increasing scrutiny by asthma researchers.

A novel tissue engineered model plus new technologies to study the human asthmatic airway

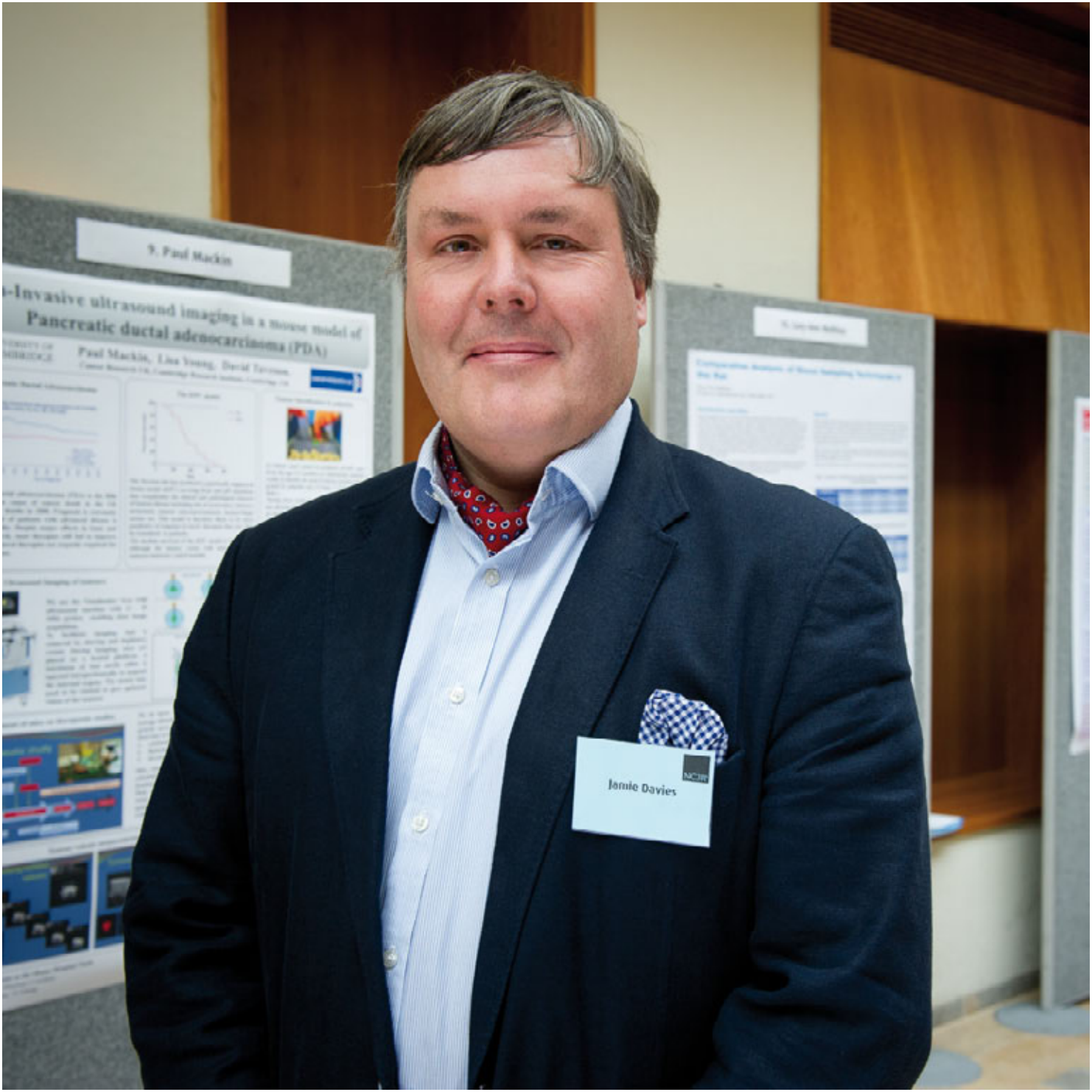
With NC3Rs research funding, Professor Donna Davies, University of Southampton, has developed tissue engineered models of the human airway, which are already providing an alternative to using animals in asthma research. These models have a number of advantages over traditional approaches in that they use human tissue and are therefore clinically more representative, particularly in terms of studying genetic and environmental influences.

The models use airway epithelia from healthy and asthmatic individuals, combined with dendritic cells – the 'sentinels' of the immune system. Recent evidence has suggested that asthma is triggered by mediators released from the airway epithelial cells in response to environmental factors such as viruses. The mediators in turn elicit an 'allergic-type' of immune response with the potential to lead to asthma. Using healthy and diseased tissue, the new models have been used to investigate this further by exposure to the common cold virus, which is a major trigger for the development of asthma in susceptible individuals.

Two new technologies have also been developed which have allowed the use of the human tissue models to be further optimised. The first is a miniaturised chip which can be used with electrical impedance spectroscopy to measure in real-time the barrier properties of the airway epithelia. The epithelium acts as a barrier preventing toxins, microbes and airborne irritants entering the lungs; the barrier is abnormal in asthmatics and this new technology will allow the effects of environmental factors, such as cigarette smoke, on the epithelial barrier function to be assessed. The electrical impedance monitoring has been also been integrated with a microfluidics system which allows continuous replenishment of the cell culture medium, improving cell viability for long term studies and enabling continuous monitoring of soluble inflammatory mediators released by cells in response to environmental insult.

Moving beyond proof of principle

This grant has provided proof of principle for an integrated system for studying asthma without the use of animals. It has resulted in three publications to date. Based on the new model, three new grants have been awarded to Professor Davies; a strategic award from the NC3Rs to further develop the microfluidic system to enable other cell types to be incorporated into the model, an MRC programme grant and an MRC industrial collaborative CASE studentship, to use the microfluidic technology to study epithelial/dendritic cell interactions in asthma pathogenesis.


9. Paul Mackin
Pancreatic ductal adenocarcinoma (PDA)
Paul Mackin, Lisa Young, David Tuveson
Jamie Davies

"The embryonic cell lines have given us a new tool to investigate kidney development without the use of mice. The ability to screen pathways and molecules *in vitro* means that we can be much more selective about what we take forward into animal experiments."

Replacing the use of animals with kidney cell lines

Kidney disease affects around 40,000 people each year in the UK

Kidney disease, the progressive loss of kidney function, affects around 40,000 people in the UK each year. In adults the most common causes are diabetes, hypertension and glomerulonephritis, that together account for 75% of cases.

The disease is classified into five stages of increasing severity. For patients reaching stage five, permanent renal replacement therapy is the only option. In the UK, 1,667 kidney transplants were performed in the last year. There is a growing gap between the requirement for transplants (currently about 7,000 people) and the availability of donor organs; as a result there is increasing interest within the scientific, clinical and patient communities in the potential of stem cells and tissue engineering to regenerate damaged kidneys or even to provide new organs.

Principal Investigator:
Jamie Davies,
Professor of Experimental Anatomy

Organisation:
University of Edinburgh

Award:
£364,044, in 2008,
over 36 months

Embryonic mouse kidneys can be grown *in vitro*

For kidney regeneration to be a viable clinical option, it is necessary to understand how the kidneys develop. This is usually studied in genetically modified mice, either in whole animals or by dissecting kidney rudiments from very early stage mouse embryos and maintaining them in organ culture.

When the kidney rudiment is isolated from a mouse embryo, just 10.5 days into gestation, it consists of three cell types only; the mesenchyme, ureteric bud tip and the ureteric bud stalk. In organ culture, kidney rudiments continue to develop and form an organ that is in most respects anatomically normal, with the ureteric bud tips and stalks branching to form a urinary collecting duct system and the mesenchyme making many excretory nephrons that connect to it. These nephrons include the structures that normally filter blood (glomerular podocytes) and the long tubules that recover valuable molecules from the urine.

An equivalent study using genetically modified mice would use around 600 animals.

Experiments in the laboratory of Professor Jamie Davies, University of Edinburgh, have previously shown that a viable kidney can be produced simply by mixing metanephrogenic mesenchyme and ureteric bud stem cells from an embryo, with no need for them to be arranged in the form of a kidney rudiment. The ability to culture rudimentary kidneys and to reconstitute by mixing different cells types allows cells from different genotypes to be used, cell movement to be tracked and the effect of different added factors (for example, drugs) to be investigated. These studies still, however, depend on isolating embryonic kidney stem cells from freshly killed

pregnant mice. It is estimated that around 15,000 female mice are used directly for this purpose in the UK each year, although the number is likely to be higher than this overall given the extensive breeding programmes required to maintain many genetically modified lines.

Conditionally-immortal kidney cell lines as an alternative to using mouse embryos

With NC3Rs funding, Professor Davies has developed a range of conditionally-immortal kidney cell lines from mouse kidney rudiments. These have been characterised for their potential as a high throughput screen of molecules and drugs that affect kidney development.

The new cell lines have the appropriate cell morphology and express the correct lineage specific markers. The ureteric bud-type cell lines have the ability to form branching tubules in three-dimensional culture, and the mesenchyme-type cell lines respond correctly to signals that alter gene expression in the early development of the natural kidney mesenchyme. Direct mixing of the different cells does not, however, result in their forming a kidney. Nevertheless, on its own each cell line behaves similarly enough to the corresponding stem cell-type in normal kidney rudiments that it has the potential to be used for high throughput screening for pathways and molecules that control specific aspects of development.

The effectiveness of this approach as a screen for pathways that control ureteric bud branching has been demonstrated in a proof of concept study. Using the ureteric bud-type cell lines in a simple two-dimensional scrape-healing assay, a set of drugs that affected collective cell movement, the two-dimensional analogue of branching, have been identified.

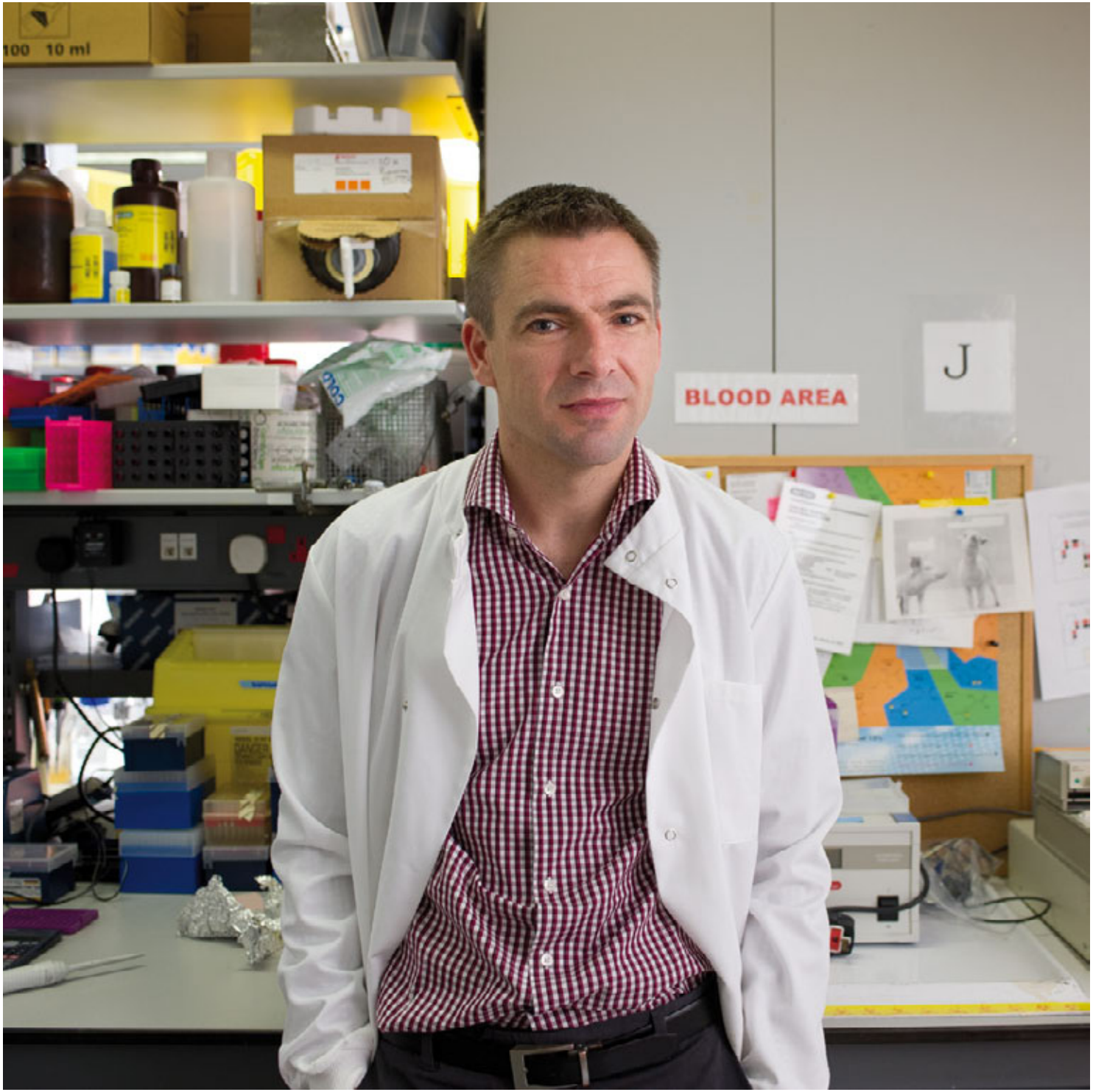
A subset of the drugs was further tested in a three-dimensional culture model, showing for the first time that the src-FAK pathway is important in the regulation of ureteric bud branching.

Identifying pathways and molecules that affect kidney development without using animals

This proof of concept study has demonstrated that the cell lines can be used as a high throughput screen, replacing the use of animals. An equivalent study using genetically modified mice would use around 600 animals; 150 for the experiments and the remaining for breeding purposes. Such studies may also be associated with suffering because of the impact of some kidney-specific knock-outs on animal welfare. Screening using cultured kidneys dissected from embryos would use approximately 140 mice.

Influencing the wider kidney research community

There have been seven publications, published or in press, arising from this grant to date. The research has also been presented at various international and national conferences including the 2010 World Biobanking and Cellular Therapy summit, the 15th UK Nephrogenesis meeting and the 2011 Harden Conference. The new cell lines are already being used by research groups in the UK and in Nice, and the techniques are being used by groups in Vienna and Brisbane, maximising the potential to further replace the use of animals.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“By using human platelets in my refined model I have been able to cut the number of mice used from 200 to 15 animals per experiment.”

Refinement of a mouse model of pulmonary embolism

New medicines are required to treat pulmonary embolism

Blood clot formation in the large veins of the legs and pelvic region is common in hospitalised patients, the elderly and occasionally following a long haul flight. These so-called deep vein thrombi can become dislodged and enter the circulation, leading to pulmonary embolism. Pulmonary embolism is caused when the thrombi become trapped in the pulmonary vessels which carry blood between the lungs and the heart, leading to vascular obstruction, cardiac dysfunction and even death. In developed countries it is estimated to affect one in 1,000 people annually with a mortality rate of one in 25,000.

Clinically, only massive (that is, obstruction of a main pulmonary artery causing systemic hypotension and decreased cardiac output) or submassive (that is an obstruction which does not cause hypotension but produces changes on echocardiography) pulmonary embolism are treated. Treatment options are

Principal Investigator:
Michael Emerson,
Senior Lecturer in Molecular Medicine

Organisation:
Imperial College London

Awards:
£149,180, in 2007, over 18 months
£204,442, in 2010, over 18 months

limited to thrombolysis which dissolves the clots. Although thrombolysis can be effective in treating massive pulmonary embolism there is controversy over its usefulness as a therapy for submassive pulmonary embolism because of the risk of bleeding. Greater understanding of the cellular and molecular mechanisms that lead to pulmonary embolism is central to the design of better treatment strategies and improved survival rates.

Mouse models of pulmonary embolism involve paralysis and death

Traditionally, the most commonly used mouse model of pulmonary embolism involves the intravenous administration of a lethal dose of clotting agents such as collagen or thrombin, which causes massive pulmonary embolism. The agent is injected into conscious mice and paralysis or death is observed in 90% of the mice within 15 minutes. This procedure is classified as substantial severity under the Animals (Scientific Procedures) Act 1986 because of the suffering caused to the animals. The effects of drugs and genetic modifications are studied by measuring their ability to significantly change the proportion of mice that develop paralysis or die. The model has provided information on cellular pathways involved in thrombosis and the effects of various compounds as treatments for pulmonary embolism. Nevertheless, the ability to study massive pulmonary embolism only, which is usually fatal in humans, limits the usefulness of the model since it cannot be used to study earlier stages of the disease and particularly those which are more amenable to therapeutic intervention. Moreover, the model is also limited by the reliance on non-specific clinical signs such as paralysis and death, which can also be caused by other factors such as shock.

With NC3Rs funding, Dr Michael Emerson, Imperial College London, has refined and reduced the use of mice in pulmonary embolism research, providing an *in vivo* model which better mimics the physiology and biochemistry of the condition in man and models the earlier stages of the disease.

A new approach to studying pulmonary embolism using anaesthetised mice

In the first grant, Dr Emerson developed a model which allows thrombus formation to be tracked *in vivo* using radiolabelled platelets isolated from the blood of donor mice. The radiolabelled platelets are injected into a recipient mouse, under terminal anaesthesia, and a sub-lethal dose of the clotting agent is administered. Platelet accumulation can then be measured non-invasively using a spectrometer connected to a gamma scintillation probe. This is a major refinement which avoids paralysis and death and has allowed the procedure to be unclassified (rather than classified as substantial severity) under the Animals (Scientific Procedures) Act 1986. The model also has a number of other advantages in that it allows measurement of platelet aggregation and disaggregation directly in real-time and takes into account non-platelet factors such as endothelial status and blood flow.

The *in vivo* model better mimics the physiology and biochemistry of the condition in man and models the earlier stages of the disease.

Using human instead of mouse platelets

In the second grant, Dr Emerson has further evolved the new model by using radiolabelled human platelets. The human platelets are then injected into NOD-SCID mice which lack T and B lymphocytes and are therefore amenable for transplantation studies. The human platelets remain viable and can be tracked in the mouse. The humanised mouse model is currently being validated by studying the anti-thrombotic effect of aspirin administered to human volunteers.

The number of mice has been reduced from 200 to 15 per study

The traditional mouse model of pulmonary embolism typically uses 200 animals per study. The refined model allows a significant reduction in animal use. By optimising the procedures for repeat administration of clotting agents, one mouse can be used several times whilst under terminal anaesthesia. This has enabled the number of animals used to be reduced to 30 per study where mice are used as platelet donors and to 15 per study where human platelets are used.

New research funding has been awarded on the basis of the refined model

There have been seven publications arising from the two grants. Based on the development of this refined model, Dr Emerson has also received grants from the Wellcome Trust and British Heart Foundation to support more basic mechanistic studies of pulmonary embolism.

The refined mouse model of pulmonary embolism has also been adopted elsewhere in the UK through collaborations Dr Emerson has established with groups at the Universities of Reading and Leicester, and the William Harvey Research Institute at Barts and The London School of Medicine and Dentistry.


Tree of Life

“The new induced pluripotent stem cell model has already reduced the number of mice used annually in my lab from 4,000 to 2,000.”

Induced pluripotent stem cells to reduce animal usage in motor neurone disease research

Motor neurone disease is a fatal neurodegenerative disease

Motor neurone disease (MND) encompasses a number of neurodegenerative disorders which affect the central nervous system, the most common of which is amyotrophic lateral sclerosis. Degeneration of the motor neurones leads to difficulty in movement, swallowing, speech and breathing. There is no cure for MND and only one drug, Riluzole, is licensed as a treatment to slow the progression of the disease.

Approximately seven in 100,000 of the UK’s population have MND. Its aetiology is unknown, with only a small percentage of patients having a family history of the disease; most cases are sporadic and there are no known predisposing factors.

Principal Investigator:
Majid Hafezparast,
Reader in Human/Mammalian Molecular Genetics

Organisation:
University of Sussex

Award:
£216,696, in 2009,
over 24 months

Mice with mutations that impair axonal transport are used to model motor neurone disease

Impaired axonal transport has been shown to play a key role in the pathogenesis of MND. Cytoplasmic dynein is a large protein involved in a range of cellular processes, including the movement of organelles, cell division and axonal retrograde transport. Mice with mutations in the cytoplasmic dynein gene lose sensory and motor neurones and are widely used as a model for studying dynein function and neurodegeneration.

Loa and *Arl* are mice with mutations in the neurone-specific functions of cytoplasmic dynein. Homozygous *Loa* mice cannot move or feed and die within 24 hours of birth. Heterozygotes, however, have a normal lifespan but show a progressive loss of locomotor function and perform significantly worse in tests of coordination and balance than wild type litter mates.

Use of the *Loa* and *Arl* derived iPS cells is being optimised and this should allow the number of mice used to be reduced to fewer than 700.

Arl mice are homozygous lethal, but can be maintained as heterozygotes. These have a neuromuscular defect that results in impaired locomotor function and an abnormal gait, with early adulthood onset.

In a typical experiment, primary motor neurones are cultured from *Loa* and *Arl* embryos. Breeding of *Loa* and *Arl* mice is classified as moderate severity under the Animals (Scientific Procedures) Act 1986. Since the lines are maintained as heterozygotes, large numbers of animals are used to provide sufficient embryos of the correct genotype as a source of primary neurones. This is compounded by *Arl* mice being poor breeders and neurones from both lines only remaining viable in culture for up to three weeks.

Induced pluripotent stem cells can replace the use of primary motor neurones

With NC3Rs funding, Dr Majid Hafezparast, University of Sussex, has developed induced pluripotent stem cells (iPS) from *Loa* and *Arl* mouse embryonic fibroblasts, providing an alternative to using primary neuronal cell cultures. In defined culture conditions, using various signalling and growth factors, the iPS cells differentiate into sensory and motor neurones which have been shown to be equivalent to those obtained from mouse embryos.

The use of iPS cells has allowed Dr Hafezparast to halve the use of mice in his laboratory to 2,000 per year. Use of the *Loa* and *Arl* derived iPS cells is being optimised and this should allow the number of mice used to be reduced to fewer than 700. The iPS cells also have other advantages. They can be further manipulated for use in molecular and cell biology studies, such as transfection with tagged genes, which is impractical in the primary neuronal cell cultures because of their low transfection efficiencies. This approach is now being used to study the role in motor neurone cell death of mutations in genes, such as superoxide dismutase 1, which have been implicated in neurodegenerative diseases.

New collaborations to extend the technology to other mouse mutants and patients

Two new collaborations have been made with UK groups, at the MRC Mammalian Genetics Unit and the Institute of Neurology, to produce iPS cells for other mouse models of neurodegenerative diseases. A collaboration has also been established with neurologists at the Brighton and Sussex Medical School to develop iPS cells from patients, which has the prospect of replacing mouse use for some studies of MND.

"We have helped ten other diabetes research groups adopt the use of our improved pseudoislet model and as a result the reduction in mouse use we have seen in our lab, by about a 1,000 animals a year, should be amplified."

Replacing animal use to study ß-cell dysfunction in diabetes

The incidence of diabetes is on the increase

There are currently around 2.8 million people in the UK who suffer from diabetes, plus an estimated 850,000 undiagnosed cases. Diabetes is a metabolic disorder affecting glucose metabolism which results from the loss of insulin production in patients with Type I diabetes and insufficient insulin production in patients with Type II diabetes. Type II diabetes accounts for between 85 to 95% of all cases. Its incidence is on the increase correlating with the rise in obesity rates.

At least 50,000 rodents a year are used to provide ß-cells for diabetes research

Insulin is produced and secreted by ß-cells in the pancreas. The ß-cells are arranged in spherical aggregates called the islets of Langerhans, which also contain other endocrine cells. Traditionally studies of ß-cell function have used rodent primary islets of Langerhans, employing a method described over 30 years ago. Rodent and human

Principal Investigator:
Peter Jones,
Professor of Endocrine Biology

Organisation:
King's College London

Award:
£387,732, in 2008,
over 36 months

islets are broadly similar and this research has yielded information on the regulation of insulin synthesis, storage and secretion by normal ß-cells, and on the processes involved in ß-cell pathophysiology. Such studies, however, require large numbers of animals. One rodent pancreas usually provides 200 to 500 islets. For a typical experiment around 100 animals are needed to provide a sufficient number of cells. Since 2000, an average of 500 papers per annum have been published on work using rodent islets; this equates to at least 50,000 rodents each year worldwide.

Many pancreatic cell lines exist but these transformed cells grow as monolayers and lack the differentiated functions of primary islets. They are therefore of limited use for many studies. Previous work by Professor Peter Jones, King's College London, has shown that when cultured on a gelatinous surface the ß-cell line MIN6 forms islet-like structures termed pseudoislets, which retain the phenotype of ß-cells.

Since 2000, an average of 500 papers per annum have been published on work using rodent islets; this equates to at least 50,000 rodents each year worldwide.

Pseudoislets can replace the use of primary rodent pancreas cells

With NC3Rs funding, Professor Jones has further improved and validated the pseudoislet model as an alternative to the use of primary islets.

Anatomically correct islet-like structures have been assembled from MIN6 cells mixed with glucagon secreting α-cells and somatostatin secreting delta cells. These mixed cell pseudoislets closely resemble mouse islets with α- and delta cells primarily located in the outer mantle and ß-cells in the inner core. The insulin content

and secretion of the ß-cells in pseudoislets in response to glucose stimulation has been shown to be equivalent to primary islets.

Pseudoislets have some advantages over mouse islets because they can be formed using cells which have been experimentally modified to assess the effects of cell/cell interactions on cell function. This has demonstrated the importance of cell adhesion proteins on insulin secretion and that the rate of ß-cell growth is not important in regulating secretory responses. These findings give insights into normal islet function and also enable improvement of the pseudoislet model.

The use of pseudoislets has reduced the use of mice in Professor Jones' laboratory by approximately 1,000 animals a year.

Pseudosislets are now used across the diabetes research community

There have been five publications arising from this grant. Professor Jones has also facilitated the adoption of the use of pseudoislets by the wider diabetes research community in the UK and elsewhere in Europe. This included an NC3Rs sponsored two day workshop in 2010 at King's College London, attended by ten different research groups. Since then there have been ten publications reporting the use of pseudoislets from the workshop's participants.


niceday

“We conquered a lot of problems. Now we work with healthier animals for longer, achieve more stable recordings and higher quality data.”

Development of a new tissue-friendly head implant for neuroscience studies with monkeys

Monkeys are used to understand the control and coordination of hand movements

An important approach for studying brain function is to make recordings of the tiny electrical signals that are generated by the single neurons that make up the brain’s functional networks. In the brain’s motor network, analyzing the spike activity of single neurons is essential to understanding the neural control of skilled hand and finger movements and their disruption by disorders such as stroke, cerebral palsy and spinal injury. For some studies, macaque monkeys are used since they provide a relevant model for investigating how the sensorimotor system controls the hands during the performance of skilled motor tasks similar to those carried out by humans.

Principal Investigator:
Roger Lemon,
Professor of Neurophysiology

Organisation:
Institute of Neurology,
University College London

Award:
£16,996, in 2005,
over 36 months

The monkey's head is restrained using a device implanted on to the skull

Stable single cell recordings from the brains of macaques performing behavioural tasks require head restraint during the period of recording. Conventionally, this is achieved by surgical implantation of an inert metal device onto the animal's skull under general anaesthesia, and training of the monkey to accept the head restraint. Although these methods reliably restrain the monkey's head, such implants are not tissue-friendly, and eventually pressure on the implant leads to inflammation and infection, bone necrosis and instability or breakage of the implant. This has a significant impact on the monkey's welfare and can require additional surgery to move or replace the implant. In addition to the animal welfare concerns, a failed implant can interrupt studies and in the longer term can lead to the decision to euthanase the monkey, wasting animals and impacting on scientific progress.

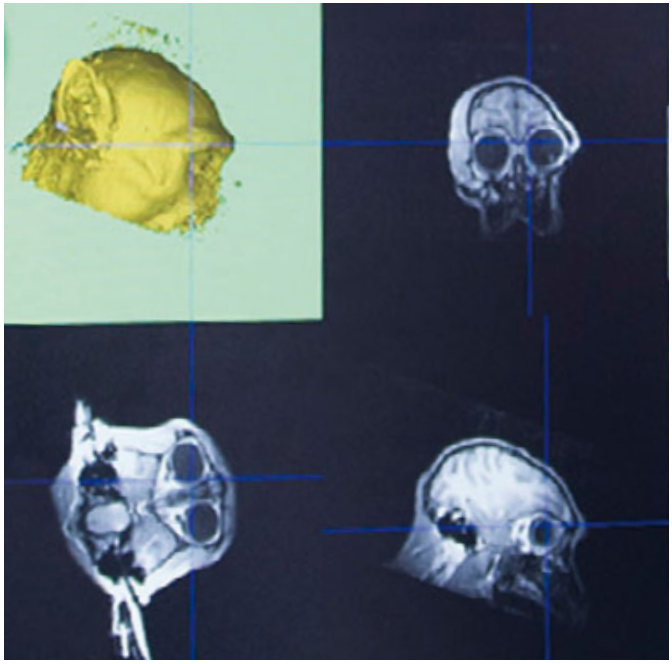
A new head implant which minimises tissue damage and infections

With NC3Rs funding, Professor Roger Lemon, Institute of Neurology, University College London, has developed and tested a refined method of head restraint which minimises the adverse impact on the monkeys and gives appreciable scientific advantages over conventional devices. Two refinements have been introduced. First, the new method employs non-invasive magnetic resonance imaging (MRI) to generate a three-dimensional model of the monkey's skull, allowing design of a custom-made implant tailored for individual monkeys. Second, it uses tissue-friendly material for the construction of the implants. Since the new method of head restraint is MRI-compatible, further non-invasive imaging can also be carried out to more accurately target brain recording and stimulation, improving the precision and amount of behavioural and electrophysiological data obtained from each macaque.

The study investigated the suitability of two tissue-friendly materials, HAPEX™ (an artificial bone analogue) and TECAPEEK™ (a thermoplastic compound). The new implants were then assessed for their mechanical stability, the level of infection in skin margins and other tissues in contact with the implant, skin recession using serial photography, and the health of the implant/bone interface.

The HAPEX™ implant became infected and had to be removed after approximately eight months of recording. This was not a long enough period to recommend it for further use. Instead, TECAPEEK™ implants were tested in three animals. To date all have remained stable, secure and relatively free of infection for the duration of the experiments (up to three years in the longest case) thus avoiding the animal welfare concerns associated with a failed head implant. The TECAPEEK™ implants also had other advantages in that they were lower in weight and easier to implant than conventional stainless steel devices and promoted an improved interface with the bone and surrounding skin tissue.

The TECAPEEK™ implants were lower in weight and easier to implant, and caused less skin recession.



More data can be obtained from monkeys with refined head implants

Five articles reporting the findings from studies using the new head restraint device have been published and MRI, photographic and electrophysiological data shared through the CARMEN project. The head restraint technique was presented at the 2008 Wellcome Trust sponsored NC3Rs workshop on refinement of the use of chronic implants. It has also been adopted by researchers at Newcastle University.

"Our *in vitro* assays for studying cancer stem cells could spell the end for most mouse xenograft studies."

Principal Investigator:
Ian Mackenzie,
Professor of Stem Cell Science

Organisation:
Queen Mary, University of London

Award:
£345,092, in 2009,
over 36 months

New *in vitro* assays for studying the biology of cancer stem cells

Cancer accounts for one in four deaths

There are over 200 different types of cancer. It is predicted that one in three people will develop some form of cancer in their lifetime. Although mortality rates from cancer have been decreasing over the past three decades, it still accounts for one in four deaths. Whilst many effective treatments have been developed, these do not always stop the spread or reoccurrence of the disease. There is a continuing need for new treatments.

Cancer stem cells are 'tumour initiators'

Recent research has shown that there is a sub-population of cancer cells that is responsible for both the maintenance and recurrence of cancers. These 'tumour initiating' cells are only a small fraction of the total cancer cell population and are termed 'cancer stem cells' because they possess characteristics of normal stem cells in that they can self-renew and give rise to other cell types. Cancer stem cells were first found in leukaemias and have

since been shown in a range of cancers including those of the breast, prostate, colon, pancreas, and brain. They have also been shown to be more resistant than other cancer cells to chemotherapy and radiotherapy.

Human cancer cells are transplanted into mice to assess if they can cause tumours

The mouse xenograft model is considered to be the 'gold standard' for identifying and studying cancer stem cells. Cells from human cancers are injected into the mouse to determine their ability to cause tumours. However, survival of the human cancer stem cells depends on a range of factors such as the strain of mouse and the site of implantation. Consequently, studies can be difficult to reproduce and information about the survival of the cancer stem cells and/or the efficacy of potential therapeutics cannot be directly evaluated. Such studies also use large numbers of mice and are associated with suffering because of the growth of tumours.

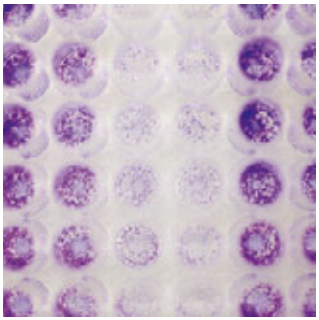
A typical xenograft study uses over 50 mice.

New *in vitro* methods for studying cancer stem cells

With NC3Rs funding, Professor Ian Mackenzie, Blizard Institute at Queen Mary, University of London, has established and validated new *in vitro* systems to replace the use of mouse xenografts for studies of cancer stem cells.

Cells from a range of cancer cell lines derived from head and neck, breast and prostate cancers have been compared with cells freshly isolated from human cancer biopsies. This has shown that the cell lines, like fresh

cancer cells, contain cancer stem cells with properties of self-renewal and resistance to apoptosis. To validate this, in collaboration with Dr Dana Costea at the University of Bergen, cell lines were transplanted into NOD-SCID mice to assess their tumorigenic potential and therefore the presence of cancer stem cells. This demonstrated that patterns of tumour formation by stem cell sub-populations were consistent with those found for the transplantation of fresh tumour cells.



These findings indicate that cell lines can form the basis of *in vitro* assays for studying cancer stem cells and for screening therapeutics. Such *in vitro* models also have other advantages over the mouse xenograft model – they allow individual cells to be studied directly and, since they are quick and easy to set up, they can be scaled up as high throughput assays – this is the next objective of the NC3Rs grant that Professor Mackenzie and his team are working on.

The potential to replace the use of thousands of mice used in xenograft studies

There is now considerable worldwide interest in the biology of cancer stem cells and, in particular, in their roles as novel and necessary targets for drug therapies. In the last three years there have been over 500 publications reporting the use of the mouse xenograft model to study the properties of cancer stem cells. A typical xenograft study uses over 50 mice and therefore the adoption of *in vitro* assays has the potential to replace the use of thousands of animals. Moreover, with the need to test both existing and new therapeutic drugs for their effectiveness in cancer stem cell elimination, there is the potential for even greater replacement by demonstrating that *in vitro* systems provide effective stem cell assays.

There has been one publication to date.

"The *in vitro* assays are now our number one research tool for optimising the manufacture of clostridial vaccines. Our next step is to get the regulators on board as ultimately the assays could replace the use of tens of thousands of mice worldwide."

Principal Investigator:
Keith Redhead,
Project Manager, Research and Development

Organisation:
Intervet

Awards:
£111,903, in 2006, over 36 months
£26,988, in 2009, over 12 months

Developing *in vitro* assays to replace animal tests for clostridial vaccine antigens

Clostridial bacteria cause a range of diseases in livestock

Bacteria belonging to the *Clostridium* genus are found widely in soil and in the faeces of animals. Some species of *Clostridium* are responsible for a wide range of animal diseases and, as such, they represent a major risk to the livestock industry. Clostridial diseases include tetanus, enterotoxaemia in lambs and goats, Braxy in young sheep and Black disease in cattle. These diseases are controlled via a range of vaccines which are some of the most common veterinary treatments.

Clostridial vaccines contain antigens consisting of the toxoid form of the toxin. This is a weakened form of the toxin which when administered to the animal stimulates antibody production against the toxin itself. During the manufacturing process, each batch of antigen is tested for the amount of toxin and toxoid to ensure the safety and quality of the final vaccine and to satisfy regulatory requirements.

Thousands of mice are used in tests on antigens for clostridial vaccines

Mice are used to quantify clostridial toxins and toxoids during vaccine manufacture. The amount of biologically active toxin is assessed in a minimum lethal dose test (MLD) and a L+ test, both of which involve substantial suffering and often death. The amount of toxiod present is measured by the total combining power (TCP) test, which can also involve the death of the mice. The three tests are classified as causing substantial suffering under the Animals (Scientific Procedures) Act 1986. Each test uses a minimum of ten mice for each antigen and more than 200 mice can be required for the full antigen testing of some multicomponent vaccines.

The *in vitro* MLD assay is up to ten-times as sensitive as the mouse assay and the L+ and TCP assays are four-times as sensitive.

New *in vitro* assays to replace the use of mice in clostridial vaccine testing

With NC3Rs funding, Dr Keith Redhead, Intervet, has developed *in vitro* assays to replace the MLD, L+ and TCP tests.

A range of cell lines were screened for Clostridial antigens. Two cell lines, VERO and MDCK were found to be sensitive to *Clostridium septicum* alpha toxin and *Clostridium perfringens* type D epsilon toxin respectively. These cell lines have been used to develop *in vitro* MLD, L+ and TCP assays, which have subsequently been validated with data from *in vivo* studies.

The assays are more sensitive and accurate than the mouse tests. For example, the *in vitro* MLD assay is up to ten-times as sensitive as the mouse assay and the L+ and TCP assays are four-times as sensitive. They are also quicker to perform, with results available in 24 hours rather than five days.

Cell lines with appropriate sensitivities to other clostridial toxins, including *Clostridium perfringens* type B/C beta toxin and to *Clostridium novyi* alpha toxin, have also been identified.

Regulatory acceptance will replace mouse use internationally

The new assays are currently used within Intervet for work on the optimisation of clostridial vaccine manufacture, reducing mouse use by several hundred animals a year. Regulatory acceptance of the assays is, however, required for the full potential of the assays to be realised in terms of replacing animal use. This could have a significant impact on numbers, replacing around 8,500 mice in Intervet alone and potentially thousands more worldwide at other veterinary vaccine manufacturers. The NC3Rs will be working with Intervet to facilitate regulatory acceptance.

“We now have transgenic mice which better mimic the human disease, but without the suffering associated with the animal model researchers have been using for the last 80 years.”

A refined mouse model to study systemic amyloidosis

Amyloid deposits are found in a range of diseases including arthritis and diabetes

Amyloidosis is a disorder of protein metabolism which leads to the extracellular deposition of insoluble amyloid fibrils. The accumulation and persistence of amyloid disrupts the structure and function of affected tissues. Amyloid deposition is also a characteristic feature of Alzheimer’s disease and type II diabetes.

Systemic amyloidosis accounts for one in every 1,000 deaths in developed countries. Amyloid A (AA) amyloidosis is the second most common type of systemic amyloidosis in humans. It occurs in up to 5% of patients with chronic inflammatory diseases such as rheumatoid arthritis. Inflammation causes increased production of the acute phase serum amyloid A protein (SAA) by the liver, which leads to amyloid A deposits and systemic amyloidosis, mainly affecting the spleen, liver and kidneys. Renal damage is usually the first clinical manifestation of the disease. There are currently no specific treatments and it is a major unmet medical need.

Principal Investigator:
Paul Simons,
Senior Lecturer in Inflammation

Organisation:
University College London

Award:
£302,128, in 2008,
over 36 months

A mouse model first developed over 80 years ago is used to study systemic AA amyloidosis

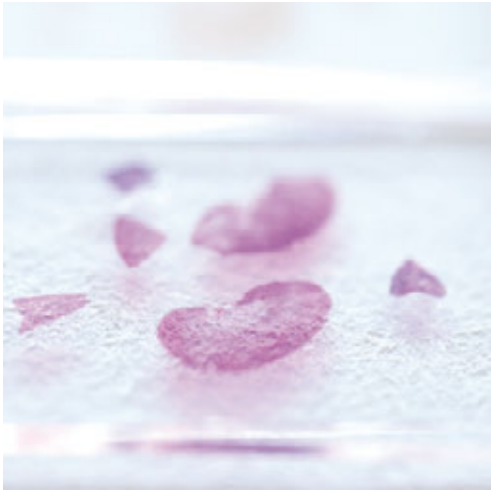
The mouse is widely used to model systemic AA amyloidosis using an approach which was developed in the early 20th century. The disease is induced experimentally by repeated injection of casein which causes persistent inflammation. The mouse model mirrors some aspects of the human disease, however, amyloid deposits are rarely seen in the kidneys – clinically the most important organ affected – and there are limited deposits in the liver. In the mouse, both development and regression of the disease can be highly variable between animals, making studies difficult to reproduce.

A transgenic mouse model which allows amyloidosis to be turned on and off

With NC3Rs funding, Dr Paul Simons, University College London, has developed and characterised a refined transgenic model of systemic AA amyloidosis which better represents the human disease.

An inducible transgenic line has been generated in which expression of the SAA gene can be controlled by the inclusion of the antibiotic, doxycycline, in the drinking water. Amyloidogenesis, that is the production of amyloid fibrils, can then be accelerated by a single intravenous injection of Amyloid Enhancing Factor.

The transgenic mice express more SAA protein than is seen in the traditional model, with amyloid fibrils being observed in the spleen, liver and kidneys. The model has further advantages in that the inducible system allows the level of SAA protein to be carefully modulated and it therefore presents the opportunity to study the spontaneous regression of amyloidosis seen in some patients. Moreover, amyloidogenesis is rapid taking six to ten days rather than two to eight weeks.



Less animal suffering by avoiding repeat injections and widespread inflammation

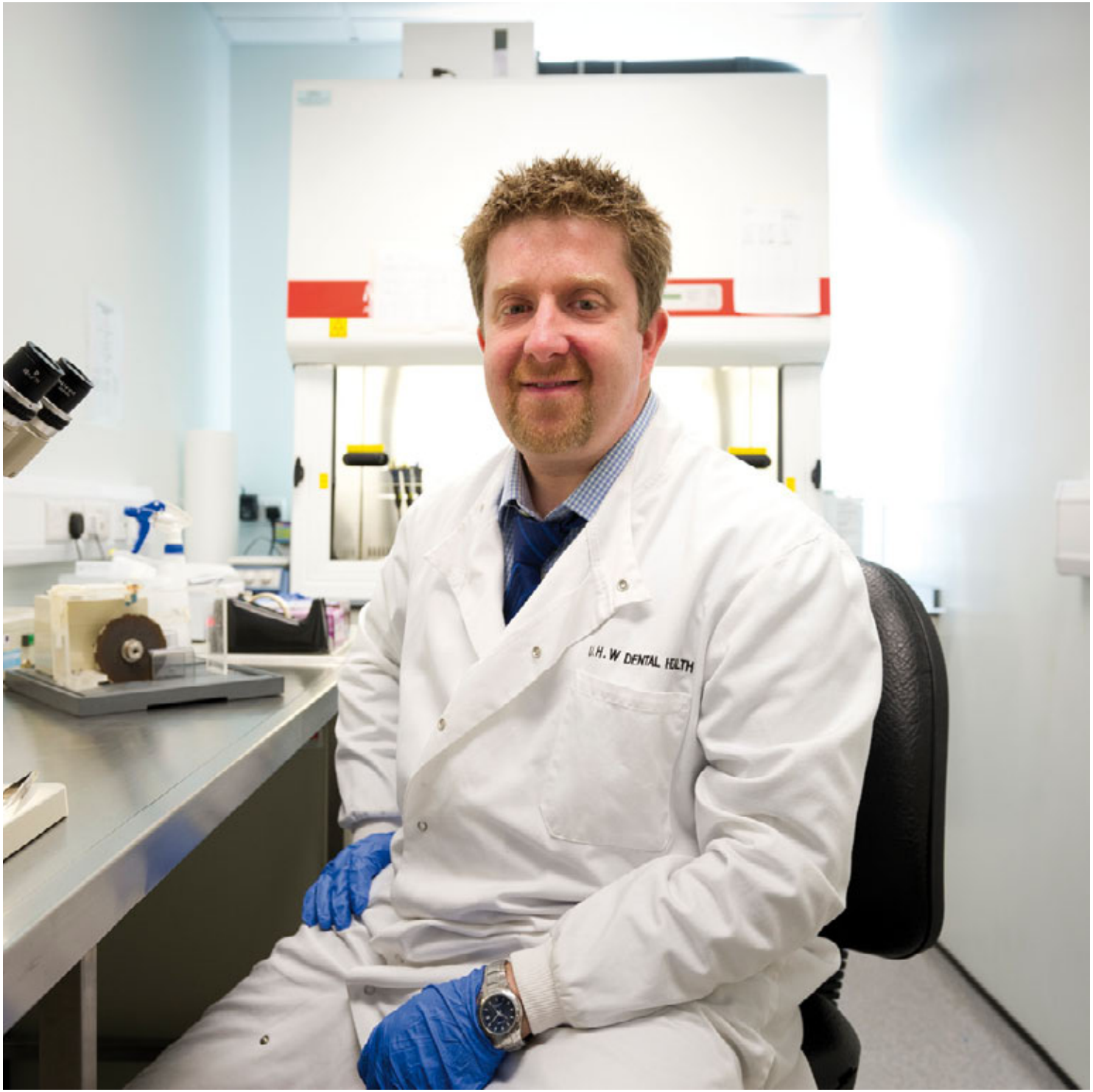
The transgenic model of systemic AA amyloidosis is a refinement over the traditional model. It avoids the daily subcutaneous injections of inflammatory agents for up to eight weeks and the concomitant systemic inflammation. Use of the transgenic mice is classified as mild severity under the Animals (Scientific Procedures) Act 1986; the traditional model involving chronic administration of casein is classified as moderate severity.

The SAA line has now been bred to homozygosity, avoiding the wastage of animals associated with other breeding strategies and the need to take biopsies to genotype. The control over the expression of the SAA protein in the transgenic mice has provided a more robust and reproducible model. As a result, group sizes for experiments have been reduced from approximately 12 mice with the standard model to eight with the transgenic mice.

New insights into the disease

Using the transgenic model it has been possible to dissociate SAA expression from inflammation. This has shown for the first time that high circulating concentrations of SAA alone, in the absence of inflammation, are sufficient for amyloidogenesis. The only essential aspect of the inflammatory response is the up-regulation of SAA expression.

This work has been presented at a range of conferences including the XIIth International Symposium on Amyloidosis in 2010. It is currently being prepared for publication.


H. W DENTAL HEALTH

"Using our *ex vivo* system, we have been able to model disease progression more accurately and investigate treatments more effectively, whilst achieving an 80% reduction in our animal use."

Development of a new *ex vivo* mouse model of periodontal disease

Over half of UK adults are affected by dental disease

Periodontal diseases are a group of chronic inflammatory diseases of the tissues which surround and support the teeth. The severity of the diseases ranges from mild and reversible inflammation of the gums to bone damage and tooth loss. Chronic inflammation of the periodontium is caused by the host immune response to the bacterial pathogens which form the subgingival biofilm (bacteria living just beneath the gums).

The most prevalent form of the disease is adult periodontitis which is the major cause of tooth loss in the developed world. In the UK, 54% of adults are affected by periodontitis with 85% of over 65s exhibiting periodontal destruction. The estimated cost to the NHS is £500 million annually. There is also a link between periodontal disease and increased risk of coronary heart disease, bacterial pneumonia and stroke.

Principal Investigator:
Alastair Sloan,
Reader in Bone Biology and Tissue Engineering

Organisation:
University of Cardiff

Award:
£193,680, in 2007,
over 24 months

A range of animals are used to study periodontal disease

A range of animals including rodents, dogs and non-human primates are used to study the pathogenesis of periodontal diseases and to develop and evaluate potential therapeutics. Disease is induced experimentally by tying a ligature around the tooth and either allowing disease to develop naturally or injecting bacteria into the ligatured area. Animal models have provided information on host/bacterial interactions, however, they are limited by differences in dental anatomy and oral microbiota and difficulties associated with translation to man. There are also animal welfare concerns, with most procedures of this type classified as moderate severity under the Animals (Scientific Procedures) Act 1986.

In vitro models using rodent cells are used to study some aspects of periodontal disease but without the complex cell/cell and host/bacterial interactions their use is limited. Given the small tissue volume available from the rodent mandible (lower jaw), obtaining a sufficient quantity of viable cells can necessitate using large numbers of animals, typically 25 to 50 mice per study.

A new three-dimensional model of the lower jaw

With NC3Rs funding, Dr Alastair Sloan, Cardiff University, has developed and validated an *ex vivo* mouse model to study inflammation and bone metabolism in periodontal disease. The model mimics what happens *in vivo*, is more representative of disease progression than *in vitro* assays, and reduces animal use.

The three-dimensional model uses a slice culture of the mouse mandible which can be maintained for up to 21 days. The model maintains the *in vivo* cellular phenotypes and tissue architecture of the mandible and tooth. This includes odontoblasts (which secrete dentin, the layer under the tooth enamel), fibroblasts of the tooth pulp and the periodontal ligament (which secrete extracellular matrix proteins), bone marrow cells (which mainly give rise to haematopoetic cells, plus osteoblast progenitor cells and bone marrow stromal cells) and osteoclasts (which resorb bone). Importantly, it also includes immune cells such as monocytes, activated macrophages and neutrophils which are crucial to the development of periodontitis.

The *ex vivo* model mimics the *in vivo* response to exogenous factors such as inflammatory cytokines, with key hallmarks of disease progression, including increased bone resorption, cell death and cytokine induction, observed. The model is now being used to investigate bone repair and cellular/extracellular matrix interactions.

An 80% reduction in the number of mice used

A typical *in vivo* study of periodontal disease with five time points uses five animals per time point. Ten mandibular slices can be obtained from a single mouse and this means that with the *ex vivo* model there is an 80% reduction in the number of animals used. The *ex vivo* model also uses 96% fewer mice than the *in vitro* assays.

Prize winning scientist

There have been two publications arising from this grant so far. In 2011 Dr Sloan was given the International Association for Dental Research's Distinguished Scientist, Young Investigator Award, in recognition of his work, including that funded by the NC3Rs.

The *ex vivo* model has been adopted by groups at the University of Alberta in Canada, Baylor College of Dentistry in the USA, and in the UK by the University of Central Lancashire. It has also been used by a large pharmaceutical company for a commercial study.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“Our bioluminescent bacteria have allowed us to refine the dose and sampling strategies we use in our mouse streptococcal infection model.”

Principal Investigator:
Shiranee Sriskandan,
Professor of Infectious Diseases

Organisation:
Imperial College London

Award:
£276,722, in 2008,
over 24 months

Exploiting bioluminescence in mouse studies of bacterial infection

There are 616 million cases of pharyngitis caused by the bacteria *Streptococcus pyogenes* worldwide each year

The bacterium, group A streptococcus (or *Streptococcus pyogenes*) causes a wide spectrum of diseases from mild skin infections and pharyngitis to life threatening invasive conditions such as sepsis and necrotising fasciitis. 20% of patients with invasive group A streptococcus infection die. Aberrant immune responses lead to some of the most feared complications, such as toxic shock, and also the heart valve-damaging disease, rheumatic fever, that can follow untreated streptococcal pharyngitis.

The main treatment for *Streptococcus pyogenes* infection is penicillin, yet despite this the infection remains hyperendemic in many communities throughout the developing world, and rheumatic valvular heart disease kills almost half a million people per year worldwide. Invasive infections often progress extremely rapidly, such that antibiotic treatment is never started, or starts too late

to be of any use. Research is therefore focused on the development of better therapeutics or a vaccine.

Bioluminescence imaging can be used to refine and reduce animal studies of bacterial infection

Studies of bacterial pathogenesis typically involve infecting the mice and subsequently euthanizing them at specific time points to determine the number of viable bacteria present in specific tissues and organs. In recent years, bioluminescence imaging has enabled bacteria, genetically modified to express luciferin or green fluorescent protein, to be tracked in mice using specialist equipment. This has provided real-time information on bacterial infection and colonisation, leading to novel scientific insights. Bioluminescence imaging also has the advantage that it is non-invasive.

The ability to monitor bioluminescent bacteria in real-time and non-invasively has provided opportunities to reduce and refine mouse studies. Mice can be serially monitored and it is therefore possible to conduct longitudinal studies without the need to euthanize groups of animals at specific time points for data collection. As a result, the number of mice used per study can be reduced typically from 18–36 to 6–8 animals. Each mouse acts as its own control, avoiding the potentially confounding effects of using different groups of animals for each time point.

The reproducibility of studies is further enhanced by the use of bioluminescence imaging to quantify the dose of bacteria given to each mouse. In conventional studies, the dose is determined by optical density and this can lead to log-fold differences in the amount of bacteria injected into one mouse compared to another. The ability to quantify the bacterial burden means that it is also possible to identify mice in which the infection is progressing rapidly, long before any adverse effects are apparent, allowing the use of humane endpoints and prevention of unnecessary suffering.

Applying the benefits of bioluminescence imaging to streptococcal research

With NC3Rs funding, Professor Shiranee Sriskandan and Dr Siouxsie Wiles*, Imperial College London, have developed a molecular 'tool kit' for the application of bioluminescence imaging to study the pathogenesis of *Streptococcus pyogenes*, focusing on the four main clinically important serotypes. Bioluminescence imaging has not previously been widely used in streptococcal research and the only commercially available bioluminescent strain is a non-clinical serotype.

Most mouse models for *Streptococcus pyogenes* result in the death of the animals within a few days. The Imperial team has recently pioneered a new mouse asymptomatic nasopharyngeal carriage model to enable better understanding of streptococcal infection. Dosing and sampling in this model have been refined using bioluminescent bacteria.

Bioluminescent *Streptococcus pyogenes* are introduced into the nostrils of the mouse resulting in nasopharyngeal infection. By quantifying the bioluminescence it has been possible to refine the volumetric dose required for nasal infection, avoiding the spread of the bacteria into the lungs. After approximately four days, however, the bioluminescent signal is too low to adequately detect in the mouse. In order to continue monitoring the mice longitudinally and non-invasively a new simple sampling method, where the mouse's nostril is gently placed on the surface of a blood agar plate, has been developed. The streptococcal bacteria can then be identified and quantified on the plate.

The ability to monitor bioluminescent bacteria in real-time and non-invasively has provided opportunities to reduce and refine mouse studies.

This proof of concept study has shown for the first time the feasibility of bioluminescent imaging in refining the study of streptococcal infections in mice. It should now be possible to extend this to also reduce the number of mice used by four-fold. Moreover, the bioluminescent imaging has already revealed evidence of bacterial transmission from the nasal site of infection, which may be of clinical relevance. There has been one publication arising from this grant to date.

*The grant was originally awarded to Dr Siouxsie Wiles and Professor Sriskandan. It transferred to Professor Sriskandan when Dr Wiles moved to New Zealand.

"We have been able reduce the mortality rate of the rats in our experiments by more than ten-fold and avoid the need for the intensive care that the animals usually require after the induction of epilepsy."

Principal Investigator:
Gavin Woodhall,
Reader in Neuropharmacology

Organisation:
Aston University

Award:
£152,048, in 2007,
over 36 months

Refinement of a rat epilepsy model

Medication does not control seizures in 30% of people with epilepsy

Epilepsy is a chronic disorder characterized by seizures (or fits) due to abnormal or excessive neuronal activity in the brain. It affects between 1 to 2% of the world's population, mainly young children and people over the age of 65. Seizures are also a major contributor to deaths following brain damage caused by stroke or head injury. For most people with epilepsy (around 60%) there is no known cause (so-called 'idiopathic' epilepsy). Around 30% of sufferers have refractory epilepsy which does not respond to treatment.

Epilepsy induced experimentally in animals has high mortality rates

Temporal lobe epilepsy is the most common form of epilepsy and the most difficult to treat. It is modelled in rats by the injection of the convulsant pilocarpine after prior injection with lithium chloride. This induces *status epilepticus* – high intensity and continuous seizure activity lasting for more than 30 minutes.

Many rat models utilise prolonged *status epilepticus* of up to six hours, although two to four hours is typical. It is followed by the development of spontaneous recurrent seizures, usually occurring every one to three days. The procedure causes behavioural abnormalities, can lead to aspiration pneumonia as a result of the inhalation of saliva, requires intensive care of the animals for up to three days and is associated with high mortality (rates greater than 50% are reported in many publications). It is classified as causing substantial suffering under the Animals (Scientific Procedures) Act 1986.

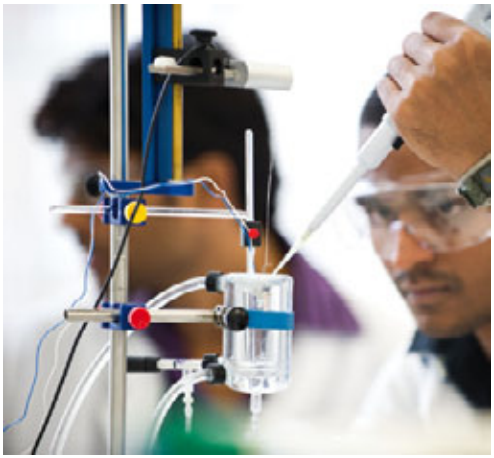
A refined rat model of epilepsy with lower mortality rates

With NC3Rs funding, Dr Gavin Woodhall, Aston University, has refined the lithium-pilocarpine rat epilepsy model by exploiting the pharmacological control of seizure activity.

The refined rat model has a number of features which improve its clinical relevance.

In the new model, *status epilepticus* is induced using low doses of pilocarpine. Once *status epilepticus* is observed, seizures are ameliorated to a lower intensity level using the alpha-2 agonist xylazine. After approximately one hour, the seizures are then terminated by administration of a multicomponent drug cocktail which blocks glutamatergic and enhances GABAergic neurotransmission. The time spent in *status epilepticus* and the intensity of the seizures is reduced and this has a number of animal welfare advantages compared to the standard rat model.

The mortality rate of the rats in Dr Woodhall's experiments has decreased from approximately 25% to less than 2%. The rats recover within six to eight hours, hand feeding and other care measures are not necessary and normal behaviour is restored within 24 hours. Recurrent seizures occur every one to seven days. These refinements should enable the severity limit of this procedure to be downgraded under the Animals (Scientific Procedures) Act 1986.



Better representation of human epilepsy

The refined rat model has a number of features which improve its clinical relevance. In humans, *status epilepticus* is rare and temporal lobe epilepsy most commonly involves brief focal seizures and limited brain damage. The standard lithium-pilocarpine model in rats, by contrast, involves global seizures and results in widespread brain damage and cell death.

The new model better mimics the development of the human disease in that there is little cell death, and there is a slow and variable time course to the development of recurrent seizures. Of particular interest is the fact that the seizure locus moves with time, which in paediatric epilepsy is known as 'migration of the irritative zone'. This has not previously been observed in rats and suggests that what is being modelled has some similarities with epilepsy in the juvenile brain.

Sharing best practice

This work has been presented at the Society for Neuroscience meeting in 2009 and the British Neuroscience Association biennial meeting in 2011. It is currently being prepared for publication. The refined procedure has also been adopted by other UK researchers.

Appendices

David Baker

Baker D, Gerritsen W, Rundle J, Amor S (2011). Critical appraisal of animal models of multiple sclerosis. *Multiple Sclerosis* 17: 647-657.

Donna Davies

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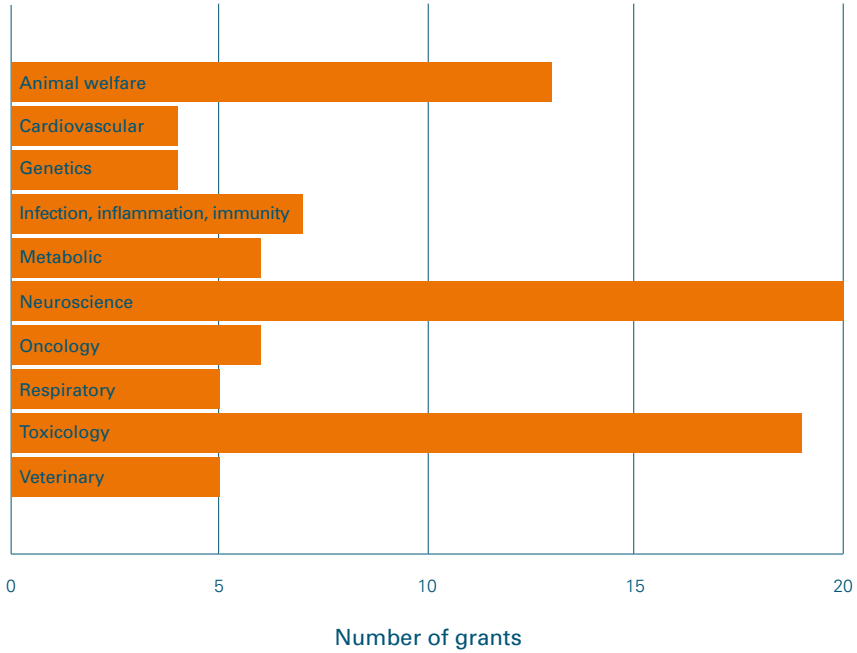
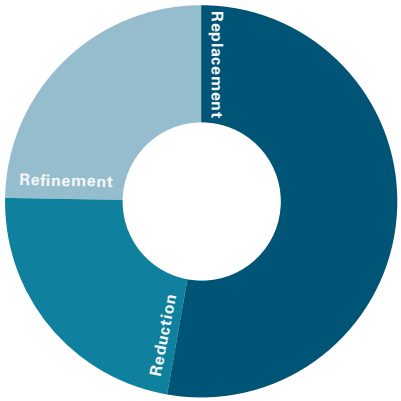
Research category



Breakdown by 'R'

Replacement	47
Refinement	22
Reduction	20



This includes project grants, pilot study grants, strategic awards and studentships from 2004 to 2011.
Further information can be found at www.nc3rs.org.uk/researchportfolio

Research organisations

Aston University	2
Brighton and Sussex Medical School	1
Brunel University	2
Cardiff University	3
Centre for Environment, Fisheries and Aquaculture Science	1
Dundee Technopole, CXR Biosciences Ltd.	1
Durham University	2
Food and Environment Research Agency	2
Hutchison-MRC Research Centre	1
Imperial College London	6
Institute of Cancer Research	1
Intervet	2
Keele University	1
King's College London	2
Lancaster University	1
Marine Scotland Science	2
MRC Human Genetics Unit	1
National Institute for Biological Standards and Control	2
Newcastle University	9
Queen Mary, University of London	4
Queen's University Belfast	1
Royal Holloway, University of London	1
St George's, University of London	1
Swansea University	1
University College London	3
University of Aberdeen	3
University of Bath	2
University of Bedfordshire	1
University of Bradford	1
University of Bristol	1
University of Cambridge	2
University of Dundee	1
University of Edinburgh	1
University of Glasgow	2
University of Hull	1
University of Leicester	1
University of Liverpool	2
University of Manchester	2
University of Nottingham	3
University of Portsmouth	1
University of Sheffield	3
University of Southampton	4
University of Stirling	1
University of Sussex	1
University of Ulster	1
University of Warwick	1
Veterinary Laboratories Agency	2

Geographic locations

Image credits

David Sayer
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Scheme: Project grants
Purpose: Response mode scheme for hypothesis driven and applied research
Duration: Up to 36 months
Amount: Dependent on the science; typically in the region of £300k
Key dates: Call opens in September; deadline in January; decisions in July
Webpage: www.nc3rs.org.uk/projectgrants

Scheme: Pilot study grants
Purpose: Response mode scheme to provide preliminary data for a subsequent, more substantive application
Duration: Up to 12 months
Amount: Up to £75k
Key dates: Call opens in September; deadline in January; decisions in July
Webpage: www.nc3rs.org.uk/pilotstudygrants

Scheme: Strategic awards
Purpose: Specific call for applications in priority areas
Duration: Up to 36 months
Amount: Dependent on the science; typical budget of £500k per call
Key dates: Usually one call per year with varying deadlines
Webpage: www.nc3rs.org.uk/strategicawards

Scheme: Studentships
Purpose: To embed the 3Rs in the training of graduate scientists
Duration: 36 or 48 months
Amount: Cash-limited award of £30k per annum
Key dates: Call opens in April; deadline in July; decisions in December
Webpage: www.nc3rs.org.uk/studentships

Scheme: David Sainsbury fellowships
Purpose: To support exceptional early career scientists (up to three years postdoctoral experience) with the transition to independent researcher
Duration: 36 months
Amount: Cash-limited award of £65k per annum
Key dates: Call opens in September; deadline in November; decisions in April
Webpage: www.nc3rs.org.uk/fellowships

Scheme: CRACK-IT challenges
Purpose: To solve business challenges with a 3Rs theme identified with industry partners
Duration: Up to 36 months
Amount: Dependent on the research area; budget for 2011 ranges from £500k to £1m per research area
Key dates: Call opens in September; deadline in November; decisions in December
Webpage: www.crackit.org.uk

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