OUR VISION
TO BE A LEADING INTERNATIONAL BRAIN RESEARCH INSTITUTE

OUR MISSION
IMPROVING LIFE THROUGH BRAIN RESEARCH

OUR VALUES
INNOVATION AND EXCELLENCE
COMMITMENT AND PASSION
INTEGRITY AND RIGOUR
COLLABORATION AND TEAMWORK

ADDICTION
ALZHEIMER’S DISEASE
CARDIOVASCULAR DISEASE
MENTAL ILLNESS
EPILEPSY
HUNTINGTON’S DISEASE
MOTOR NEURON DISEASE
MULTIPLE SCLEROSIS
PARKINSON’S DISEASE
SCHIZOPHRENIA
STROKE
TRAUMATIC BRAIN AND SPINAL CORD INJURY

‘BRAIN IN A DISH’. HUMAN EMBRYONIC STEM CELLS GROWN IN THE LABORATORY
FORM A ‘NEURAL TUBE’ LIKE STRUCTURE IN MUCH THE SAME WAY AS THE HUMAN
NERVOUS SYSTEM NORMALLY DEVELOPS. THE CELLS HAVE BEEN ENGINEERED TO
EXPRESS GREEN FLUORESCENT PROTEIN SO THEY CAN BE TRACKED IN NEURAL
TRANSPLANTATION EXPERIMENTS. OTX2 SHOWN IN RED, DAPI IN BLUE.
The Florey has given me a fantastic opportunity to start my own independent career... working with colleagues in very different and exciting fields... it’s an outstanding environment for my research, for collaboration and for bringing my interests closer to the clinic.

Dr Stephanie Bissiere who has joined us from the University of California in Los Angeles.

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### Global Collaboration

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Florey scientists collaborate with colleagues all over the world.
The initiative comes at a critical time for Australian research. This is for two reasons. Firstly, there is a serious mismatch between the growth in capital infrastructure in most states and the static nature of funding available from the NHMRC and ARC for staff and infrastructure support. The gap cannot be bridged by philanthropic funding in the current economic climate, so a serious investment increase by governments is needed. This would take advantage of the ‘once in a generation’ opportunity to boost our intellectual capital significantly with top flight investigators from other parts of the world as they grapple with the fallout from the global financial pressures from which we have been relatively spared. Secondly, the career path for scientists has, in recent years, become too unstable. A streamlined process needs to be put in place to ensure a career structure which promotes excellence and progression while providing a reasonable level of security. This will demonstrate to young scientists that a career in science, while exhilarating, competitive and dynamic, has the stability to be worth pursuing. If these issues can be successfully addressed in the review and subsequent implementation, we will have a vibrant research sector contributing to society in a way that makes us a global leader.

The Florey Neuroscience Institutes has experienced an extraordinary time of growth and scientific endeavour during the last two years. There is energy and enthusiasm, a resilience and commitment which can largely be attributed to two magnificent new buildings and spacious laboratories now equipped in a way to take advantage of the latest advances in technology. The juxtaposition of scientists with similar interests, even though they may be from different institutions, is producing a work environment that promotes a spirit of collaboration and excellence.

The common theme is that all are working to improve the human condition. While political and financial climates in Australia and around the world continue to be volatile, we are aiming to provide a stable but dynamic and productive environment for our staff so that they can continue to contribute to the global world of science discovery and translation. We must be able to demonstrate that we are contributing to the nation’s wealth and to international global health.

An important component of this is to persuade our governments to continue to recognise the long-term value of a stable and increasing investment in science. Indeed, it is sobering to realise that some 39,000 people are employed in science in Australia. As a group they are improving health and wealth through therapeutic developments and more efficient health care. It’s worth remembering that NHMRC funding produces eight patents per million dollars spent and some 45 per cent of all Australian patents are for medical research innovations.

Harnessing these discoveries within the health care framework can sometimes be challenging, so we welcomed the initiative of the federal and state governments to create advanced health care networks to better integrate the research and delivery elements of the health care continuum. This certainly is in accord with the Florey philosophy to “discover and translate”.

We were also pleased to hear the announcement by the Minister for Mental Health and Ageing, Mark Butler, that the 2011 Australian of the Year, Simon McKeon, would chair an independent review of health and medical research in Australia. Mr McKeon has been asked to recommend a 10-year strategic health and medical research plan for the nation.
OUR SCIENCE AND PEOPLE

To expand upon the research funding theme, our scientists have also been extremely successful in competitive NHMRC and ARC project grants for 2011 with a funding success rate over double the national average. This is an impressive result, particularly given the static pool of funding from which to draw, as mentioned earlier. Our philanthropic grant success was also high, reflecting both the hard work of our fundraising and marketing team and the talent of our scientists. On behalf of the Board, I congratulate and thank them for their achievements.

I am also pleased to report that three senior research fellows were reappointed and two new fellows were admitted bringing our total to 23 senior staff who mentor and develop the next generation of scientists.

We have been actively recruiting internationally and, armed with our magnificent new facilities, have managed to lure several very talented scientists to Melbourne.

Professor Martin Pera has returned from California to head Stem Cells Australia and Dr Ben Emery has returned after five years at Stanford University. With the return to Australia of the Florey’s Dr Daniel Scott, we can be reassured that the Florey is making its contribution towards reversing the brain drain that has seen so many of our most talented scientists take their skills overseas. As a part of this influx, we also welcome Dr Mathias Dutschmann and Dr Hari Subramanian from Leeds University in the UK. Dr Stuart McDougall has also joined us from the UK to work with our systems neurophysiology team.

They are joined by young Swiss researcher Dr Stephanie Bissiere who has come to work with the Neuropeptides and Behavioural Neuroscience divisions as a senior research fellow. Stephanie comes from the Department of Psychology and Brain Research Institute at UCLA, USA. Her fascinating research involves the way mammals store fear memories and the way they might impact on mental disorders, particularly through the involvement of electrical synapses.

The Florey offers an exceptional mentoring system for young researchers, helping them prepare grant applications through our well developed “grant assist” program and building up their publications and curricula vitae. Probably as a result of these and other factors, we continue to expand and punch well above our weight on the international stage.

I would like to thank the entire Institute for their efforts this year. Our core business is research, supported by staff ranging from cleaners to laboratory assistants, administrative staff and board members. You have all contributed to this report and its underlying activities, either directly or indirectly and justly deserve the success which has been achieved in setting us on a path to becoming one of the most significant research institutes on the international scene.

GEOFFREY A DONNAN
DIRECTOR
The Australian community faces a bill for over $300 billion in the next decade because diseases of the brain and mind will account for nearly half of all illnesses.

Dr Henry De Aizpurua, Deputy Director and Head of Business Development

Our Divisions
SNAPSHOT OF OUR STUDIES

Our research focuses on understanding the interaction between genes and environment, how experience can change the brain, and how disease develops in mouse models of brain disorders such as Huntington’s disease, schizophrenia and autism spectrum disorders. We particularly want to understand how specific cognitive and psychiatric disorders arise and develop. We use a range of techniques to understand how genes and environment (‘nature versus nurture’) combine to affect specific aspects of brain function and dysfunction.

Further study of how various environmental manipulations (such as environmental enrichment and exercise interventions) can delay disease progression might provide direction for improved therapeutic approaches like ‘enviromimetics’, drugs which mimic or enhance the beneficial effects of environmental stimulation. These could be of great clinical value for many brain diseases.

RESEARCH HIGHLIGHT

We have discovered changes in a mouse model of Huntington’s disease (HD) that may explain why mood disorders (e.g. anxiety and depression) are so common in this disease. There are specific abnormalities in the way the mice respond to stress which closely model the changes found in human depression. Furthermore, not only do the symptoms in these mice respond to a clinically effective antidepressant drug (sertraline), but they also respond to increased voluntary physical exercise. These new findings provide insights not only into HD, but depression in the wider population, and may lead to new therapeutic approaches.

SENIOR STAFF

Assoc Prof Anthony Hannan
Dr Terence Pang
Dr Thibault Renoir
Dr Mark Ransome
Dr Emma Burrows
Monique Howard

This image from the Neural Plasticity laboratory demonstrates the recording of a male mouse singing in response to the alluring aroma of a female mouse. Florey researchers are amongst the best in the world at deciphering mouse vocalisation and other behaviours - a key to understanding brain disorders involving speech, communication and cognitive abnormalities, such as autism.

RESEARCH OVERVIEW

The human cerebral cortex contains billions of neurons that are interconnected by trillions of synapses to form functional networks underlying our most complex brain functions. It is only after birth, when environmental stimuli induce patterned neural activity via the sensory pathways, that diverse cortical functions begin to emerge. One of our general aims is to gain insight into the way in which the genetic program regulating maturation and function of the cortex is dynamically moulded by environmental stimuli. Our exploration of normal brain development and function has provided new information on mechanisms of experience-dependent ‘plasticity’ that may underlie higher brain functions such as learning and memory. One particular aspect of our research is focused on understanding gene-environment interactions in disorders involving disruption of normal cortical development, plasticity and/or function, such as those associated with Huntington’s disease (involving abnormal cortical/striatal function and plasticity) and schizophrenia (involving abnormal cortical maturation and plasticity). Using a range of techniques, including molecular biology, protein chemistry, neuroanatomy and mouse behavioural analysis, we are attempting to understand how genes and environment combine to affect specific aspects of brain and behaviour.

Further study of the beneficial effects of various environmental manipulations might provide direction for therapeutic approaches of clinical value for brain diseases. Ongoing research aims to investigate gene-environment interactions and their impact on brain function and behaviour, not only to elucidate disease mechanisms, but also to use our approach as a tool to identify molecular targets for future development of novel therapeutics. We have discovered molecular pathways that process information from the environment and induce experience-dependent changes in the structure and function of neurons in the cerebral cortex.
To better understand the complex nature of gene-environment interactions, we have begun to compare the effects of complex sensory and motor stressors, as well as simple voluntary physical exercise (wheel running) in mice. Our work on gene expression and profiling studies are identifying novel molecular targets whose regulation is disrupted in HD but may mediate the beneficial effects of environmental enrichment. We now have evidence for molecular changes in synapses (synaptic plasticity) which occur in distinct regions of the cerebral cortex, as well as other brain areas, after exposing mice to different forms of environmental stimulation. This link between environmental stimulation and changes in the synaptic connections we observed that environmental enrichment, which induces increased neuronal plasticity. In addition to altered synaptic plasticity, we also have evidence for molecular changes (for example those involving the serotonergic system and neurotrophins) and their wild-type littermates. These findings not only have implications for our understanding of the molecular mechanisms underlying not only have implications for our understanding of the molecular mechanisms underlying normal and abnormal maturation and cellular plasticity, but also how it might impact on adult neurogenesis. We are also exploring how environmental stimuli, as well as the gene-environment interactions, play a significant role in modulating the disease process.

The influence of environmental factors in HD has been the focus of many laboratories. HD environmental enrichment can reverse schizophrenia-like symptoms in HD mice. This has also allowed us to develop research into how environmental factors influence, during postnatal and early postnatal development, the complexity of the interactions between environmental factors, and the HD gene and their wild-type littermates. This has inspired new international research into how environmental stimuli, as well as the gene-environment interactions, play a significant role in modulating the disease process.

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ILLAWARA HEALTH AND MEDICAL RESEARCH INSTITUTE, UNIVERSITY OF WOLLONGONG, NSW (2010)


Keynote Speaker, BioAutism, Melbourne (2011)

Plenary Lecture, Academy for Psychiatry of Old Age, University of Melbourne, 16th Annual Symposium, ‘Late life mental disorders – between concept and reality’, Melbourne (2011)

Cognitive Neuroscience Mini-Symposium, University of Melbourne (2011)

Department of Physiology, Monash University, Melbourne (2011)

School of Medical Sciences Seminar Series, University of New South Wales, Sydney (2011)

International

Anthony Hannan

Invited seminars

Faculty of Medicine, University of Lund, Sweden (2010)

Department of Experimental Psychology, University of Oxford, UK (2010)

Cambridge Institute for Medical Research, University of Cambridge, UK (2010)

Department of Physiology, University of Otago, New Zealand (2010)

Young Investigator Colloquium Speaker at the 9th Biennial Meeting of the Asia-Pacific Society for Neurochemistry (APSN), Phuket, Thailand (2010)

Invited Workshop Participant, Cure Huntington’s Disease Initiative (CHDI) Workshop on Exercise-Drug Synergy: Neuroprotection and Neural Plasticity in Huntington’s Disease, New York, USA (2011)

Johns Hopkins University, Baltimore, USA (2011)


World Congress on Huntington’s Disease, Melbourne (2011)

Symposium Speaker, ‘New neurons for diseased brains: Functional significance and perspectives for brain repair’ (sponsored by the French Society for Neuroscience and Italian Neuroscience Society), IBRO World Congress of Neuroscience, Florence, Italy (2011)

Symposium Speaker, ‘Plasticity, structure and function of the brain (Festschrift in honour of Colin Blakemore)’, Physiology Conference, Oxford, UK (2011)

Plenary Lecture, Inaugural New Zealand Stroke & Applied Neurosciences Conference (NZSANC), Auckland, New Zealand (2011)

TRENTE PANG

Federation of European Neuroscience Societies, Amsterdam, Holland, 2010

World Congress on Huntington’s Disease, Melbourne (2011)

Thibault Renoir

Federation of European Neuroscience Societies, Amsterdam, Holland, 2010

World Congress on Huntington’s Disease, Melbourne (2011)

Emma Burrows

Federation of European Neuroscience Societies, Amsterdam, Holland, 2010

Mark Ransome

World Congress on Huntington’s Disease, Melbourne (2011)

Xin Du

Federation of European Neuroscience Societies, Amsterdam, Holland, 2010

World Congress on Huntington’s Disease, Melbourne (2011)

Christina Mo

World Congress on Huntington’s Disease, Melbourne (2011)

Michelle Zajac

Society for Neuroscience, San Diego, USA, 2010

AWARDS

Thibault Renoir

NHMRC-INSERM Fellowship

Caitlin McOmish

NHMRC CJ Martin Fellowship

Michelle Zajac, Mari Kondo, Emma Burrows, Christina Mo, Xin Du

FNI Student Awards

Annabel Short

Australian Postgraduate Award

MAJOR COLLABORATIVE LINKS

National

Prof Patrick Tam and Dr Greg Peika
Children’s Medical Research Institute and University of Sydney, Sydney
Characterisation of a mouse model of Rett syndrome

Prof Edna Harderman and Dr Stephen Palmer
University of New South Wales, Sydney
Characterisation of a mouse model of Williams syndrome

A/Prof Maarten van den Buisse
Mental Health Research Institute of Victoria
Gene-environment interactions in a mouse model of schizophrenia

Dr Erica Fletcher
Department of Anatomy and Cell Biology, University of Melbourne
Retinal abnormalities in a mouse model of HD

A/Prof. Xiao-Jun Du
Baker IDI, Melbourne
Cardiovascular abnormalities in a mouse model of HD

Dr Elisa Hill and Prof. Terry O’Brien
Children’s Medical Research Institute and University of Melbourne
Behavioural analysis and environmental and pharmacological manipulations in a mouse model of autism

Dr Danny Hatters
Bio21 Institute, Department of Biochemistry, University of Melbourne
Molecular pathogenesis in Huntington’s disease

International

Dr Laurence Lannfume
INSERM UMR 677, Paris, France
Gene-environment effects on monoaminergic systems

Dr Ghazaleh Sadri-Vakili
Massachusetts General Hospital, Harvard University, Boston, USA
Epigenetics in the healthy and diseased brain

Dr Caitlin McOmish
Columbia University, USA
Behavioural and pharmacological analysis of a model of schizophrenia

MCRI, Royal Childrens Hospital, Melbourne
Experience-dependent epigenetics in a mouse model

Dr Trent Woodruff and A/Prof. Peter Noakes
University of Queensland, Brisbane
Immune dysfunction in Huntington’s disease

Dr Daniel Lévesque
University of Montreal, Canada
Interaction and pharmacological modulation of specific G-protein coupled neurotransmitter receptors of relevance to psychiatric disorders

Prof. Daniel Luvesque
we now have no doubt that addiction is not a character flaw. It’s a brain disorder and it’s costing Australia $50 billion a year in health costs. In the next 10 years we will see a doubling of people over 50 seeking help for addiction.

**WE NOW HAVE NO DOUBT THAT ADDICTION IS NOT A CHARACTER FLAW. IT’S A BRAIN DISORDER AND IT’S COSTING AUSTRALIA $50 BILLION A YEAR IN HEALTH COSTS. IN THE NEXT 10 YEARS WE WILL SEE A DOUBLING OF PEOPLE OVER 50 SEEKING HELP FOR ADDICTION.**

**PROF ANDREW LAWRENCE, CO-DIVISION HEAD, BEHAVIOURAL NEUROSCIENCE**

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SNAPSHOT OF OUR STUDIES

Our laboratory investigates the neural pathways implicated in drug-seeking behaviour, and in addition, the association between mood disorders, stress and drug-seeking behaviour. For example, alcohol is one of the most widely used and abused drugs in society, with immense social, medical and financial impact. The neuropharmacological basis of alcohol reward is an ongoing research area in this laboratory, with a major focus on the development of genetic animal models of addiction, including inbred strains of rats and knockout/transgenic mice. We measure drug self-administration and behavioural parameters to assess the influence of specific receptors and emotional states such as anxiety and depression on drug-seeking behaviour. These behavioural approaches are complemented with neurochemical, anatomical and molecular research strands to provide a multidisciplinary strategy.

We also use genetic approaches to investigate the neural substrates of drug-seeking, drug-induced plasticity and relapse. This latter aspect is of critical importance, as the defining feature of addiction is its chronic and relapsing nature. To facilitate these studies, we apply the technique of intravenous self-administration of drugs of abuse in rats and mice; current projects involve the self-administration of opiates, cocaine and nicotine. The use of different rodent strains along with relevant paradigms has enabled us to address key questions relating to addiction, such as identifying the factors implicated in behavioural responses to drugs of abuse, and also the chemistry of relapse.

OUR RESEARCH HIGHLIGHTS

Nicotine addiction, through smoking cigarettes, is a major contributor to the global burden of disease and a leading cause of preventable death. Our group has established a model of nicotine self-administration in mice, and is combining this with molecular genetic approaches to examine the role of specific nicotinic receptor subunits in nicotine reward, plasticity, locomotor behaviour and anxiety. Using these paradigms, we have demonstrated that while nicotinic receptors containing the α4 subunit are critical for the acute locomotor effects, they are sufficient, but not necessary, for the reinforcing effects of nicotine. Parallel pharmacological studies with the nicotinic antagonist mecamylamine further support the notion that nicotinic receptors of different configurations are primarily responsible for mediating these effects. Thus, different aspects of nicotine’s effects are mediated by receptors with different subunit configurations, an important consideration for investigations into the development of targeted, subunit-specific modulators as drug therapies for nicotine cessation.

SENIOR STAFF

Professor Andrew J Lawrence
Dr Jhodie Duncan
Dr Jee Hyun Kim
Dr Robyn Brown
Dr Heather Madsen
Mrs Elena Krstew

This image was produced by Heather Madsen as part of her PhD studies and involves the deletion of a molecule in the brain.
Glutamate, drug-seeking & extinction

Andrew Lawrence, Jee Hyun Kim, Robyn Brown, Cameron Adams, Michael Bird, Rose Chessworth, Sophie Lukinga, Peter Dodd (UQ) & Kevin Pfeifer (UWA)

Drug-seeking & extinction: Relapse is a defining feature and major clinical obstacle in addiction. We, and others, have shown a role for glutamate and glutamate receptors in the competing processes of drug-seeking and staying abstinent. In animals, we can model this facet of addiction by a method known as extinction-reinstatement. Extinction refers to the decrease in fear/drug-seeking responses expressed to a stimulus due to repeated exposure to that stimulus without any reinforcement. Because extinction is an active learning process, it involves many of the neurobiological substrates that subserve normal learning and memory, including increased transmission and plasticity at central glutamatergic synapses. A particular receptor for glutamate, the mGlu5 receptor, is found on both the drug-seeking and extinction circuits in the brain. At present, we are using a combination of genetic and neuropharmacological approaches to delineate the precise anatomical loci for these receptors. This receptor is regulating these two opposing processes.

Neuropeptide involvement in drug & alcohol-seeking

Andrew Lawrence, Bianca Jupp, Phil Ryan, Betty Knivid & Elena Kniew, Robyn Brown, Nicola Chen, Shaun Khoa & Hanna Kastman

We have developed a model of relapse to alcohol-seeking after extended periods of abstinence, demonstrating the utility of animals to study the enduring propensity to relapse that is characteristic of human addicts. Importantly, we have examined patterns of neural activation following cue-induced relapse immediately after extinction training (a rodent equivalent of rehab) compared to relapse that occurs when extinction is followed by an extended period of abstinence. Immediate reinstatement increased neural activation in a range of cortical and sub-cortical structures implicated in drug/alcohol-seeking and while delayed reinstatement showed a broadly similar pattern, these markers were further elevated in cortical structures. These data suggest that a base network of interconnected circuitry is implicated in relapse-like behaviour, although following prolonged abstinence the way the brain integrates the salience of cues that previously signalled drug availability may change. Notably, functional blockade of the orexin system essentially prevented the expression of relapse-like behaviour at both time points. This would suggest that the orexin system in the brain is involved in cue-driven reward-seeking, irrespective of a period of abstinence. By combining pharmacological blockade of the orexin system with the relapse paradigm and subsequent assessment of neural activation we have identified putative anatomic loci where the orexin system may be acting to influence reward-seeking. These target structures are the current focus of studies identifying where in the brain this peptide system acts to modulate relapse. In addition, we have recently demonstrated that the orexin system is implicated in the motivational properties of alcohol to a far greater extent than it is for food rewards, a useful property for developing potential therapeutics.


SNAPSHOT OF OUR STUDIES

Our group is interested in the cognitive impact of neurological disease, especially stroke and focal onset dementias. We are also interested in the cognitive outcomes following stroke, both short and long-term. Recently developed imaging methods can now give us new insights into these brain diseases.

In the short-term (in collaboration with Dr Loetscher at Flinders University), we are using novel testing paradigms in association with advanced imaging techniques to investigate paralysis after stroke. We have previously examined the association between brain perfusion and carotid arterial disease with cognitive profiles and cortical utilization. Over the longer term, we are examining the effect of stroke on longitudinal brain volume and cognition. In this project, we take a detailed history and cognitive assessment of a group of prospectively recruited stroke patients following acute stroke, and make serial assessments over a one year period. We hope to be able to extend this study over 3 years, and plan to image their brains with a PET marker sensitive for amyloid, the protein deposited in the brain of Alzheimer’s disease patients.

Dr Brodtmann is also director of the Eastern Cognitive Disorders Clinic at Box Hill Hospital, a specialist focal onset dementia service that is the largest in Victoria. This group has focused on carer support services, setting up the first frontotemporal dementia (FTD) support group in Melbourne, and collaborates with the FRONTIER group (Prof John Hodges) in Sydney.

RESEARCH HIGHLIGHT

We were delighted to receive both a Brain Foundation award and JO & JR Wicking Trust grant as seeding funds for our longitudinal brain volume study after acute stroke. This funding will allow us to follow a group of patients after their stroke, investigating their brain volumes and cognition with advanced imaging techniques. Our aim is to investigate whether stroke is associated with brain degeneration, something that has not been explicitly examined in prior studies.

We also received a Telematics Trust grant which, together with further support from the Ross Trust, has allowed us to develop a website-based educational package for health professionals and carers of patients with FTD. This is an international first, and was launched at a symposium in October 2011.

SENIOR STAFF

Dr Amy Brodtmann
Dr Toby Cumming
Dr Tobias Loetscher (at Flinders University)
Is stroke degenerative?
A longitudinal study of cognitive and changes regional brain volume following stroke

Amy Brodtmann, Toby Cumming, Heath Paradowski

Alzheimer's disease (AD) is the most common form of dementia. While the causes are not well understood, we know from post-mortem studies that certain proteins (known as plaques and tangles) are found in the brains of patients with AD. These proteins act to accelerate cell death in the brain, which has the effect of reducing brain volume over time, a process known as atrophy. Stroke occurs when blood supply to the brain is disturbed, either by a blocked or a burst blood vessel. Historically, AD and stroke have been seen as separate entities. There may be reason to believe, however, that stroke has a role in the progression of AD. Dementia caused by problems in the brain’s blood vessels (vascular dementia) often co-exists with dementia caused by AD. Research has shown that AD patients with a history of stroke had poorer cognitive function than those who had not suffered stroke. The question remains as to whether stroke can trigger progressive dementia and ongoing cell death in the same way as AD. In this project, we recruit stroke patients after their acute event and follow them with serial cognitive testing and brain volume estimation.

Cortical remodelling associated with carotid stenosis
Amy Brodmann, Fernando Calamante, Alan Connelly, Shawna Farquharson, David Abbott, Geoffrey Donnan

Cortical remodelling has been demonstrated following stroke, often via fMRI studies. It is not fully understood if some of these changes are compensatory or correlate with functional and cognitive impairments. There is evidence that the hypoperfusion caused by vascular disease drives the recruitment of other areas of the brain not affected by arterial disease. We have studied a group of patients with unilateral carotid arterial disease, and found that extensive remodelling is occurring in a region supplied by the narrowed artery, the motor cortex. This study has provided new and important information on the role of normal vascular perfusion on brain reorganisation, and further information on the effects of hypoperfusion on cortical remodelling. Information on cortical perfusion is critical, as it appears to be one of the most important determinants of cortical plasticity. In addition, understanding how the brain responds to chronic ischaemic stress may contribute to our understanding of vascular cognitive impairment. These findings formed the basis of a larger longitudinal study examining cognition and brain volume after stroke.

Representational neglect following acute stroke
Tobias Loetscher, Amy Brodmann, Mike Nicholls

Spatial neglect is a debilitating, but relatively common, clinical disorder resulting from stroke. Neglect prevents the patient from attending equally to both sides of space – often resulting in bizarre symptoms such as ignoring people standing on the left and dressing just the right side of the body. Intriguingly, in addition to behaving as if the left side of the world ceased to exist, these patients also ignore the left side of imagined space. Whereas neglect research in the last decades has mainly been focused on spatial deficits in visuospatial function, in this project we examine spatial impairments in imagined space. Using simple behavioural tasks, distortions in the representation of imagined space is assessed in patients suffering from neglect.

Developing a web-based educational tool-kit for health professionals and carers of patients with FTD
Together with DBMAS and AAV, we have commenced work on a unique specialist set of modules that will be freely available from the ECDC website. This was launched at the first Melbourne symposium on FTD, hosted by Dr Brodmann and her staff at ECDC, in October 2011.

Analysis of speech in FTD syndromes using a computerised paradigm
In collaboration with Dr Adam Vogel at the University of Melbourne, we plan to analyse the speech characteristics of patients with various FTD syndromes in order to improve diagnostic accuracy.

Functional Neuroanatomy of Discourse
Leasha Lillywhite, Michael Saling

Behavioural work on discourse production suggests a role for the temporal lobe in message-level representation. A study of the impact of temporal lobe epilepsy on this network is in progress, as well as examining patients with acquired aphasia syndromes, particularly those with primary progressive aphasia, part of the frontotemporal dementias.

Alexia without agraphia
Tobias Loetscher, Shawna Farquharson, Leasha Lillywhite, Amy Brodmann

Frontotemporal dementia registry
Amy Brodmann, Dennis Velakoulis, Richard Cotton, Colin Masters, Tiffany Cowie, David Darby

In consultation with FTD stakeholders, we are working toward a Victoria wide FTD registry. We aim to ally this with the establishment of the Australian branch of the International FTD Association.

Early detection of Alzheimer’s disease in a community setting using computerised testing COGSTATE
David Darby, Michael Woodward, Lynette Moore, Chris Rowe, Amy Brodmann

Changes in bone structure, lean mass, and glucose metabolism within the first six months post-stroke: a prospective study
Karen Borschmann, Marco Pang, Sandra Illiano-Burns, Elif Ekinci, Amy Brodmann, Julie Bernhardt

Progressive aphasia and amyloid deposition: a multidisciplinary approach to improving dementia diagnosis
John Hodges, Christopher Rowe, Olivier Piguet, Kenyn Pike, Kime Ballard, Victor Villeneuve
SNAPSHOT OF OUR YEAR

In 2010, the Florey established a Division of Neuro-ethics to broaden our scope and to complement our clinical and basic scientific investigations. The division promises to add a layer of philosophical rigor to our wide-ranging research.

The division is led by philosopher Professor Neil Levy, who specializes in philosophy of mind and ethics. He focuses especially on how cognitive science – neuroscience, social and cognitive psychology – illuminate traditional philosophical questions, such as the nature of the control we have over our actions and the extent to which our common sense conception of ourselves needs to be revised. He is especially interested in self-control: how and why it is lost and how we can prevent such losses, both in pathological cases (eg addiction) and ordinary failures of the will (eg, overeating). These questions are tackled in a multi-pronged manner. Some of the work is traditional philosophy: argument and conceptual analysis. Some is empirical: bringing the details of work in cognitive science to bear on traditional philosophical questions such as the nature of free will. And some of the work is experimental, examining the ways in which the resources needed for self-regulation may be reduced in the short term when they are called upon for cognitively demanding tasks.

This work is conducted in collaboration with University of Melbourne psychologists and Florey neuroscientists. Prof Levy has a growing interest in the philosophical role of consciousness: how and to what extent does being conscious of information increase our control over our behaviour?

HIGHLIGHTS OF THE LAST TWO YEARS

In 2011, Prof Levy published Hard Luck, a new book on the nature of free will, with Oxford University Press. A review of Hard Luck in Mind, one of the most prestigious philosophy journals in the world, called the book “important and challenging”.

Florey Neuroscience Institute Annual Report 2011

PUBLICATIONS

SNAPSHOT VIEW OF OUR STUDIES

We want to understand how brain cells are properly assembled during development, more specifically, how individual neurons in the embryonic brain know where to go and what to become. We also want to learn which genes drive neuronal migration and test their functions in the intact brain. For instance, how do these genes alter the way cells are born, change shape, migrate and adopt different neuronal identities? These studies will provide new information on how genes have shaped brain evolution, and may explain the genetic causes of mental illnesses such as autism, schizophrenia and epilepsy. Our group is also actively involved in discovering how to prolong the survival of neurons after injury or stress following stroke or brain trauma. We have discovered a number of neuroprotective proteins and aim to uncover their mechanisms so we can devise methods for delivering them to stressed neurons.

RESEARCH HIGHLIGHT OF OUR YEAR

Following brain injury, neurons die for days and even weeks after the event. Some neurons die as a consequence of the injury (e.g. trauma, stroke) but neurons in adjacent areas die from uncontrolled release of toxic substances and excessive firing activity. The brain tries to protect itself from this secondary cell death by increasing Ndfip1, a protein that is normally present at low levels but increased by stress. Neurons that increase Ndfip1 are protected from death, but unfortunately this protective mechanism appears to be limited to a small number of participating neurons. The reason for this is unknown but it opens up a therapeutic opportunity. We have been researching how brain Ndfip1 protects neurons from death, and thereby devising therapeutic methods of amplifying the Ndfip1 response to cover other neurons that normally succumb to death.

OVERVIEW

Our work is directed at discovering how newly-born neurons are properly assembled, interconnected and electrically activated. In particular, we are interested in understanding how immature brain cells in the embryonic brain know where to go, what to become, and what other cells they should be connected to. These issues are supremely important for the proper assembly of the brain, with particular consequences for the prevention of congenital brain disorders. We are particularly interested in understanding how inhibitory neurons of the cortex find their destinations after they are born. Inhibitory neurons, also known as interneurons, are disturbed in many neurological conditions including epilepsy, schizophrenia, and autism. Our recent work is focussed on studying the ways that they move through the immature brain, and the factors that influence their departure and arrival times in different brain layers. We have also been studying the rules that dictate the movement of excitatory neurons, the major population of nerve cells in the cortex. We found that Reelin, an extracellular protein, is an important player right at the beginning of the migratory journey, much earlier than was thought possible.

In parallel, we have also been exploring the mechanisms behind the branching of neurons after they stop migrating. Neuron branching is an important step for brain wiring by establishing the territories of nerve cell connectivity. We have been working on Ndfip1, a key protein involved in controlling signals within nerve cells with outcomes for branching and establishment of terminal points. These events underscore the establishment of nerve cell networks required for proper electrical activity.

Ndfip1 is also very important for neuron survival. We have conducted experiments to understand why neurons that over-express Ndfip1 are able to withstand death. Our work has identified that Ndfip1 improves neuron survival, by hitch-hiking mechanisms used in cancer cells to avoid death. This discovery opens up new vistas for understanding the signaling mechanisms underlying both neuroprotection and cancer. We have also synthesized new drugs for increasing Ndfip1 content in nerve cells to improve survival during stress events.

SENIOR STAFF

Professor Seong-Seng Tan
Dr Jenny Gunnerson
Dr Joanne Britto
Dr Vicki Hammond
Dr Jason Howitt
Dr Ulrich Putz
Migration of immature neurons

Reelin activity during the high-resolution confocal microscopy to monitor the movement of neurons in real-time. This technique is a valuable tool in revealing the migratory behaviour of neurons from birth to a layer position and has allowed us to discover the unique susceptibility of migrating immature neurons to the absence or presence of Reelin. We focused on the migratory activity in the germinal zones to observe the events that occur after a neuron is born. In the absence of Reelin, the immature neurons still maintain an ability to migrate, however, there is a loss of directionality and a tendency to meander rather than follow a straight radial path. Compared to the normal situation, this wayward migration may explain the increase in cell dispersion and abnormalities in cortical layering observed in the absence of Reelin.

Reelin dosage is critical for layer organization of the developing cortex

Vicki Hammond, Jenny Gunnersen, Seong-Seng Tan

Reelin is an important protein that is indispensable for cortical lamination. In the absence of Reelin, cortical layers fail to form due to inappropriate neuron migration and positioning. The inversion of cortical layers is attributed to failure of neurons to migrate past earlier-generated neurons although how Reelin-insufficiency causes this is unclear. The issue is complicated by recent studies showing that very little Reelin is required for cortical layering. To test how variation in the number of Reelin-producing cells is linked to cortical lamination, we have employed Reelin-/- Reelin-/- chimeric mice in which the number of Reelin-expressing neurons is adjusted. We found that the Reeler phenotype was rescued in chimeras with a large contribution of Reelin-/- neurons; conversely in chimeras with a weak contribution by Reelin-/- neurons, the mutant phenotype remained. However, increasing the number of Reelin-/- neurons beyond an unknown threshold resulted in partial rescue, with the formation of a corrected-layered secondary cortex lying on top of an inverted mutant cortex. Therefore, the development of cortical layers in the correct order requires a minimal level of Reelin protein to be present although paradoxically, this is insufficient to prevent the simultaneous formation of inverted cortical layers in the same hemisphere.

Cellular upregulation of Ndfip1 using low levels of bioactive cobalt complexes

Christine Schieber, Jason Howitt, Ulrich Putz, Seong-Seng Tan

The delivery of metal ions using cell membrane-permeable metal complexes represents a method for activating cellular pathways. Here, we report the synthesis and characterisation of new Co(III) (acac) complexes capable of upregulating the ubiquitin ligase adaptor protein Ndfip1. Ndfip1 is a neuroprotective protein that is upregulated in the brain after injury and functions in combination with Nedd2 ligases to ubiquitinate harmful proteins for removal. We previously showed that Ndfip1 can be increased in human neurons using CoCl2, that is toxic at high concentration. Here we demonstrate that a similar effect can be achieved by low concentrations of synthetic Co(III) complexes that are non-toxic and designed to be activated following cellular entry. Activation is achieved by intracellular reduction of Co(III) to Co(II) leading to release of Co(II) ions for Ndfip1 upregulation. The cellular benefit of Ndfip1 upregulation by Co(III) complexes includes demonstrable protection against cell death in SH-SYSY cells during stress. In vivo, focal delivery of Co(III) complexes into the adult mouse brain was observed to upregulate Ndfip1 in neurons. These results demonstrate that a cellular response pathway can be advantageously manipulated by chemical modification of metal complexes, and represents a significant step of harnessing low concentration metal complexes for therapeutic benefit.

Ndfip1 is important for the development of healthy neurons

Vicki Hammond, Jenny Gunnersen, Seong-Seng Tan

To maintain healthy cells, it is necessary to break down excess or damaged proteins. One means of tagging proteins for removal is by adding ubiquitin - a process termed ubiquitination. Since aberrant ubiquitination of proteins is a prominent feature of many neurological disorders and ubiquitination is required for developing neurons to establish their polarity, branching and connections, we have been studying these processes in brain development. As the brain matures, neurons extend processes to make connections with other neurons and form functional circuits. Correct development of branches and connections, or synapses, is crucial for normal cognition. Specifically, fewer or smaller synapses are indicative of abnormal synaptic function which, in turn, contributes to mental retardation.

Recently, we produced an Ndfip1 knockout mouse with an inactive Ndfip1 gene and we have analysed the consequences for neuron development. This work has revealed that Ndfip1 is important for maintaining the branching and synaptic connections that allow effective communication between neurons. We are now working to elucidate the molecular targets of Ndfip1 which, when misregulated, cause developing neurons to malfunction. With this knowledge, we hope to gain a better understanding of how Ndfip1 can rescue damaged neurons in the adult brain.
PUBLICATIONS


2. Hammond VE, So E, Cate HS, Britto JM, Gunnersen JM, Tan SS. Cortical layer development and orientation is modulated by relative contributions of reelin-negative and -positive neurons in mouse chimeras. Cereb Cortex. 2010 Sep;20(9):2017-26.


MAJOR COLLABORATIVE LINKS

National
Prof Penny Bartlett
Queensland Brain Institute, University of Queensland
Generation of neurons in the adult and embryo
Prof Pankaj Sah
Queensland Brain Institute, University of Queensland
Electrophysiology of brain cells lacking Sea-6
Prof Shanad Kumar
Hanson Centre, IMVS, Adelaide
The role of Ndfip1 in brain injury
Assoc Prof David Walker
Monash Medical Centre, Melbourne
Effects of pre-term injury on cortical development
Dr Leigh Johnston
NICIDA Victoria Research Laboratory, Department of Electrical and Electronic Engineering, University of Melbourne
Mathematical modelling of neuronal migration
Dr John Power and Prof Pankaj Sah, Queensland Brain Institute
Electrophysiological analyses of neurons in mouse mutant models of developmental disease

Students Graduated
PhD - Jenny Lackovic
Title “The role of Nedd4 family interacting protein 1 (Ndfip1) in neuronal survival following cerebral ischemia”
BSc Honours – Hui-Xuan Ng
Title “Positioning of interneurons during cortical development”
Grade: H1

EDITORIAL POSITIONS
Seong-Seng Tan
Journal of Neuroscience (USA)
Experimental Neurology
Journal of Anatomy
Anatomy & Cell Biology (Korea)
OUR GREATEST STRENGTH IS OUR COLLABORATIVE TRANSLATIONAL RESEARCH. MANY OF OUR DISCOVERIES WOULDN’T HAVE BEEN POSSIBLE WITHOUT THE TEAM’S COLLECTIVE ABILITIES. WE’VE LEARNED IT TAKES TIME FOR BASIC SCIENTISTS TO ‘GET INSIDE THE HEAD’ OF CLINICAL RESEARCHERS AND VICE VERSA.

PROF GRAEMLJ JACKSON, CO-DIVISION HEAD, EPILEPSY
EPILEPSY

ION CHANNELS AND HUMAN DISEASE

SNAPSHOT OF OUR STUDIES

Epilepsy is a debilitating and very common neurological disorder affecting some 2% of the population. Many of these patients are unable to control their seizures despite the development of new epilepsy medications. A deeper understanding of the neurobiology and physiology of epilepsy is needed in order to develop much needed new strategies for treatment or cure. It is now very apparent that our genes play a major role in determining whether we get epilepsy with current estimates suggesting that 70% of all epilepsies have a genetic basis. Our research is squarely aimed at developing a mechanistic understanding of the sequence of events that occur from gene mutation to behavioral seizure. To achieve this we employ a range of approaches that target the particular “brain scale” under investigation: from single molecule studies of ion channel function to synaptic biology, neural network function and whole animal behavior. By “connecting the dots” in this way, new understanding of the epilepsy syndromes will emerge that will create opportunities for diagnosis, prognosis and therapy.

RESEARCH HIGHLIGHT

Genetic epilepsy has the scope to impact a range of fundamental neurobiological functions and at present there are more genetic mutations than there are epilepsy syndromes. Part of our research is to search for points of convergence where multiple genetic pathways converge on a limited number of physiological pathways. In this way it may be possible to understand on what brain level a particular syndrome emerges. To this end we have completed a large study that showed that a particular part of a neuron may actually represent such a point of convergence and that dysfunction of this compartment, by a range of genetic mechanisms, may result in a more limited number of epilepsy syndromes. This has implications for therapy as it may identify a new opportunity for designing effective therapies.

SENIOR STAFF

Associate Professor Steven Petrou
Dr Christopher Reid
Professor James Wiley
Dr Alison Clarke
Dr Elena Gazina
Dr Ben Gu
Dr Marie Phillips
Dr Kay Richards
Dr Robert Richardson
Dr Evan Thomas
Dr Ernesto Vargas
Dr Verena Wimmer

OVERVIEW

Anti-epileptic drugs have significant shortcomings and almost a third of patients are unable to achieve adequate seizure control. At present, surgery is the main recourse for these patients, who often live for 10 or more years with untreatable epilepsy prior to surgical intervention. New therapeutics are urgently needed to not only treat patients that don’t respond to current anti-epileptic drugs but to also reduce the serious side effects of these medications.

Our laboratory has taken the approach that novel opportunities for therapeutic intervention will arise by; 1) the creation of syndrome-specific epilepsy models based on human genetic lesions and; 2) a detailed analysis of the fundamental mechanisms that underlie disease genesis and progression in these models.

To achieve these goals we employ a multidisciplinary approach that combines molecular biology, biophysics, computer modelling, single cell and brain slice electrophysiology, macro and micro histological digital imaging, EEG, unit recording and in vivo patch clamp in brains and behavioural analysis of mice. By studying the effects of epilepsy gene mutations at several levels of functional organisation, we can evaluate our models against the human conditions and then delve into the mechanisms of seizure genesis. We have strong commercial links so that discoveries in the lab can more readily benefit patients.
MAJOR RESEARCH PROJECTS

Development of syndrome specific models of human epilepsy

Syndrome specific models of epilepsy are the key to understanding the fundamental mechanisms of the disease and to the development of better therapeutic treatments. The goal of this project is to develop genetically modified animal models of specific human epilepsy syndromes. These studies begin with the function validation of epilepsy causing gene mutations found in patients in collaboration with a team led by Prof Samuel Berkovic. The majority of these mutations are found in a class of genes coding for ion channels and a range of cell biological methods. Once disease causing mutations are identified in human studies, mouse models are created that harbour the identical mutations. Validation of these models requires studies which worry scientists, the molecular, neuronal, network and whole animal level. We search for similarities in cellular behaviour and seizure activity between our mice and the patients with the mutation as this provides us with greater confidence that fundamental mechanisms of seizure genesis may be shared.

Our search for the mechanism of epileptogenesis requires the use of miniaturised single neuron recording devices for use in freely moving or anaesthetised mice. We couple these studies with EEG and behavioural tests as our markers of syndrome type.

We have developed two models which warrant further study in life. More work is currently under progress to study the possible developmental effects of this mutation. In the other model, we are characterising expression of the mutant gene in the CNS to determine the neuronal components that drive seizure phenotypes. Mutations lead to reduced expression in excitatory neurons (EMK1) and a separate line that express only in inhibitory neurons (DLX) are currently being bred with our conditional model and testing of seizure susceptibility is underway. We are also investigating whether HCN expression is altered in other seizure models to be tested using this approach. We are using this method to determine if changing HCN expression can produce or ‘rescue’ epilepsy. We have now validated the HCN shRNA tool in vitro, and in vivo studies are underway at how reducing the HCN1 channel changes neuronal network properties. Also, together with our collaborators we are the first to find a mutation in the HCN gene that may act to increase the susceptibility of an individual’s likelihood of getting epilepsy. Using a medium-throughput electrophysiological approach we have demonstrated that this variant changes channel function. We are using this validated assay to determine if other HCN variants alter channel function. The research aim is to determine the impact of this mutation on channel function and how this may change seizure thresholds.

Hyperpolarisation activated cation current in familial and acquired epilepsy

Christopher Reid, Alison Marie Philips, Verena Wimmer, Steven Petrov

There is an obvious clinical need for improved treatments for epilepsy, but only will be realised by a better understanding of epilepsy. A key goal in our understanding of this disease is to define the molecular mechanisms involved in the epileptogenic process and attribute functional consequences to them. One candidate class of protein is the hyperpolarisation activated cation current (HCN). We are currently investigating the heterogeneity of the GABA receptor gene and wish to understand how two different mutations in the same gene can cause two very different phenotypes. We have made a mouse model with the mutation that gives rise to the less severe forms of epilepsy and we are now making a second mouse model with the GABA receptor mutation that gives rise to SMEI. Future work on these mouse models will help us identify the pathways leading to different types of epilepsy. Identification of the point of physiological divergence that results in kindling seizures may yield important clues as to the fundamental mechanism of epileptogenesis in these different syndromes.

Sodium channels splicing and seizure susceptibility

Elena Gazza, Steven Petrov

Clinical data have identified connection between the SCN2A protein (a sodium channel protein) and childhood epilepsy. The SCN2A protein has 2 isoforms, neonatal and adult. Our data also show that when we are currently being bred with our conditional model and testing of seizure susceptibility is underway. With the creation of this novel model, we now have the ability to gain precise control over temporal and spatial aspects of epilepsy gene expression.

Dissecting the mechanisms of clinical heterogeneity in familial epilepsy

Verena Wimmer, Chris Reid, and Steven Petrov

The genetic basis of epilepsy is known to be highly complex. It displays genetic heterogeneity where different genes can cause the same epilepsy phenotype as well as clinical heterogeneity where the same gene can cause different epilepsy phenotypes. In the GABA receptor gene, two mutations have been found that result in a different phenotype. One mutation is seen in patients with childhood absence epilepsy with febrile seizures, which are relatively mild forms of epilepsy, and in one family a mutation in the same gene results in severe myoclonic epilepsy of infancy (SMEI) which is an extremely severe condition with a poor prognosis. We are interested in the heterogeneity of the GABA receptor gene and wish to understand how two different mutations in the same gene can cause two very different phenotypes. We have made a mouse model with the mutation that gives rise to the less severe forms of epilepsy and we are now making a second mouse model with the GABA receptor mutation that gives rise to SMEI. Future work on these mouse models will help us identify the pathways leading to different types of epilepsy. Identification of the point of physiological divergence that results in kindling seizures may yield important clues as to the fundamental mechanism of epileptogenesis in these different syndromes.

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Environmental influences of seizure susceptibility:

Chris Reid & Steven Petrov

Absence epilepsies are highly heritable, but it is well known that environmental effects such as decreased vigilance and voluntary hyperventilation may induce absence seizures. However, the usual physiological or environmental precipitants of absence seizures are not fully understood. There has been renewed interest in the relationship of glucose concentration to absence seizures with the discovery that there may be different generalized seizure types, occur in patients with impaired transport of glucose across the blood brain barrier. There is also a role for GLUT1 that encodes GLUT1, the major glucose transporter. These seizures occur in the classical severe GLUT1 encephalopathy, but also in milder cases that have otherwise typical idiopathic generalised epilepsy associated with absence mutations. Interestingly, patients with GLUT1 deficiency respond to oral glucose with significant improvement in both clinical seizures and electroencephalographic activity. Blood glucose levels fluctuate naturally and whether reductions in levels influence absence seizure threshold is unclear. The project is to investigate interneuron properties in an epileptic mouse model using immunohistochemical and electrophysiological techniques. Our laboratory has produced, and is characterising, the first transgenic (knock-in) mouse model carrying a human epilepsy mutation (R43Q) on the GABA receptor γ2 subunit (GABRG2/R43Q). GABAergic interneurons make up around 20% of neurons in the brain and are important in controlling the spread of seizures. Reducing glucose levels fluctuate naturally and whether reductions in levels influence absence seizure threshold is unclear. The project is to investigate interneuron properties in an epileptic mouse model using immunohistochemical and electrophysiological techniques. Our laboratory has produced, and is characterising, the first transgenic (knock-in) mouse model carrying a human epilepsy mutation (R43Q) on the GABA receptor γ2 subunit (GABRG2/R43Q). GABAergic interneurons make up around 20% of neurons in the brain and are important in controlling the spread of seizures. Our laboratory has produced, and is characterising, the first transgenic (knock-in) mouse model carrying a human epilepsy mutation (R43Q) on the GABA receptor γ2 subunit (GABRG2/R43Q). GABAergic interneurons make up around 20% of neurons in the brain and are important in controlling the spread of seizures. Our laboratory has produced, and is characterising, the first transgenic (knock-in) mouse model carrying a human epilepsy mutation (R43Q) on the GABA receptor γ2 subunit (GABRG2/R43Q). GABAergic interneurons make up around 20% of neurons in the brain and are important in controlling the spread of seizures.
incorporating this mutation developed by our group recapitulated this epileptic condition, confirming the role of the mutation results in impaired GABAA receptor trafficking, which results in a deficit in cortical inhibition. Our work now focuses on relevant neuronal networks and in vivo in order to relate these molecular deficits with the clinical pathology of the disease. In families harbouring the GABA receptor 2 (R43Q) mutation, the presence of the mutation is linked to Childhood Absence Epilepsy. However, there is a strong clinical heterogeneity and not all subjects with the mutation exhibit an epileptic phenotype. In order to investigate the influence of genetic background on the final phenotype of this mutation, the mutation was bred into different genetic backgrounds; the C57/B6 and the DBA/2J mice strains. The genetic background of the animal harbouring the mutation also exerted a strong influence on the epileptic phenotype. In the C57/ B6 mice only exhibited 4-10 seizures per hour with each seizure lasting only 0.5–1s. In contrast, mutant mice in the DBA/2J background exhibited 40-150 seizures per hour with each seizure lasting 1-3s. To investigate the physiological difference between these two strains responsible for contrasting seizure susceptibility, a technique called Patch Clamp recording was used to record synaptic signals in brain slices to characterise how different cells communicate with each other. We focused our efforts on the cortex and the thalamus, two large brain regions known to play an important role in Absence epilepsy. Interestingly, the DBA strain showed a large increase in synaptic inhibition in the thalamus but not the cortex. Inhibition in the thalamus is essential in driving this region of the brain into synchronized epileptic activity. It is inherent difference between the mice strains may therefore be responsible for the different seizure severity they develop as a result of the mutation. This suggests that although a single mutation may be a primary cause for the development of epilepsy, other genetic factors strongly influence the final clinical outcome. We are taking a genetic approach to determining what differences exist between the C57 and DBA strains that define seizure susceptibility. DBA by C57 crosses that harbour the 2 (R43Q) mutation have varied seizure susceptibility potentially allowing us to map to genes of interest. An extension of this approach is currently being completed by our collaborator Prof. Wayne Frankel (Jacksons Laboratory, USA). Here the R43Q mutation is being bred into a range of different mice species and seizure susceptibility measured. Both approaches hope to discover new seizures modulating genes that will give us insight into the basis of clinical heterogeneity.

Connecting the dots from molecular lesion to network seizure in epilepsy

Evan Thomas, Steven Petrou

Recent advances in the genetics of epilepsy have led to the discovery of many mutations that cause seizure syndromes. Almost without exception these mutations are in ion channels – molecules that reside in the cell membrane and conduct current that is the basis of electrical signalling in the nervous system. In our laboratory we use a variety of techniques to understand how these molecules are different from those in humans without epilepsy. One of these is to artificially express these ion channels in model cell lines and measure the differences in the electrical properties compared to ion channels from healthy people. These differences are small and subtle and it is often difficult to see how they cause the brain to become prone to seizure. One way to make the casual connection between molecular deficit and changes in brain dynamics is through the use of computer models. Data collected from cell line studies can be converted to mathematical models of the ion channel in question. These models can then be incorporated into models of single neurons and used to predict differences in sensitivity of these neurons to interactions from other neurons. Then these neuron models can be incorporated in network models with thousands of neurons to try to understand what triggers seizures. Understanding why some brains are more liable to seizures than others will enable us to develop better treatments for seizure prevention and also help us to understand why brains become epileptic in the first place.

New methods for exploring complex models

Jordan Chevrette, Steven Petrou, Evan Thomas

Many diseases, including epilepsy, are caused by subtle biophysical changes in the response of proteins. Thus, in order for computational models to be predictive they must capture sufficient detail that these subtle changes are evident. This poses a new problem in that these models then have large numbers of parameters, many of which are not well established or not mutually consistent. New methods are needed to explore large computationally demanding models. To develop these methodologies, we are using a well respected thalamocortical model that already exists. We have taken the NWB model (an open source simulation package) port of this model and updated the model to include significant new findings published last 5 years. Briefly, the model consists of 13 neurons types from the cortex and thalamus, with realistic morphology and connectivity. The network models express a realistic complement of voltage and calcium sensitive ion channels, AMPA, NMDA, GABAA mediated synaptic conductances and gap junctions. While this is not a complete model of the cortex and thalamus, it contains the important components believed to play a significant role in the generation of epileptic seizures. Indeed, the original model and our updated model (from preliminary results) are able to display both normal oscillations and epileptic oscillations.

The goal of this project is to use techniques to intelligently explore the parameter space of the model and to use this data to explore how the model captures the range of seizure phenotypes. The model contains several thousand parameters describing neuron morphology, probabilities of connections between different neuron types, biophysical properties of ion channels, distribution of ion channels, and dynamics of postsynaptic responses. Some of these parameters are perturbed by the disease. Experimental studies indicate that epileptic oscillations are caused by deficits in inhibitory transmission in the cortex and changes in the distribution of inhibitory interneurons in the cortex and thus provide important clues as to which subset of parameters is important. Exactly which parameters are perturbed by the disease is unknown, but these data reduce the number of potentially important parameters to around 40. This parameter space will be explored using the Nimrod Toolkit (http://message.lab. monash.edu.au/nimrod) for workflow management of complex parametric experiments. This is a unique tool for working with parametrically complex models and we believe this is the first application of these techniques to questions in neuroscience. We will use the Nimrod/E Tools to create a fractional factorial design for the parameter space search. This works as follows: If N values of each parameter are to be tested then a brute force approach will require N^P runs to test every parameter combination. A factorial design is based on the idea of first testing single parameter changes, followed by two simultaneous parameter changes, then three and so on. Typically higher order interactions have negligible effect. In this way, the number of runs can be reduced to around 20,000 to examine 1, 2 and 3-way parameter interactions. Exploring a complete set of 4-way interactions is not computationally feasible, however data from this project will be used to determine if 4-way interactions are likely to be important and to specify a subset of interactions that may be worth pursuing.

Multi-modal imaging analysis of mouse models of human epilepsy syndromes

Kay Richards, Gary Egan, David Reutenus, Steven Petrou

How does a gene mutation, representing dysfunction at the molecular level, lead to altered whole brain function such as epilepsy? To begin to tackle this question we are utilising mouse models of inherited epilepsy syndromes, combined with multi-modal imaging. Our research goal is to identify macroscopic and microscopic structural changes in the brain which may result in the generation of seizures. Our methodology consists of three parts: 1) MR 2) Histology and 3) High resolution 3D brain reconstruction. Part 1 involves analysis of gross anatomical structure of whole mouse brain using magnetic resonance imaging (MRI). Evidence from human MRI studies of patients who carry the ion channel mutation linked to 3}
Altered temperature sensitivity of familial sodium channel mutations in epilepsy as a unifying mechanism forgenesis of febrile seizures

Evan Thomas, Steven Petrou

Generalised epilepsy with febrile seizures plus (GEFS+) type 1 is characterised by a C121W point mutation in the 1 subunit of the voltage gated sodium channel. People afflicted with this condition display seizures initiated by fever up to six years of age, and then display febrile or afebrile seizures into adulthood. Previous published studies using cell lines have shown that this sodium channel mutation alters the functionality of the channel. The aim of this project is to study the sodium channel’s kinetics properties with changes in temperature that correspond to both normal and fever body temperatures in humans. Data so far show changes in channel kinetics as well as differing temperature sensitivities of the mutated channel compared to the control channel. Preliminary data at fever temperature indicate that changes in temperature sensitivity may cause a higher susceptibility to seizures. Future work will entail action potential firing property studies of neurons in C121W mouse models as well as investigating a new technique called Dynamic Clamping that may enable action potential firing property mutations of mutated sodium channels to be understood in simple cultured cells such as human embryonic kidney (HEK292) cells instead of neurons.

Kinetic models of ion channel gating for gene environmental interactions

Evan Thomas, Steven Petrou

A growing area of interest is pharmacoresistance, with variation in the patient’s genome prevents the symptoms from being controlled with drugs. Some pharmacoresistance will be due to variation in ion channel responses to drugs, indeed this may be the same variation that caused the disease. Other environmental interactions, such as temperature and pH, are triggers of seizures in individuals with heightened sensitivity. In order to incorporate these effects into a predictive single neuron and network model, high quality mathematical models of the ion channels response to both endogenous and exogenous stimuli are needed. This is surprisingly difficult to do properly, even in the case of Hodgkin-Huxley models. The reasons are that gradient following algorithms suffer from two problems. Firstly, they are easily fooled by local minima which is a point in parameter space that only minimizes the error in a small region. Secondly, they handle discontinuities and singularities poorly, both of which are present in exponential based rate functions. To overcome these difficulties, a strategy is needed to develop a good first guess of the solution. The strategy used is to initially fit a Hodgkin-Huxley model, convert that to its right equivalent kinetic model, remove the relationship between rate functions and finally use a pattern search algorithm to perform the final fit.

High content high throughput analysis of gene variation in neurons

Evan Petrou, Evan Thomas, Samuel Benkovic

The aim of this study is to understand the functional consequences of genetic variation found in sodium channel alpha subunits in order to develop a deeper understanding of genetic risk in familial epilepsy. The recent explosion of variant data coming from patients with epilepsy has identified sodium channels, and in particular SCN1A, as a high level function and impact risk. Using this combined approach we hope to develop a more thorough and predictive understanding of the genetic risk for epilepsy.

Ion transport blockers as antivirals

Elena Gazina, Steven Petrou

We have demonstrated that a number of amiloride derivatives have antiviral activity in tissue culture against viruses belonging to two medically important genera of the Picornavirus family: coxsackievirus B3 (CVB3, causes myocarditis) and human rhinovirus 2 (HRV2, a common cold virus). The compounds were more effective against CVB3 than against HRV2. Our data suggested that the antiviral activity against coxsackievirus B3 was due to inhibition of viral RNA replication, with the antiviral target likely to be a viral protein. The virus release from infected cells was also inhibited. We have generated amiloride resistant CVB3 mutants by passing an in-frame deletion in the presence of amiloride. We are currently sequencing the genomes of the resistant virus isolates to identify mutation(s) causing resistance, and thus identify the viral protein(s) targeted by amiloride and its derivatives. Once the protein is identified, the studies will be conducted to elucidate the detailed mechanism of antiviral activity. We have analysed anti-CVBS3 activity and cytotoxicity of 29 compounds belonging to a large group of chemicals, sulphonamides, which exhibit antiviral activity. The resulting structure-activity data were used to delineate medicinal chemistry strategy aimed at improving the antiviral activity and cytotoxicity of the compounds.

The Role of PZK7 Receptors in Neuroinflammation and Degeneration

Ben Gu, Steven Petrou, James Wiley

The P2X7 receptor is a ligand-gated cation channel highly expressed on monocytes, macrophages and microglial cells which play an important role in the inflammatory effects of extracellular ATP. Prolonged exposure of these cells to ligands leads to channel dilatation and massive K+ efflux which in turn is a co-stimulus for the secretion of cytokines, interleukin-1 and interleukin-18 which perpetuates inflammation. Whole animal evidence suggests that P2X7 receptors on microglial cells in the brain and spinal cord play a major role in amplifying the damage due to inflammatory or traumatic lesions. Several polymorphic variants of the P2X7 gene are associated with conditions linked with the permeability responses of the receptor and these polymorphic variants have been associated with greater inflammation in such conditions as multiple sclerosis, Sjogren’s Syndrome. Other reports have associated bipolar disorder with a gain of function polymorphism in the P2X7 gene which is consistent with the current concept of bipolar disorder as an ion channelopathy. While there is a clear role for P2X7 in inflammation, emerging evidence suggests a second unsupervised function for P2X7 in innate immunity. Thus macrophages or other cell types transfected with P2X7 can recognize and engulf foreign particles, gram positive and negative bacteria and even lymphocytes. This phagocytic function of P2X7 is only observed in the absence of ATP and allows for the removal of foreign and apoptotic debris in the absence of inflammation. This unique property of the P2X7 receptors may be attributed to an array of anti-parallel beta-sheeted sheets in the extracellular domain providing an extensive interacting surface with the particle to be engulfed. However, the significance for the nervous system of these findings remains to be elucidated.

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MAJOR COLLABORATIVE LINKS

National
Prof Sam Berkovic, Austin Hospital Analysis of gene mutations in familial epilepsy
Prof John Mulley, Women’s and Children’s Hospital, Adelaide Validation of epilepsy causing mutations
Prof David Reuters, Monash Medical Centre Construction of a 3D probability map of the mouse brain
Prof Graeme Jackson, Florey Neuroscience Institutes In vivo imaging of animal models of epilepsy
Prof Stan Skalidis, University of Melbourne Novel sensors for measuring brain and neuronal function
Prof Lloyd Hollenberg, University of Melbourne Quantum decoherence methods for optical imaging of neuronal activity

International
Prof Matt Jones, University of Madison, Wisconsin, USA
Biophysical analysis of GABAA receptor function in brain slices in the R434 mouse model of generalised epilepsy
Prof Kai Kaia, University of Helsinki
pH dependence of axon initial segment function
Prof Taille Baram, University of California, Irvine, USA
Analysis of if3-mutants in epilepsy
Prof Holger Lerche, University of Ulm, Germany
Functional analysis of genetic variation in IGE
Prof Wayne Frankel, Jackson Laboratories, Bar Harbour, USA
Genetic and functional analysis of epilepsy in the mouse model
Prof Heinz Beck, University of Bonn
Medical Centre, Germany
Axon initial segment dysfunction in epilepsy
PUBLICATIONS


THE MELBOURNE BRAIN CENTRE – AT HEIDELBERG AND PARKVILLE
There is a relatively high prevalence of epilepsy in the general population (it has been estimated that about 1 in every 140 people suffer from epilepsy). Despite this, the mechanisms of seizure generation remain unclear. A significant proportion of people with continuing seizures are resistant to antiepileptic drugs; no viable treatment options exist for some patients.

Using combinations of neuroimaging techniques at the Florey’s Heidelberg campus, we have made significant breakthroughs to help us understand the mechanisms of seizure generation. One of the keys helping us unlock these secrets is functional magnetic resonance imaging (fMRI) and simultaneously acquired electroencephalography (EEG). The method that allows this technology to be used simultaneously has been developed by the neuroimaging team, and is the subject of a current patent application. Structural and physiological MRI methods are also being developed and applied to investigate changes in brain networks that occur in epilepsy.
What is the sequence of events that lead to a clinical seizure? Another promising approach is to investigate neurophysiological changes immediately before the onset of a seizure. It is known that changes in brain activity can occur minutes (or even hours) prior to clinical symptoms. We have successfully used MRI to demonstrate that these changes in activity may continue many minutes prior to seizure onset. Our results indicate that there are changes in the lead-up to a seizure in areas that include (but are not limited to) the presumed seizure focus. The relationship of these signal changes to seizure outcomes might help to localise the epileptic network and ‘seizure focus’ for surgical treatment is a complex question that we are actively pursuing.

Brain networks
Richard Masterton, David Abbott, John Archer, Graeme Jackson
fMRI was first demonstrated in humans in the early 1990’s, and has most often been used to identify focal regions of the brain associated with a particular cognitive function. However, fMRI can also reveal changes in the networks of activity. Studies of so-called “functional connectivity” are helping us map complex networks of interacting brain regions in healthy people and in people with epilepsy. We are interested both in the technical aspects of the method, as well as its potential application in clinical research.

Building global models of the brain’s functional networks
Richard Masterton, Graeme Jackson
At a global level, the brain is a single highly interconnected network that integrates separate functions distributed across different specialised brain regions and sub-networks. Normal brain function requires synchronisation and segregation of activities within different parts of this network. However, in this process it is thought to be associated with many neurological conditions such as epilepsy.

We are using functional connectivity to probe the brain’s functional organization and build complex models of its global network topology based upon observed synchronisation and interaction between brain regions. We are developing new tools for creating and analyzing these large network topologies. We have conducted work that demonstrates that the brain’s network topology changes during maturation from a centralized to a distributed organization. Continuing work is to investigate how different epilepsy syndromes - which often have specific age-dependent onset and remission phases - may interact with or be affected by these brain network changes.

Measuring changes in cortical excitability in patients with epilepsy
Radvica Badayev, Richard Macdonell, Samuel Berkovic, Graeme Jackson
At a biological level, epilepsy can be understood as a network of abnormally communicating brain areas, with a net increase in brain cell activity. This theory of increased excitability in epilepsy is mainly derived from animal studies and experimental data, and was until recently unproven in humans because of a lack of the means to study this non-invasively. Transcranial magnetic stimulation (TMS) is a safe and non-invasive tool that can be used to investigate the activity of brain cells and provide inferences on the excitability of the brain in humans during the resting state (between seizures). It is an excellent tool used to measure human brain cell excitability and the various circuits contributing to this. Most of the previous TMS studies in epilepsy showed conflicting results because they investigated patients with chronic epilepsy who were taking many anti-epileptic drugs. By studying patients with new onset epilepsy who were not taking medication, we found that there is an alteration in brain cell activity associated with epilepsy with a net increase in brain cell excitability. The pattern of this disturbance depended on the type of epilepsy, as it was on both sides in generalized epilepsies, and confined to one side of the brain in focal epilepsies. We were also able to demonstrate that there was a cycle of change in excitability, as there was an additional increase in excitability for 24 hours after a seizure before it returned to baseline levels. Excitability was also influenced by many factors known to provoke seizures. Our studies showed direct evidence of increased excitability early in the morning, pre-menstrual phase and sleep deprivation. When the patients started anti-epileptic medication, baseline excitability normalized only if patients stopped having seizures. Patients who continued to have seizures showed progressive alterations in brain excitability. These findings provide novel human insights into the mechanisms underlying epilepsy. Furthermore they are currently being translated into clinical practice, both in the diagnosis of new onset seizures and also to monitor responsiveness to treatment.
school children, and is characterised by focal epileptic discharges (spikes) without frequent seizures or apparent brain pathology. Children with BECTS also experience cognitive difficulties, but the relationship between the cognitive problems and the ‘spikes’ was previously unknown. Participants in our study completed a battery of neuropsychological tests assessing intelligence, receptive and expressive language, primary memory, new learning, academic attainment, and executive function. In addition, neuroimaging measures including structural, functional (including simultaneous EEG/fMRI) and physiological (T2 relaxometry) were acquired. Last year we published our observation that language-related activation measured with fMRI is less lateralized to the left hemisphere in anterior brain regions in patients relative to the control group. This is consistent with our observation of decreased performance in the BECTS group compared to the control group on the neuropsychological measure most dependent on the integrity of anterior aspects of the language axis, namely; sentence production. This year we published an analysis of the simultaneous EEG/fMRI study of BECTS patients, which revealed a focal region of increased brain activation associated with the patients’ centro-temporal spikes (CTS). This was localised to the post-central gyrus, clearly posterior to the central sulcus. Furthermore, we found that the average fMRI response to CTS had marked differences to the canonical haemodynamic response function (HRF) that is typically assumed for analysis of the group-average decrease (Figure 3). This has important implications for analysis methodology for EEG-fMRI studies. A localisation analysis using the group-average response provided increased sensitivity and specificity compared to the canonical HRF.

How does disease affect brain function in absence epilepsy? Patrick Carney, Richard Masterton, Simon Harvey, Ingrid Schiffer, Sam Benkovic, Graeme Jackson. Another example of our endophenotyping studies is that of absence epilepsy. We used EEG-fMRI to study epileptiform activity in a cohort of untreated children with typical absence seizures (AS). We were able to identify a core network of structures involved in generalized epileptiform activity consisting of increased activity in the thalamus and decreased activity in the lateral and mesial parietal lobe, caudate nuclei, and additionally the brainstem reticular formation (Figure 4). Furthermore, we identified changes in parietal BOLD signal (but not in other cortical regions) which precede the onset of epileptiform activity. suggesting the parietal cortex has a role in the initiation of epileptiform activity in this patient cohort.

Functional organisation of language in children with focal epilepsy Regula Everts*, Simon Harvey*, Leasha Lillywhite*, Jacques Wernnal*, David Abbott, Linda Gonzalez*, Michael Kean*, Graeme D. Jackson and Vicki Andersson* (Royal Children’s Hospital, Melbourne). We have used fMRI to map language activity in a group of children with epilepsy and a group of healthy children (some studied at the Florey’s Heidelberg campus, others at the Royal Children’s Hospital in Melbourne). We found a correlation between language lateralization (i.e. the side of the brain most involved in language function) and verbal memory performance in patients with left-sided epilepsy in the three main areas of the brain responsible for language. Bilateral or right-sided language lateralization was associated with better verbal memory performance. Verbal memory performance made the largest contribution to language lateralization, whereas handedness and side of seizures did not contribute to the variance in language lateralization. This finding reflects the association between neocortical language and hippocampal memory regions in patients with left-sided epilepsy. Atypical language lateralization is advantageous for verbal memory performance, presumably a result of transfer of verbal memory function. In children with focal epilepsy, verbal memory performance provides a better idea of language lateralization, than handedness and side of epilepsy.

Analysis methods development: language lateralisation David Abbott, Tony Waten, Leasha Lillywhite, Graeme Jackson. A difficulty that arose in our studies of language lateralisation was the degree of variability observed in the quantitative lateralisation index as a function of the statistical threshold chosen. We therefore undertook a development project that aimed to test the robustness of adaptive thresholding of fMRI data to yield a fixed number of active voxels, and to develop a largely threshold-independent method of assessing when individual patients have statistically atypical language lateralization. Simulated data and real fMRI data in healthy controls and selected epilepsy patients performing a verbal fluency language fMRI task were used. Dependence of laterality on the thresholding method was demonstrated for simulated and real data. Simulated data were used to test the hypothesis that thresholding based upon a fixed number of active voxels would yield a laterality index that was more stable across a range of signal strengths (study power) compared to thresholding at a fixed p-value. This stability allowed development of a method comparing an individual to a group of controls across a wide range of thresholds, providing a robust indication of atypical lateralization that is more objective than conventional methods. Thirty healthy controls were used as normative data for the threshold-independent method. An individual with atypical language lateralization compared to the control distribution is shown in Figure 5. The method can also be used more generally to assess relative regional distribution of activity in other neuroimaging paradigms; for example, one can apply it to the assessment of lateralisation of activation in a memory task, or to the assessment of anterior-posterior distribution rather than laterality.
How does disease affect the structure of the brain?
Heath Pardoe, David Abbott, Gaby Pell, Graeme Jackson.

Structural neuroimaging using MRI is an important diagnostic and research tool, non-invasively providing a three-dimensional view of the internal structure of the living human brain. In some brain diseases there are obvious structural abnormalities that can be seen using MRI, but in many other diseases we are discovering that there are subtle changes in brain structure that cannot be seen by eye.

Researchers at the Florey's Heidelberg campus are using MRI to investigate how brain structures may be subtly altered in people with conditions such as obstructive sleep apnoea and epilepsy, to assist in understanding and better treating these conditions. For example, a deep-brain structure often damaged in epilepsy is the hippocampus, and the volume of that structure in a patient compared to healthy controls can be a phenotypic marker of disease.

The outcomes of our structural imaging research will enable the scientific community to map patterns of structural change in brain tissue, and determine the relationship between these structural changes and altered function of pathological tissue.

Is obstructive sleep apnoea associated with changes in brain morphology?
Heath Pardoe, David Abbott, Gaby Pell, Fenghal O’Donoghue (Austin Health), Melinda Jackson (Austin Health), Mary Morrell (Imperial College, UK), Doug Garfield (Keele University, UK), Graeme Jackson.

Many of the neuroimaging methods developed and applied by scientists in the Epilepsy Imaging group are also useful for studies of other conditions. Collaborative projects have therefore been established that leverage the expertise of our group to help tackle other questions in neuroscience. Some, such as investigating the mechanisms of recovery after stroke, are collaborations with other Florey divisions, and more detail can therefore be found elsewhere in this research report. Others are external collaborations with universities and other research institutes. One such collaboration is in the area of obstructive sleep apnoea. This is a disorder characterized by obstructed breathing during sleep. The disorder causes intermittent hypoxia and frequent arousals from sleep, which can have a considerable negative impact in lifestyle. Moderate sleep apnoea has been estimated to affect up to 24 percent of middle-aged men. Although there is a well-recognized link between obstructive sleep apnoea and cognitive dysfunction, there has been considerable debate about the mechanism linking these conditions. A collaborative study was established between our group, the department of Respiratory and Sleep Medicine at the Austin Hospital, and the Sleep and Ventilation unit at the Royal Brompton Hospital in the UK. We used an image analysis method known as voxel-based morphometry to investigate changes in brain structure associated with obstructive sleep apnoea. The voxel-based approach was modified (using an approach pioneered by us to allow for comparison of MRI-data acquired from multiple imaging centres. The results of the study suggested that the structure of the cerebellum can be affected in people with obstructive sleep apnoea.

Neuroanatomical changes in childhood-onset epilepsy
Heath Pardoe, Gaby Pell, David Abbott, Anne Berg (Northern Illinois University), Graeme Jackson.

The prognosis for childhood-onset epilepsy is complex. Many patients with childhood-onset epilepsy stop having seizures for an extended period, only to have seizure activity re-occur several years later, which then requires surgical treatment. These cases are rarely imaged early in the course of their epilepsy, so little is known about which ones may be assisted by early intervention to prevent the re-occurrence of seizures.

In this study, we are using advanced image analysis techniques to identify neuroanatomical changes in over 300 people with childhood-onset epilepsy. The use of quantitative analysis of MRI-based neuroimaging data will help to determine how changes in brain structure related to epilepsy affect social, educational and health-related outcomes in these patients.

Image analysis methods utilised in this study include:
- Voxel-based morphometry
- T2-relaxometry
- Cortical thickness analysis

Our involvement in such a unique large-scale study of childhood epilepsy provides valuable insight into the mechanisms by which epilepsy can affect the quality of life of people burdened with this disease.

Quantitative imaging of cortical abnormalities in extratemporal epilepsy
Heath Pardoe, Graeme Jackson.

Epilepsy is often associated with abnormalities in cortical structure. The identification of abnormal cortex is important in the pre-surgical assessment of intractable epilepsy patients because the chances of seizure freedom following surgical resection are substantially higher if a lesion has been identified on neuroimaging. In this project, we developed a method to identify cortical abnormalities in patients with extratemporal epilepsy using cortical thickness analysis of structural MRI, alignment of the structural MRI scans using high-dimensional non-rigid registration, and statistical analysis to objectively identify cortical abnormalities in individuals. This method was successfully used to objectively identify cortical abnormalities such as focal cortical dysplasias and glomas in epilepsy patients.
PUBLICATIONS


PROFESSOR INGRID SCHEFFER has won a coveted global award for work that has changed the way the world understands epilepsy. Her triumph... as Asia-Pacific L’Oreal-UNESCO Women in Science 2012 Laureate, honours her ground-breaking genetic research into many forms of genetic epilepsy.

MICHAEL SHORT, THE AGE, DECEMBER 5, 2011
The Genomic Disorders Research Centre was formed to lead the world in research on genetic mutations and their effect on human well-being. It was the first and remains the only centre to focus on gene mutation, its cause, documentation, collection and consequences. The centre coordinates national and international activities such as the Human Variome Project, along with courses, workshops, and the high profile genetics journal Human Mutation.

The Human Variome Project is the global community effort to collect, curate and make accessible information on all genetic variations. The project's consortium of researchers and healthcare professionals works to establish and maintain the standards, systems and infrastructure necessary for the routine sharing of genetic variation information.

One of the major initiatives of the Human Variome Project is its neurogenetics consortium which collects genetic data implicated in many neurological disorders.

The Human Variome Project consortium continues to grow. Twelve countries have now joined the project as country nodes: Austria, Australia, Belgium, China, Cyprus, Egypt, Greece, Kuwait, Malaysia, Nepal, Spain and Vietnam.

The project has also welcomed its first gene/disease specific database partner: the International Society for Gastrointestinal Hereditary Tumours. This new partnership solidifies the ongoing collaboration between the project and InSiGHT.

During 2011, the centre continued work on developing software and systems for the Human Variome Project Australian node. This work is funded by a federal government national e-architecture taskforce grant scheme. The project will enable gene variation data to be collected from Australian laboratories, allowing enhanced diagnostic abilities for Australian clinicians treating patients with inherited cancers and other debilitating disorders. It is intended to form a model for data collection elsewhere.

In 2011, the centre held the Human Variome Project Beijing meeting, continuing the valuable relationship between the project and China.

The centre also organised the International Mutation Detection meeting in Santorini and attended meetings in Amsterdam, Montreal and Dubai to speak about the Human Variome Project.

Research assistants, Heidi Ho and Kate Sidon, at work in the new microscopy suite at Heidelberg.
The Advanced MRI Development physics team at the Florey Heidelberg campus has an international reputation for innovative methodological development in a number of areas of magnetic resonance physics and of MR signal processing. One of the principal interests of this team is translational research, with the aim of applying new methods to important clinical and neuroscientific problems, in particular in the field of epilepsy (see Major Program in Epilepsy Imaging section). Significant breakthroughs have been achieved in the field of diffusion MRI and perfusion MRI, and many ongoing projects are developing these strengths further.

**SENIOR STAFF**

- Prof Alan Connelly
- Dr Fernando Calamante
- Dr Donald Tournier
- Dr Lisa Willats
- Dr Xiaoyun Liang

Shawna Farquharson, our chief research radiographer, runs the two ‘research dedicated’ 3Tesla MRI scanners on the Florey’s Heidelberg campus. The state-of-the-art MRI facility is used by scientists across Australia to perform world-leading clinical neuroscientific research and technological development.
MAJOR RESEARCH PROJECTS

Diffusion MRI: How can we measure structural connectivity in the brain?
Donald Tournier, Fernando Calamante, Alan Connelly

Diffusion MRI is an imaging technique that is unique in its ability to probe tissue microarchitecture at the cellular level non-invasively. It is increasingly used to investigate brain white matter and its disorders, providing a wealth of important information that cannot be obtained by any other method. White matter consists of myelinated axonal fibres that connect cortical regions, where the processing of information takes place. These connections are essential for normal brain function.

White matter abnormalities have been implicated in many disease states, including multiple sclerosis, stroke, epilepsy, tumours, dementia, and in a range of mental health disorders (e.g. schizophrenia). The pathophysiological basis of many of these disorders is thought to be related to abnormalities in the structural connections between different areas of the brain. Furthermore, knowledge of white matter organisation is essential for neurosurgery, to ensure the preservation of the patient’s most important functions. There is therefore enormous interest from neuroscientists, psychiatrists and neurosurgeons in using this technique.

One important aspect of our research is the development of improved acquisition and processing methods for diffusion MRI, with special emphasis in its role to study brain connectivity.

Inferring white matter connectivity using diffusion-weighted imaging

Diffusion-weighted MRI is sensitive to the microscopic motion or diffusion of water molecules in the brain along a given direction. In the white matter, which consists of tightly packed bundles of neuronal axons, the regular arrangement of fibres introduces a directional dependence of the image intensity (i.e. anisotropy). This information can be used by the so-called ‘‘fibre-tracking’’ algorithms, to track the path of the fibres and to infer the structural connectivity. However, the model currently widely used (known as the diffusion tensor model) is invalid in regions containing multiple white matter tracts that cross or pass very close to each other. This is a serious problem since, as has been shown recently through collaborative work between our team and colleagues in Europe, around 90% of image voxels within white matter contain more than one fibre population. This can lead the fibre-tracking algorithm either to fail to identify existing connections, or to infer connections that do not exist in reality.

We have developed an alternative analysis method to address this limitation, known as the spherical deconvolution technique. We have demonstrated that this approach provides fibre orientation distribution (FOD) estimates that are robust to the presence of multiple orientations within a voxel, and are not based on any assumptions regarding how many fibre orientations are present. The particular spherical deconvolution approach that we have developed (known as constrained spherical deconvolution or CSD) permits the use of super-resolution, whereby more FOD parameters are estimated than were actually measured, improving the angular resolution of the results. The method provides very well defined fibre orientation estimates (Figure 1), which enable tractography algorithms to be able to track reliably through crossing fibre regions. Used in conjunction with advanced tracking algorithms, in particular those which adopt a probabilistic approach, the CSD method can generate either whole brain fibre maps (Figure 2) or images of individual fibre bundles of interest (Figure 3). By minimising the confounding effects of crossing fibres, the CSD method provides an important tool for neuroscientific and clinical investigations of brain connectivity. During 2009, the software that we developed both to perform spherical deconvolution (SD), and to perform in vivo white matter fibre tracking using the fibre orientations generated by SD, has been available via the BRI website as a software package (MRtrix) that has been downloaded widely (>4300 times) by researchers working in this field internationally.

Perfusion MRI: How can we measure cerebral blood supply?
Fernando Calamante, Lisa Willats, Xiaoyun Liang, Alan Connelly

Perfusion MRI is a non-invasive imaging technique for measuring cerebral perfusion (blood delivery to brain tissue per unit time). Blood delivers oxygen and nutrients to the tissue, which are necessary for cellular metabolism. The survival of the brain is dependent on a continuous and adequate supply of blood, and failure of the cerebral circulation can result in cell death. Similarly, some clinical conditions are associated with a hyperperfusion status (such as epilepsy and tumours) due to their increased energy demand. For these reasons, the ability to measure perfusion accurately, noninvasively, and with good spatial resolution would offer the chance to identify and characterise abnormal tissue in many clinical conditions.

A significant expansion in the availability of MRI scanners has taken place in the last decade, and perfusion MRI has become an important diagnostic technique. Every major MRI scanner manufacturer provides imaging sequences for perfusion MRI, but their product analytical software is still somewhat rudimentary. Improved methods to quantify perfusion and characterise the vascular networks in the brain is an important aspect of research at the BRI.
Brain perfusion quantification in patients with stroke

Bolus-tracking MRI is the most commonly used perfusion MRI technique in clinical studies. It involves injection of a bolus of contrast agent in the arm, which can be used to measure blood flow in the brain. Perfusion quantification requires measurement of the arterial input function (AIF), which describes the input of contrast agent to the tissue. Although this function can vary throughout the brain, a single (i.e., global) AIF is commonly used in practice. However, the presence of vascular abnormalities (e.g., arterial stenosis, occlusion, or collateral supply) may cause distortions in the bolus (delay and dispersion), which we have shown can lead to severe local underestimations in perfusion quantification.

One solution to this delay and dispersion problem involves estimation of a local AIF from a small vessel closer to the tissue of interest. Although this can vary throughout the brain, a single (i.e., global) AIF is commonly used in practice. However, the presence of vascular abnormalities (e.g., arterial stenosis, occlusion, or collateral supply) may cause distortions in the bolus (delay and dispersion), which we have shown can lead to severe local underestimations in perfusion quantification.

We have recently developed a modified 3-dimensional GRASE (gradient and spin-echo) ASL method to allow imaging of the whole-brain (which is essential for a full characterization of the networks in the brain). We evaluated this new method to study resting state functional networks in a group of healthy subjects (see figure 5).
What is the blood flow pattern in the brain vasculature?

Another important aspect of understanding cerebral perfusion is to determine the arterial origin for the blood supplied to the different regions of the brain, i.e. the vascular network. This information, known as the cerebral arterial territories, can play a key role in the management of stroke patients in the differential diagnosis of haemodynamic border zone infarction and thromboembolic ischemia. However, individual arterial territories are difficult to identify in vivo, and interpretation of acute stroke subtype and underlying pathogenesis, based on the topographic patterns alone, is often inaccurate. Therefore, there is a need for the development of new techniques for the mapping of arterial territories. The currently limited understanding of the collateral circulation in the human brain can be greatly enhanced through the development of vascular models, providing the basis for future therapeutic and prognostic applications.

As part of a collaboration with Dr. J.R. Cebra (George Mason University, USA) and Dr Roland Bammer (Stanford University, USA), we have developed a novel method to create a subject-specific vascular model, which is based on combining high-resolution MR images and MR angiography with computational fluid dynamic modeling (Figure 4). These vascular models can greatly enhance the current limited understanding of the collateral circulation in the human brain, providing the basis for future therapeutic and prognostic applications.

Track-density imaging (TDI): super-resolution white matter imaging using whole-brain track-density mapping

Fernando Calamante, Donald Tournier, Graeme Jackson, and Alan Connelly (FNI)

Neuroimaging advances have given rise to major progress in neurosciences and neurology, as ever more subtle and specific imaging methods reveal new aspects of the brain. One major limitation of current methods is the spatial scale of the information available. We have developed an approach to gain spatial resolution using post-processing methods based on diffusion MRI fibre-tracking, to reveal structures beyond the resolution of the acquired imaging voxel; we termed such a method super-resolution track-density imaging (TDI) (Figures 7 and 8). A major unmet challenge in imaging is the identification of abnormalities in white matter as a cause of illness; super-resolution TDI is shown to produce high-quality white matter images, with high spatial resolution and outstanding anatomical contrast. A unique property of these maps is demonstrated: their spatial resolution and signal-to-noise ratio can be tailored depending on the chosen image resolution and total number of fibre-tracks generated. Super-resolution TDI should greatly enhance the study of white matter in disorders of the brain.

As part of a collaboration with the Max Planck Institute for Human Cognitive and Brain Sciences (Leipzig, Germany), we used high-resolution diffusion MRI data acquired at 7 Tesla to validate the super-resolution properties of this novel technique. More recently, we assessed the high anatomical contrast of the TDI maps by comparing them to histological sections (myelin and Nissl staining) from ex vivo mouse data (Figure 9); this study was part of a collaboration with The University of Queensland and Neuroscience Research Australia, using ultra-high field 16.4 Tesla MRI data.

ADDITIONAL RESEARCH PROJECTS

Development of a new fibre tracking algorithm for visualising white matter fibre tracts in the brain

Alan Connelly, Fernando Calamante, Donald Tournier (FNI), Robert Smith (PhD student)

Whole brain tracking datasets (consisting of 1 million tracks or more) present a significant problem in terms of their size, to the extent that no current fibre-track clustering algorithm can come close to dealing with such large amounts of data. It is desirable therefore to develop a method by which such large datasets can be clustered into biologically meaningful fibre bundles. We are undertaking to find a solution to this problem, capable of automated clustering of very large probabilistic track data sets at any chosen cluster scale. This would provide an invaluable tool for the investigation of structural connectivity within the abnormal brain.
will benefit group based clinical and neuroscience studies, as well as automatic atlas based anatomical labelling, which are increasingly important application areas for diffusion MRI.

Identifying brain damage underlying speech deficits in children following traumatic brain injury: A new model of diagnosis and prognosis

Alan Connelly, Donald Tourmier (FNI), Angela Morgan, Sheena Reilly, Vicki Anderson (MCR), Frederique Liegeois (University College London)

Our aim is to link state-of-the-art MRI findings to quantitative speech data to determine the association between regions of brain damage and speech disorder-post TBI; examine neural mechanisms controlling speech prognosis by determining regions of brain damage associated with persistent vs recovered speech; and develop a world-first speech diagnostic system for children with speech disorder-post TBI.

Physiological significance of the Time-to-Maximum (Tmax) parameter in perfusion MRI

Fernando Calamante, Alan Connelly (FNI), Søren Christensen, Patricia Desmond, Stephen Davis (Royal Melbourne Hospital), and Leif Østergaard (Århus University Hospital, Denmark)

Many perfusion-related MRI parameters are used to investigate the so-called penumbra in stroke (i.e. a region of hypoperfused tissue surrounding an infarct core that is at risk of proceeding to infarction). Although time-to-maximum (Tmax) of the residue function has been suggested as a very promising parameter, its physiological meaning and sensitivity to experimental conditions are not well-understood.

This study will use simulations to further our understanding of the practical meaning of Tmax, including its dependence on such conditions as delay, dispersion, and mean transit time. Our aim is to provide recommendations for its use in clinical investigations independent of the model used (e.g. Q-ball or CSD). More accurate spatial normalisation will benefit group based clinical and neuroscience studies, as well as automatic atlas based anatomical labelling, which are increasingly important application areas for diffusion MRI.
To apply super-resolution track-density imaging methods for the analysis of the MR perfusion data from patients with stroke.

Dr. Iven Mareels, Prof. Lex Doyle, Prof Stephen Davis, Dr. Angela Morgan, Dr. David Thomas, and Dr. Alfred Anwander

To develop improved perfusion MRI methods for the analysis of the MR perfusion data from patients with stroke.

Dr. David Thomas

University of London, UK.

Development of improved method of quantifying cerebral blood flow using arterial spin labelling.

Dr. Chin-Po Lin

National Yang-Ming University, Taipei, Taiwan.

To investigate MRI methods to identify white matter fibre orientations in the brain using experimental water phantom.

Dr. Robin Heidemann and Dr. Alfred Anwander

Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany.

To validate super-resolution track-density imaging methods.

Prof. Zang-Hee Cho

Neuroscience Research Institute, Gachon University of Medicine and Science, Incheon, Korea.

To apply super-resolution track-density imaging methods as a tool for the construction of a brain atlas.

Prof. Geoffrey A. Donnan and A/Prof Helen Dewey, (FNI).

Characterization of the penumbra in patients with acute stroke using advanced perfusion MRI techniques.

Dr Amy Brodtmann, (FNI)

To establish whether arterial stenoses can cause cortical remodelling within brain regions supplied by the affected vessel.

Prof. Sheena Reilly, Dr. Angela Morgan

Murdoch Children’s Research Institute.

To determine the structural-functional abnormalities in children with developmental language disorders.

Dr. Mark Mackay

Murdoch Children’s Research Institute.

Characterisation of the ischaemic penumbra in children with stroke.

Prof Stephen Davis, Dr Patricia Desmond, Dr Soren Christensen

Royal Melbourne Hospital.

To develop improved perfusion MRI methods for the analysis of the MR perfusion data from patients with stroke.

Prof. Iven Mareels, Dr. Leigh Johnston

Department of Electrical and Electronic Engineering, University of Melbourne.

Development of improved methods of measuring white matter connectivity using diffusion MRI.

A/Prof. Gavin Fabinyi, Austin Health

Investigation of the efficacy of diffusion based MRI white matter fibre tracking in predicting the visual field deficits commonly experienced by patients undergoing temporal lobe surgery.

Dr. Torsten Baldeweg, Dr Frederique Liegeois, Dr Brigitte Vollmer

Institute of Child Health, University College London and Karolinska Institute, Stockholm, Sweden.

Combined neurophysiological and neuroimaging study of speech and language function in preterm children with perinatally acquired brain lesions.

Dr. Martin King, Dr Brigitte Vollmer, Dr Samuel Gросche

Institute of Child Health, University College London and Karolinska Institute, Stockholm, Sweden.

Neuroimaging study of normal brain development from infancy to adulthood.

Prof Lex Doyle, Dr Peter Anderson, Prof Terrie Inder, Prof Jeff Neil

Royal Women’s Hospital, Melbourne and St Louis, USA.

Determining the mechanisms leading to long term impairment in very preterm children.

Dr. Olivier Salvado, Prof Stuart Crozier

CSIRO, University of Queensland.

To develop methods to spatially normalise white matter fibre orientation images across subjects and perform group analyses of high angular resolution diffusion weighted MRI data.

EDITORIAL POSITIONS

Prof Alan Connelly, Epilepsia.

GRANTS

S.E.Berkovic, G.D. Jackson, J.C. Mulley, D.C. Reynolds, S. Petrou, I.E. Scheffer, J. Gecc, and A. Connelly

Epilepsy: Molecular basis and mechanisms in the era of functional genomics. National and Health Medical Research Council (Program Grant). 5 years (2008-2010). $215,076.

A. Morgan, A. Connelly, and F. Liegeois


H. Calamante, A. Connelly, and H. F. Elek


G. Donnan, S. Davis, and A. Connelly


F. Calamante, G. D. Jackson, and A. Connelly

Functional and structural brain networks in epilepsy. Foundation for Imaging Research. 2 years (2010-2011). $200,000.

F. Calamante

National Health and Medical Research Council Career Development Award. 5 years (2007-2011). $445,000.

PUBLICATIONS


Myelin is an insulating coat that enables neurons to efficiently transmit electrical signals throughout the central nervous system. The multiple sclerosis division studies how myelination develops and how it is lost during disease. To do this, we focus on oligodendrocytes, the cells responsible for producing myelin within the central nervous system. We study how this cell is generated, the external and intrinsic factors that stimulate its capacity to myelinate, the growth factors that maintain its viability during immune-mediated attack, and how it is stimulated to repair itself during demyelination. We also study interactions between these cells and the immune system, how the immune system works, and its capacity to either cause damage or induce repair.

Additionally, we study the effect of genes on MS; in 2010, we identified that MerTK is a susceptibility gene for MS, and that MerTK and its partner receptors are important biomarkers of early disease activity. Our applied work also extends to identifying measures of disease severity, including diffusion tensor imaging to assess damage after optic neuritis and serum-based markers to assess the extent of neuronal damage from a global perspective.

SNAPSHOT OF OUR RESEARCH

Finding new ways to aid repair of the central nervous system is one of the holy grails of neuroscience. We have made significant advances in this important area, having identified a potential way to improve repair in MS. A set of proteins known as BMPs actively inhibit repair in an animal model of multiple sclerosis by blocking the maturation of oligodendrocytes. We found that injecting an inhibitor of the BMPs (known as Noggin) both increases the numbers of mature oligodendrocytes and enhances myelin repair after myelin damage. We are now investigating how to apply this finding to improve the treatment of MS.

Members of the Florey team also contributed to one of the largest human genetic studies ever undertaken which identified the major common genetic variants involved in MS. The results of this study were published in Nature in 2011, and represent years of work by the International Multiple Sclerosis Genetics Consortium involving more than 250 researchers in 15 countries. The study confirmed the presence of up to 57 MS genetic loci, with a remarkable pattern suggesting that MS susceptibility is enhanced by subtle, inherited differences in immune function. The next step is to rigorously assess hundreds of patients to see how these newly identified genes function and contribute to the development of MS.

SENIOR STAFF

Prof Trevor Kilpatrick
Dr Helmut Butzkueven
Dr Holly Cate
Dr Simon Murray
Dr Toby Merson
Dr Ben Emery
Dr Junhua Xiao
Dr Judith Field
Dr Michele Binder
Dr Melissa Gresele
Dr Vilija Jokubaitis

Dr Ben Emery has returned to the Florey’s multiple sclerosis team from Stanford University in California.
Overview

Multiple sclerosis (MS) is the most common neurodegenerative disease affecting young Caucasian adults. It is considered to be an autoimmune disease, but its severity depends on the nature of the nervous system's response to injury. Our Division strives to understand the cause of MS, and to develop better therapies to treat it. To achieve these aims, we continue to focus on the genetic determinants of MS, the potential of neuroprotective and regenerative medicine, and the development of experimental animal models.

Our earlier genetics research has identified two novel loci that confer susceptibility to the disease. One of these influences the expression of the CD40 protein, which regulates immune function, and which has also been implicated in the pathogenesis of other autoimmune diseases such as Grave’s disease and rheumatoid arthritis. The other encodes a region of the genome that encompasses 17 genes, including key candidate genes involved in Vitamin D metabolism, which we and others have previously shown to be potentially important in the susceptibility to MS.

Our focus on regenerative medicine follows on from successful experiments in our laboratory and we and others performed some years ago which identified stem cells within discrete regions of the adult mammalian brain, in particular the subventricular zone (SVZ). By studying the molecular response of the SVZ to demyelination, we have identified candidate genes that are potentially important in modulating regeneration. One important set of candidates is the bone morphogenic protein family, and we have explored the influence of this series of factors on precursor cells and their progeny during demyelination.

We are also seeking to understand how the process of myelination is regulated, both within the oligodendrocytes themselves, and by elucidation of the signaling events that occur between neurons and the oligodendrocytes. Previous research by Dr Ben Emerson identified a gene (now known as Myelin Gene Regulatory Factor or MRF) that has a vital role in coordinating the myelination process during development. Mice lacking a functional copy of the MRF gene are unable to form myelin, conversely, forcing the expression of MRF can accelerate aspects of the myelination process. Ben and his team are currently using a wide variety of techniques including biochemical analysis, bioinformatics, in vitro cultures of oligodendrocytes and genetically modified mice to investigate how MRF and other proteins regulate the myelination process.

An additional avenue of research seeks to understand the pathological consequences of oligodendrocyte apoptosis, a process thought to initiate the development of new MS lesions. We have demonstrated that the loss of key sympathetic interactions between the oligodendrocyte and the axons they ensheath has profound functional consequences that occur prior to the removal of the myelin membrane, that is, in the absence of demyelination. Our findings illustrate that the genesis of axonal pathology in the absence of demyelination in recent onset disease could be induced by oligodendrocyte death or dysfunction.

Another area of interest is to develop strategies to optimise the survival of the oligodendrocyte, the myelinating cell of the central nervous system. Our more recent work has identified that the TAM family of receptor tyrosine kinases and their ligands Gas6 and Protein S are expressed in the central nervous system (CNS), including in oligodendrocytes, the myelin-producing cells of the CNS, namely oligodendrocytes and neuronal stem/progenitor cells. Previous work from our laboratory has shown that loss of Gas6-dependent TAM receptor signalling negatively affects the survival of oligodendrocytes during cuprizone challenge, an experimental mouse model of demyelination. In this model, the loss of Gas6, and subsequent decrease in oligodendrocyte survival, was accompanied by an increase in the response of the microglia, the innate immune cell of the CNS. We have now also shown that, in the absence of Gas6, the repair process known as remyelination is also compromised, with loss of Gas6 leading to a delay in remyelination. The delay in remyelination was accompanied by a reduction in oligodendrocyte numbers. We further found that, in vitro, Gas6 can increase mitosis in a dose-dependent manner. The reduced rate of remyelination of Gas6 KO mice could thus result from a lack of Gas6 at a critical time during myelin production after injury.

We have now extended our work from mouse studies to human disease. We conducted an association study to identify single nucleotide polymorphisms (SNPs) within genes encoding the TAM receptors and their ligands associated with MS. We identified polymorphisms within the MERTK gene associated with the risk of developing MS, thereby corroborating the relevance of our animal studies to the human disease.

Neuroprotective strategies to optimise the survival of oligodendrocytes during myelin injury and also in modulating innate immune responses upon aspects of learning and memory, in particular, cue-mediated spatial navigation.

Role of TAM receptor signalling in central nervous system demyelination

Michele Binder, Holly Cate, Junhua Xiao, Dennis Gern Merlo, Trevor Kilpatrick

The TAM family (Tyro3, Axl and MerTK) of protein tyrosine kinase receptors play pivotal roles in the processes of cell survival and proliferation, activation, endocytosis, modulation of the immune response, and the removal of dead cells from tissue. Dysregulation of these processes is central to both the initial development, and the subsequent clinical course, of demyelinating diseases such as MS. All three receptors, and their ligands Gas6 and Protein S, are expressed in the central nervous system (CNS), including in oligodendrocytes, the myelin-producing cells of the CNS, namely oligodendrocytes and neuronal stem/progenitor cells.

To investigate the consequences of oligodendrocyte loss upon axonal injury, we have generated a transgenic mouse model of conditional oligodendrocyte ablation. In this model, oligodendrocytes are rendered selectively sensitive to exogenously administered Diphtheria toxin (DT), by inducing expression of the diphtheria toxin receptor (DTR) specifically in oligodendrocytes. Administration of DT to MBP-DTR mice resulted in severe clinical dysfunction with an ascending spinal paralysis resulting in finally in respiratory impairment necessitating euthanasia within twenty-two days of DT challenge. Pathologically, at this time-point, mice exhibited a loss of approximately 26% of oligodendrocyte cell bodies throughout the CNS. Oligodendrocyte cell body loss was associated with moderate microglial activation, but not by widespread myelin degradation, as assessed both immunohistochemically and ultrastructural. In contrast, acute axonal injury, characterised by structural and biochemical alterations at nodes of Ranvier and reduced action potential conduction velocity, as measured by somatosensory evoked potentials (SSEP), was prominent. In summary, we have shown that a death signal instigated within oligodendrocytes can result in subcellular changes and loss of key symbiotic interactions between the oligodendrocytes and the axons it ensheathes resulting in profound functional consequences, all of which occur prior to the removal of the myelin membrane, that is, in the absence of remyelination. These findings could have profound implications for the understanding of the pathogenesis of diseases of the central nervous system such as multiple sclerosis in which the oligodendrocyte is potentially targeted.

We have also utilised DT-mediated targeted cell ablation of neuronal stem/progenitors in the adult mouse brain to investigate the functional/behavioural implications of suppressing adult neurogenesis in adult mice. Ablation of hippocampal progenitor cells was achieved by crossing two strains of transgenic mice, Nestin-CreER2 and iDTR, to enable tamoxifen-mediated induction of DTR expression in neural stem/progenitor cells in the subventricular zone and hippocampal dentate gyrus. In response to DT challenge, the double transgenic mice exhibited a 60% reduction in neurosphere-forming cells and a similar reduction in doublecortin-expressing neuroblasts assessed in vitro and in vivo, respectively. Thus, we have established a robust model for the ablation of neuronal progenitors that enables selective depletion of adult-born hippocampal granule neurons at a specific stage in their post-mitotic maturation. We are now applying this experimental model to investigate the consequences upon aspects of learning and memory, in particular, cue-mediated spatial navigation.

Bone morphogenic protein signalling alters the oligodendrocyte progenitor cell response during CNS demyelination.

Jennifer Sato, Tim Aumann, Daniel Merlo, Trevor Kilpatrick, Holly Cate

Oligodendrocyte cell death is a key pathological event in CNS demyelination. Oligodendrocytes regenerate naturally by differentiation of oligodendrocyte progenitor cells (OPCs) located within and surrounding myelin lesions, but this process is limited. Enhancement of oligodendrocyte regeneration is a promising strategy for remyelination. Myelin injury induces several growth factors that could modulate oligodendroglialogenesis and OPC differentiation. Bone morphogenic proteins (BMP) are one such class of factors that have been shown to inhibit the differentiation of OPCs into mature oligodendrocytes.
Holly S. Cate, Jennifer K. Sabo, cuprizone-induced demyelination in the subventricular zone during astrogliosis and oligodendroglia BMP signalling alters numbers of mature oligodendrocytes. The adult subventricular zone (SVZ) is a potential region for the production of myelin genes, with its expression acting as a key checkpoint to coordinate the myelination process. Given MRF is expressed outside the CNS in cell types other than oligodendrocytes, it is unlikely MRF acts in isolation to promote myelin gene expression in oligodendrocytes. We are therefore taking several approaches to try to identify other proteins that may act in conjunction with MRF to control myelination. The first approach, in conjunction with Dr. Moshe Olshansky and Prof. Terry Speed at the Walter and Eliza Hall Institute, is to screen the MRF bound DNA regions for other known transcription factors. The second approach is to use a proteomics approach to identify other proteins that physically interact with MRF in cells.

The role of the neurotrophins in regulating peripheral nervous system myelination Junhua Xiao, Melanie Willingham, Tony Hughes, Trevor Kilpatrick and Simon Murray We continue our characterisation of the influence that the neurotrophin BDNF exerts on peripheral myelination by Schwann cells. Following up on our seminal discovery, published in the Journal of Neuroscience in 2009, demonstrating that BDNF exerts contrasting influences upon peripheral myelination depending on the complement of BDNF receptors expressed by the peripheral neuron, we are currently investigating the influence of selective activation of only one BDNF receptor. In collaboration with Assoc. Prof. Tony Hughes (Dept. of Pharmacology, University of Melbourne), we are developing the use of small molecular weight peptide mimetics of BDNF, designed to selectively activate the BDNF receptor p75NTR, for the analysis of peripheral myelination in vitro. These data are looking extremely promising, suggesting that selectively targeting p75NTR activation can uniformly promote myelination of all peripheral neurons.

In novel work, PhD scholar Melanie Willingham has discovered a new role for the precursor form of BDNF, known as proBDNF, in peripheral myelination. She has identified that proBDNF plays a key role in promoting the cholesterol biosynthesis program in Schwann cells. This is particularly exciting as cholesterol is a vital component of myelin, and its synthesis is absolutely required for myelination. The data also suggest that BDNF and proBDNF could play distinct but complementary roles in promoting the peripheral myelinating process. We continue to utilise in vitro techniques, including live in vitro myelination assay (Figure 4), compartmentalised Campenot chambers, viral-based shRNA approaches to specifically knock down or activate specific genes, and the use of chemical/biochemical techniques we have previously used in our study of MRF binding in the SVZ. Most recently we have used a toxin-based mouse model of demyelination, the cuprizone model, to explore the role of BMP signalling in central demyelination. The (SVZ) was isolated using laser capture microdissection and gene expression was assessed by real time PCR, which identified the upregulation of BMP4 and the BMP receptors, BMPRα and BMPRβ, in the SVZ during central nervous system demyelination. We examined the effects of exogenous application of BMP4 on primary cultures of SVZ-derived adult mouse NPCs. In the presence of BMP4, astrocyte production increased 4-9 fold and oligodendrogliaogenesis decreased by 12% of control. Upon examination of BMP signalling activation in vivo, we found a significant increase in BMP signalling in the SVZ and this activity was present in oligodendrocytes and astrocytes. Upregulation of BMP4 signalling coincided with a 28% increase in astrocyte numbers in the SVZ. Micro-avasor pumps were used to infused Noggin, and endogenous antagonist of BMP4, or vehicle into the brains of mice during acute demyelination induced by cuprizone challenge. Noggin infusion significantly decreased BMP signalling, suggesting that Noggin was active in vivo. We found that Noggin infusion significantly decreased the number of astrocytes and increased the number of oligodendrocytes. Our previous work has demonstrated that the transcription factor MRF is vital for the production of myelin during development. It is currently unknown whether MRF is also required in the adult CNS for the maintenance of myelin. To investigate this we are using a sophisticated genetic approach to order to inactive the gene encoding MRF in the adult mouse CNS. We have found that following the inactivation of MRF the mice rapidly lose the expression of myelin genes, ultimately causing a gradual breakdown of CNS myelin. This demonstrates that MRF is required on an ongoing basis to maintain the myelin sheath. These findings also demonstrate that maintenance of the myelin is an active process, with the myelin sheaths requiring ongoing support from the oligodendrocyte. We are currently using animal models to study whether promotion of MRF expression is a potential therapeutic strategy to improve the efficiency of myelin repair in human diseases such as MS.

Molecular mechanisms of the regulation of myelination Melanie Willingham, Helena Bujalka, Stacey Jackson, Ben Emery, Moshe Olshansky and Terry Speed (from The Walter and Eliza Hall Institute of Medical Research) Using Chromatin-immunoprecipitation (a technique in which proteins are co-purified by the DNA they bind to) we have identified a large number of genomic sites that MRF binds to. Importantly, these regions cluster around genes with known roles in myelination. Using in vitro and biochemical techniques we have confirmed that MRF binding to these regions promotes the expression of the adjacent genes. These results confirm that MRF has a direct role in controlling a cohort of myelin genes, with its expression acting as a key checkpoint to coordinate the myelination process. Given MRF is expressed outside the CNS in cell types other than oligodendrocytes, it is unlikely MRF acts in isolation to promote myelin gene expression in oligodendrocytes. We are therefore taking several approaches to try to identify other proteins that may act in conjunction with MRF to control myelination. The first approach, in conjunction with Dr. Moshe Olshansky and Prof. Terry Speed at the Walter and Eliza Hall Institute, is to screen the MRF bound DNA regions for other known transcription factors. The second approach is to use a proteomics approach to identify other proteins that physically interact with MRF in cells.

In vitro. Recent work in our laboratory has identified that BMP signalling is increased in lesions during cuprizone-induced demyelination in mice. We have monitored BMP signalling during demyelination by using anosing mini-pumps to infuse either BMP4, its endogenous antagonist Noggin or vehicle into the brains of cuprizone-challenged mice. Mice infused with BMP4 during demyelination exhibited increases in proliferating OPCs, microglia and astrocytes in the demyelinated lesion compared to vehicle infused mice, while there was no significant difference between BMP4 and vehicle mice. In addition, Noggin infused mice had increased myelination compared to vehicle infused mice as assessed by myelin staining and electron microscopy after 1-week recovery. Thus, our results suggest that exogenous BMP signalling transiently increases the proliferation of glial cells, which are rapidly cleared following recovery from a demyelinating insult. On the other hand, inhibition of endogenous BMP signalling during demyelination promotes the regeneration of mature oligodendrocytes.

BMP signalling alters numbers of astrocytes and oligodendroglia in the subventricular zone during cuprizone-induced demyelination Holly S. Cate, Jennifer K. Sabo, Daniel Merlo, Victoria Peneau, Trevor J. Kilpatrick Chronic demyelinating diseases of the central nervous system are characterised by the death of oligodendrocytes. The adult subventricular zone (SVZ) is a potential source of precursors to replace neuronal cells lost during demyelination. Bone morphogenic proteins (BMPs) have previously been implicated in the inhibition of oligodendrocytes production; however, the regulatory role of BMPs in the injured adult brain has not been well examined. We have used a toxin-based mouse model of demyelination, the cuprizone model, to explore the role of BMP signalling in central demyelination. The (SVZ) was isolated using laser capture microdissection and gene expression was assessed by real time PCR, which identified the upregulation of BMP4 and the BMP receptors, BMPRα and BMPRβ, in the SVZ during central nervous system demyelination. We examined the effects of exogenous application of BMP4 on primary cultures of SVZ-derived adult mouse NPCs. In the presence of BMP4, astrocyte production increased 4-9 fold and oligodendrogliaogenesis decreased by 12% of control. Upon examination of BMP signalling activation in vivo, we found a significant increase in BMP signalling in the SVZ and this activity was present in oligodendrocytes and astrocytes. Upregulation of BMP4 signalling coincided with a 28% increase in astrocyte numbers in the SVZ. Micro-avasor pumps were used to infused Noggin, and endogenous antagonist of BMP4, or vehicle into the brains of mice during acute demyelination induced by cuprizone challenge. Noggin infusion significantly decreased BMP signalling, suggesting that Noggin was active in vivo. We found that Noggin infusion significantly decreased the number of astrocytes and increased the number of oligodendrocytes. Our previous work has demonstrated that the transcription factor MRF is vital for the production of myelin during development. It is currently unknown whether MRF is also required in the adult CNS for the maintenance of myelin. To investigate this we are using a sophisticated genetic approach to order to inactive the gene encoding MRF in the adult mouse CNS. We have found that following the inactivation of MRF the mice rapidly lose the expression of myelin genes, ultimately causing a gradual breakdown of CNS myelin. This demonstrates that MRF is required on an ongoing basis to maintain the myelin sheath. These findings also demonstrate that maintenance of the myelin is an active process, with the myelin sheaths requiring ongoing support from the oligodendrocyte. We are currently using animal models to study whether promotion of MRF expression is a potential therapeutic strategy to improve the efficiency of myelin repair in human diseases such as MS.

Molecular mechanisms of the regulation of myelination Melanie Willingham, Helena Bujalka, Stacey Jackson, Ben Emery, Moshe Olshansky and Terry Speed (from The Walter and Eliza Hall Institute of Medical Research) Using Chromatin-immunoprecipitation (a technique in which proteins are co-purified by the DNA they bind to) we have identified a large number of genomic sites that MRF binds to. Importantly, these regions cluster around genes with known roles in myelination. Using in vitro and biochemical techniques we have confirmed that MRF binding to these regions promotes the expression of the adjacent genes. These results confirm that MRF has a direct role in controlling a cohort of myelin genes, with its expression acting as a key checkpoint to coordinate the myelination process. Given MRF is expressed outside the CNS in cell types other than oligodendrocytes, it is unlikely MRF acts in isolation to promote myelin gene expression in oligodendrocytes. We are therefore taking several approaches to try to identify other proteins that may act in conjunction with MRF to control myelination. The first approach, in conjunction with Dr. Moshe Olshansky and Prof. Terry Speed at the Walter and Eliza Hall Institute, is to screen the MRF bound DNA regions for other known transcription factors. The second approach is to use a proteomics approach to identify other proteins that physically interact with MRF in cells.
The role of the neurotrophins in regulating central nervous system myelination

Jinhua Xiao, Agnes Wong, Anita Ferrer, Trevor Kilpatrick and Simon Murray

We are also characterising the influence that the neurotrophin BDNF exerts on central nervous system myelination by oligodendrocytes. A number of lines of evidence suggest that BDNF plays a key role in promoting central nervous system myelination. The BDNF knockout mouse is lethal in the early postnatal period, and we have assessed several mice that survived up to three weeks indicated a hypomyelinated CNS. In data currently being prepared for a manuscript, our analysis of BDNF heterozygous mice, which live a normal lifespan, indicate they exhibit delayed CNS myelination, suggesting that the amount of BDNF present is an important factor in regulating oligodendrocyte myelination. To complement this, in data we published in the journal Neuron, we demonstrated that BDNF exerts a strong promyelinating influence in vitro. Using pharmacological and genetic approaches, our analyses further identified that BDNF directly activates the receptor tyrosine kinase TrkB expressed on oligodendrocytes to promote myelination. In order to demonstrate this in vivo, we have imported TrkB floxed mice from Prof. Luis Parada (University of Texas Southwestern, USA), and generated mice with an oligodendrocyte-specific deletion of TrkB. PhD scholar Anita Ferrer will continue this analysis in 2012, to identify both the key signaling pathways activated and ultimately also the protein targets of these kinases, that BDNF utilises to promote oligodendrocyte myelination.

Collectively, these projects outline a detailed cellular, molecular and genetic investigation into the mechanisms that control myelination. Such investigations will provide new insight into, and increase our understanding of the molecular processes that govern the myelination process. This allowed a critical step in establishing how these responses might ultimately be targeted and modulated for therapeutic benefit in the context of demyelinating disease.

The role of ceruloplasmin regulation in CNS autoimmunity

Melissa Gresele, Stella Alexandrou, Trevor Kilpatrick and Helmut Butzkueven

We conducted a microarray study to identify genes that were differentially regulated in mice with experimental autoimmune encephalomyelitis (EAE), a mouse model of induced, MS-like autoimmunity. A total of 181 genes with at least a 2-fold increase in expression were identified, and ceruloplasmin (Cp) was one of the most highly up-regulated (132.2 fold increase). Cp protein is a ferroxidase that converts toxic ferrous iron to its non-toxic ferric form and also promotes the efflux of iron from astrocytes in the CNS. Expression of this protein is known to be increased in response to several neurological conditions, however, the role of Cp regulation during acute neuro-inflammation is not known. To investigate the role of Cp, we induced EAE in Cp gene knockout, heterozygous and wild-type mice. Cp knockout mice were found to have slower disease evolution relative to the wild-type mice (EAE stage 13 to 17; P<0.05). Surprisingly, iron levels in EAE affected spinal cord tissue assessed by mass spectrometry were significantly higher in Cp knockout mice than in Cp knock-in mice, indicating a significant increase in the number of reactive astrocytes in early EAE and not present in wild-type mice at the same stage of disease. As Cp is known to have pro-oxidant sites, we propose that the increase in Cp may contribute to tissue damage in the early stages of EAE. In addition, Cp could contribute to the regulation of astrocyte activity in this disease.

The role of Db2 in modulating microglial CNS response in autoimmunity

Vilija Jokubaitis, Melissa Gresele, Bill Doherty, Trevor Kilpatrick and Helmut Butzkueven

Resident immune cells within the brain known as microglia have an important role in play in MS. They have been shown to mediate both damage and repair processes. Working together with A/Professor Helmut Butzkueven and Professor Trevor Kilpatrick, Vilija Jokubaitis and Melissa Gresele have demonstrated that the protein Disabl2 (Db2) is expressed by microglia during demyelinating disease in both mice and humans, and that deletion of Db2 in mice diminishes disease severity. Recently our team, including Dr Melissa Gresele (Distance Learning Institute), have identified that the protein Disabl2 expressing microglia occurs via the nitric oxide biochemical signalling pathway. In addition, we have been able to show that this phenomenon is independent of another immune cell type, the T-cell. Our research has provided us with a potential therapy for the treatment of multiple sclerosis, indicating that modulating Db2 levels within microglia could aid in reducing brain damage (in particular axonal injury) in MS.

Endogenous leukemia inhibitory factor signalling reduces axonal damage and modulates macrophage activity in mice with experimental autoimmune encephalomyelitis

Helmut Butzkueven, Melissa Gresele, Stella Alexandrou, Trevor Kilpatrick and Simon Murray

The leukemia inhibitory factor (LIF)/ciliary neurotrophic factor (CNTF) signalling pathway is an endogenous protective pathway that is regulated during neuro-inflammatory diseases such as MS. The LIF and CNTF are members of the interleukin-6 family of cytokines, which signal through the common receptor subunit, glycoprotein 130 (gp130). It has been demonstrated previously that the therapeutic administration of recombinant LIF can reduce disease severity in murine experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, and that gp130 dependent astrocyte activation is protective in this disease. Using knockout mice, we showed that endogenous LIF and CNTF act together to reduce disease severity and prevent inflammatory mediated axonal damage in both EAE and MS. Additionally, from EAE day 18-21, MRI scans were performed to measure water diffusivity along the mouse optic nerve (ADC), a non-invasive indicator of axonal integrity; we also sampled the axon degradation product pNF-H, in the blood, and conducted detailed histological evaluations for axonal loss. In the absence of CNTF, the genetic deletion of LIF was associated with increased levels of decreased ADC parallel in optic nerve MRI and optic nerve and spinal cord axon density reduction. In addition, deletion of the gp130 receptor subunit from macrophages and microglia, but not oligodendrocytes, resulted in significantly worsened EAE disease severity through acute stages of the disease.

These results suggest that endogenous LIF has neuroprotective properties in mice with acute neuro-inflammatory disease, and could provide a novel therapeutic target for minimising disability progression in MS. Importantly, in addition to its effects on astrocyte activity, we show that macrophage/microglia specific gp130 signalling reduces EAE severity, perhaps by mediating endogenous LIF/CNTF signals, such as reduction of TNF production.

The extracellular matrix response in the CNS in acute EAE

Anna Jonas, Melissa Gresele, Trevor Kilpatrick and Helmut Butzkueven

The majority of EAE inflammation is spinal, and affects axonal projections from large motor neurons in the frontal motor cortex. We hypothesised that these motor neurons respond to the axonal injury, and therefore conducted microarray studies on motor cortex enriched tissue, comparing EAE control mice. Approximately half of the up-regulated mRNA species in EAE frontal cortex, surprisingly, encoded for extra-cellular matrix (ECM) or ECM-associated proteins. One of these genes, matrilin-2 (Matn2), was found to be highly correlated with EAE disease severity scores (r=0.001), whereas disease-induced Matn2 gene expression changes were not observed in control tissue from the occipital and cortices. Matrilin-2 was found to be specifically localised to the cytoplasm of cortical layer 2/3 and 5/6 neurons, and was highly up-regulated in and around inflammatory lesions of the white matter in EAE spinal cord. We observed three distinct patterns of spinal cord, first, a decrease in Matn2 expression in the spinal cord (perivascular, linear peri-axonal and diffuse intra-lesional) at peak disease, most likely produced by reactive astrocytes, followed by the amplification of this region using the technique of long-range PCR, as well as pooling of DNA samples from MS cases and controls, which has been followed by specific sequence capture methods for this 300 kb region. These two methods of DNA enrichment have resulted in DNA enrichment that has now been sequenced interrogated for novel rare variations that can be analysed for association with MS risk.

The CD40 locus has previously been associated with the risk of autoimmune disease development, particularly Graves’ disease and rheumatoid arthritis. The variation associated with MS encompasses 17 genes. Our analysis of MS DNA that has now been sequenced associated with the risk of developing MS, and has directed us to further analysis of MS genetic risk, and the functional consequences of these genetic changes.

The chromosome 12 locus identified as associated with increased risk of MS encompasses 17 genes. Our aim is to determine which of these genes contains variation associated with risk of MS, with the ultimate goal of ascertaining the functional consequence of these genetic changes. This has involved the method of deep sequencing of the entire 300 kilobase (kb) region of DNA associated with MS, which has been followed by further genetic studies associated with the risk of developing MS, and has directed us to further analysis of MS genetic risk, and the functional consequences of these genetic changes.

A follow up to the ANZGene GWAS for multiple sclerosis

The Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZGene) including Trevor Kilpatrick, Helmut Butzkueven and Judith Field.

The recent genome-wide association scan (GWAS) performed by the Australia and New Zealand MS Genetics Consortium (ANZGene) led to the identification of a new risk SNP associated with the risk of developing MS, and has directed us to further analysis of MS genetic risk, and the functional consequences of these genetic changes.

The chromosome 12 locus identified as associated with increased risk of MS encompasses 17 genes. Our aim is to determine which of these genes contains variation associated with risk of MS, with the ultimate goal of ascertaining the functional consequence of these genetic changes.
There is a fantastic concentration of neuroscientists at the Florey and in Melbourne. It’s a great environment to continue my research and build up a lab.

Dr Ben Emery, on returning home from Stanford University in California.

Senior Staff
Prof Trevor Kilpatrick
Dr Helmut Butzkueven
Dr Holly Cate
Dr Simon Murray
Dr Toby Merson
Dr Ben Emery
Dr Junhua Xiao
Dr Judith Field
Dr Michele Binder
Dr Melissa Gresle
Dr Vilija Jokubaitis

Of these changes in expression are in relation to the function of the individual’s immune system. This analysis shows a genotype-dependent decrease in CD40 protein expression in individuals which carry the CD40 MS risk-associated SNP in monocytes and B cells, and work is continuing to further refine the cell subtypes showing differences in expression, as well as any differences in expression between MS patients and controls.

Finally, using the ANZgene GWAS data we investigated the association of single nucleotide polymorphisms (SNPs) across the human leukocyte antigen (HLA) complex in 1618 MS cases and 3418 controls of European ancestry. A total of 1927 SNPs within the HLA complex were interrogated, with logistic regression analysis resulting in the identification of 7 SNPs independently associated with MS conditional on the others ($p < 4 \times 10^{-4}$). The association of these 7 SNPs with MS was independently replicated in a cohort 2212 MS cases and 2251 controls ($p < 0.001$), with a combined p-value of $p < 6 \times 10^{-4}$. The rs9277535 SNP was found to be strongly associated with risk of developing MS in the GWAS discovery data set ($p < 9 \times 10^{-9}$) and this was independently replicated ($p < 7 \times 10^{-4}$; combined $p < 2 \times 10^{-10}$). The rs9277535 SNP identifies the HLA class II DPB1 allele DPB1*0301, which has previously been associated with MS in smaller samples of MS cases and controls. We have therefore identified an independent MS-susceptibility locus with genome-wide significance in the HLA class II DPB1*0301 allele.
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RESEARCH OVERVIEW

The broad interests of our group relate to Parkinson’s Disease (PD), Motor Neuron Disease (MND) and the role of dopamine on frontal lobe function. There are four general themes running the research: The pathophysiology of PD and MND, Cell therapies (CT) for PD, the role of dopamine in fore brain function and the use of scaffolds for brain repair.

The pathophysiology of PD and MND. PD and MND are both characterised by abnormal aggregation of protein in the cell. Our research in these two conditions is travelling parallel paths. In MND the focus is on the role of aberrant proteins in ER stress, the movement of vesicles and other cargo in the cell and secretion of SOD1 and related proteins. In PD, the interest is also on secretion and how abnormal secretion acts as a marker of disease and also points to the locus of pathology. Work related to this aim are detailed below.

Cell therapies for PD. Our interest here relates to how the new dopamine cells are recruited into brain circuitry and what factors regulate this. Our interest are in both the well recognised midbrain-striatal circuitry as well as in the pathway form the Sub Ventricular Zone to the olfactory Bulb.

The role of dopamine in fore brain function. We have shown that when disabling movement disorders such as dyskinesias occur in PD, the dopamine levels in the striatum are similar to those produced by cocaine. Our hypotheses is that the dyskinesias are produced by the same molecular and structural changes as those that cause addiction. We are examining Cell therapy tools for ameliorating dyskinesias and both characterised by abnormal aggregation of protein in the cell. Our research in these two conditions is travelling parallel paths. In MND the focus is on the role of aberrant proteins in ER stress, the movement of vesicles and other cargo in the cell and secretion of SOD1 and related proteins. In PD, the interest is also on secretion and how abnormal secretion acts as a marker of disease and also points to the locus of pathology. Work related to this aim are detailed below.

The use of scaffolds for brain repair. In collaboration with the Materials Engineers at Monash University (John Forsythe), we are examining how to use artificial scaffolds to improve cell survival, axon growth and appropriate cell connectivity. These will be used as improved tissue culture media as well as transplantation devices.

α-Synuclein in human plasma

Roger Tinsley, Xu-Xin Chia, Katya Kotchet and Malcolm Home in collaboration with Philip Nagley, Monash University, Gerald Shaw, University of Florida and Myles Prince, Peter MacCallum Hospital.

α-Synuclein is a key protein in the pathogenesis of Parkinson’s Disease (PD). It is the predominant protein in Lewy Bodies, which are intraneuronal inclusions found in the substantia nigra in PD. Mutations in the α-synuclein gene also cause heritable forms of PD. It is likely that obtaining a better understanding of the function of α-synuclein in healthy and pathological neurons would lead to insights into the pathological process of PD as well as highlighting therapeutic possibilities. Recently it was discovered that α-synuclein is secreted, and can be found in both cerebrospinal fluid and blood plasma. We found that the levels of circulating α-synuclein in PD and age–matched controls is different. Using Western Blotting we found that the α-synuclein monomer is lower in Parkinson’s Disease subjects than in age matched controls. We then developed a new antibody for α-synuclein and found that total α-synuclein was high in people with Parkinson’s Disease, even though the monomer was low, suggesting abnormal secretion in Parkinson’s Disease. About 40% of people with Parkinson’s Disease have α-synuclein levels that are higher than the 90th percentile of the normal population. These patients have a worse form of Parkinson’s Disease than those with low levels.

The amount of α-synuclein found in plasma is too high to be from brain via CSF and as blood cells express α-synuclein, they are a potential source. We have examined α-synuclein levels in people undergoing bone marrow ablation of blood cell cancers. In these people α-synuclein levels are similar to those produced by PD and MND. When we examined some of these blood cells we found that they have a shorter survival than the same cells from control subjects. We argued therefore if the levels of α-synuclein in the blood are altered, it suggests that PD has interfered with the secretion of α-synuclein by these cells and that it may also be affecting their longevity. This suggests that the same process affecting α-synuclein secretion in blood may also be affecting neurons and their survival.

Our current studies are directed toward understanding how and why α-synuclein is secreted and how this may affect neuronal function. The long term aim is to understand what the change in α-synuclein level can tell us about the disease process. We are also interested in whether α-synuclein levels can be used as a risk marker for Parkinson’s Disease especially in people with presymptomatic disease. These findings may lead to biomarkers and to high through put screens for drugs to be used in Parkinson’s Disease.
MND-linked misfolded proteins in exosomes

Brad Turner, Malcolm Horne in collaboration with Julie Atkin (LaTrobe University), Kevin Talbot (University of Oxford) and Andrew Hill (University of Melbourne)

MND is characterised by paralysis which begins focally and disseminates, suggesting a spread of degeneration in the nervous system. We recently found three proteins pivotal to MND pathogenesis - SOD1, TDP-43 and FUS in exosomes. These are small secretory vesicles derived from endosomes and released by cells. These exosomes were isolated from motor neuronal cultures and rodent cerebrospinal fluid. Since these proteins form core components of pathologic inclusions found in MND, their secretion by exosomes may contribute to disease propagation in spastic MND. The finding of exosome-associated TDP-43 and FUS is also surprising since these are DNA/RNA binding proteins, suggesting novel extracellular functions for these conventional nuclear proteins. There is reduced accumulation of these proteins in neurally-derived exosomes is reduced when mutations in SOD1, TDP-43 and FUS linked to inherited MND are present. There was an associated defect in the function of the early endosome in our cell models of inherited MND. This points to abnormal protein secretion in MND and when considering in the context of our previous discovery of endoplasmic reticulum (ER) stress and ER-Golgi transport failure in MND models are arguments that motor neurons may be particularly susceptible to vesicle trafficking dysfunction as part of the pathogenesis of MND. Our current studies are aimed at deciphering the sequence of ER, Golgi and endosome abnormalities in MND to better understand pathological pathways and define new therapeutic targets. We are also addressing whether altered exosome secretion of SOD1, TDP-43 or FUS occurs in MND patients and correlates with disease severity to determine the potential biomarker value of exosomes.

ER to Golgi trafficking is disrupted in Motor Neuron Disease

Brad Turner, Malcolm Horne in collaboration with Julie Atkin (LaTrobe University), Paul Gleeson (University of Melbourne), and Prof Philip Nagley (Monash University)

We were one of the first laboratories to demonstrate that endoplasmic reticulum (ER) stress occurs early and is a key point in the pathophysiological cascade that causes MND. Proteins synthesised in the ER are transported to the Golgi apparatus for transport to other parts of the cell. When transport of proteins from the ER to Golgi fails, the Golgi becomes fragmented, leading to ER stress. With their long axons, cellular transport is crucial in motor neurons which bear the brunt of MND. We are proposing that the common and early event in MND is aberrant binding of MND proteins to proteins involved in cellular transport (such as dynein). This will disrupt ER to Golgi transport, axonal transport and impaired vesicle trafficking, leading to failure of protein secretion, ER stress and consequently cell death. In support of this proposal, a long list of vesicle, endosome and intracellular transport proteins have been linked to diseases of motor neurons. Our evidence demonstrates that proteins linked to MND: mutant SOD1, FUS and TDP-43 (but not wild-type SOD1) disrupts ER to Golgi trafficking by abnormally to proteins such as dynein, Rab1 and COP. This interaction between the “MND proteins” and dynein might disturb efficient transport either by competing with other essential cargo for transport steps or by diminishing dynein function. Over-expressing a component of dynein prevents inclusion formation and improved cellular survival. COP is required for proteins to exit the ER. COP is not only co-immunoprecipitates with the “MND proteins” but it is also up-regulated and mis-localised into inclusions. Rab1 is required for involved in the docking of vesicles trafficking between ER and Golgi and the “MND proteins” bind to Rab1, presumably reducing its function. Together these data suggest that transport from the ER to the ERGIC compartment is blocked in cells expressing mutant “MND proteins”. In summary, ER-Golgi trafficking is impaired in MND and proteins linked to MND bind to proteins involved in transport, suggesting disruption to ER to Golgi transport function in MND.

ADDITIONAL RESEARCH PROJECTS

Mechanisms of Dyskinesia
Davor Stanic, Doris Tomas, Wah Chin Boon and Malcolm Horne

Artificial three dimensional scaffolds for Growth of neurons and brain repair
Rogan Tinsley, Clare Parish and Malcolm Horne in collaboration with Denise Fon, Hangqing Feng and John Forsythe (Monash University) and David Finkelstein (Mental Health Research Institute)

Building cell models of neurodegenerative disease
Julie Atkin, Rogan Tinsley, Brad Turner, Mohammad Gadi, Adam Walker, Manal Farg and Malcolm Horne, in collaboration with He Ling Ng and Philip Nagley (Monash University)

Genes in Parkinson’s disease
Justin Rubio and Malcolm Horne in collaboration with Katya Kotschett (St Vincent’s) and (Menzies Research Centre, Hobart)
RESEARCH OVERVIEW
The focus of the Neuropharmacology group is on the cellular consequences of injury processes such as excitotoxicity, oxidative stress and inflammation, all of which contribute to brain pathologies. Overstimulation of receptors for the brain’s major excitatory neurotransmitter, L-glutamate, is termed “excitotoxicity” and arises when astrocytic glutamate transporters fail to remove the transmitter from the extracellular space. Subsequent rises in intracellular calcium activate cellular death cascades and there are concurrent disruptions of cellular energetics, which lead to the generation of toxic reactive oxygen and nitrogen species. Gial cells are also intimately involved in the maintenance of normal synaptic transmission and during brain injury become activated, changing their phenotype and energetics, and synthesizing inflammatory and trophic factors. Not only is astrocyte biology actively under study, but neuronal injury particularly caused by excitotoxic and oxidative stressors continue to be major focus of attention. Our studies of neuronal injury have concentrated on elucidating the contributions of two relatively unknown forms of programmed cell death, autophagy and programmed necrosis.

MAJOR PROJECT
Astrocyte biology, glutamate transporters and bioengineering
Linda Lau, Ross O’Shea, Rebecca Sheean, Michelle Kovacevic, Malcolm Horne, Victoria Perreau, Holly Cate, and Philip Beart in collaboration with Steve Cheung (Deakin University), Leanne Bischof (CSIRO Mathematical & Information Sciences), David Nisbet (Australian National University) and John Forsythe (Monash University) 

Glia outnumber neurons and we have focused on their contributions to injury processes. We have evidence that astrocytes adapt their morphology and hence their biology in inflammatory events, with glutamate transporters (EAATs, Excitatory Amino Acid Transporters) being especially involved. In pure astrocyte cultures, Drs Linda Lau and Ross O’Shea dissected the temporal sequence of changes in EAAT abundance and function, and related them to astrocytic phenotype. Here we manipulated the actin system, known to be a key determinant of the astrocytic cytoskeleton, and believed to be linked to EAATs via various scaffolding proteins. Since the Rho family small GTPases regulate actin dynamics, we used Rho kinase (ROCK) inhibitors such as HA1077 (Fasudil™) and Y27632 to induce stellation of cultured astrocytes and found a doubling in the cell-surface expression of EAAT2 over 24 hrs. The effectiveness of the ROCK inhibition in altering astrocytic morphology was shown by cytochemistry and image analysis, with significant conversion of filamentous (F)-actin to monomeric globular (G)-actin. These morphometric studies we greatly advanced by the design and implementation of a unique form of advanced image analysis developed jointly with Dr Leanne Bischoff (CSIRO, Sydney).

In other work Prof Beart’s team find homeostasis of EAAT2, and believe such maintenance of function reflects its importance, comprising 1% of the proteome. In research work driven by PhD student, Rebecca Sheean, no evidence was found of internalization of EAAT2 with the PKC inhibitor, rottlerin, which inhibited glutamate uptake. Indeed drugs interfering with the endoplasmic reticulum, Golgi apparatus and endosomes (brefeldin, nocadazole), intracellular compartments classically linked to trafficking/internalization, were found to be ineffective. However, monensin, an activator of Na+ ionophore-mediated cellular influx accelerated recovery from the inhibition of uptake induced by rottlerin suggesting a critical role for intracellular Na+. Rebecca Sheean examined the involvement of Na,K-ATPase subunits by analysing 86Rb uptake and found de-coupling of EAAT function and intracellular Na+. We believe this phenomenon is the basis of the short term, Vmax adaptive responses and that long term changes involve transcriptional regulation of the type found with ROCK inhibitors. Since the ROCK inhibitors are being clinically explored to manage the glial scarring, which hinders recovery in brain and spinal cord trauma, we undertook gene profiling on astrocytes treated with Fasudil™. This programme was undertaken in collaboration with Dr Steve Cheung (Deakin University) and drew upon the special skills of Drs Vicky Perreau and Holly Cate of the National Neuroscience Facility and the MS Division, respectively. Our data add new dimensions to the concept of protein-protein interactions or a signalosome linking EAATs to metabolism and trophic factors. We found up-regulation of EAAT2 activity by Fasudil™ produced notable alterations in the expression of genes linked to the regulation of EAAT function - the FIYD2 and ATP1B1 subunits of Na,K-ATPase, NHRF1, sphins and endothelin receptor B. All these proteins have been previously to be suggested to be associated with EAAT function and led us to hypothesise regarding members of the EAAT signalosome complex. Another major outcome from this study of the astrocyte transcriptome, and confirmed by RT-PCR, was evidence of a “healthy” astrocytic phenotype wherein we noted elevations in the expression of BDNF, anti-oxidant defence mechanisms and glycolytic metabolic enzymes at strategic time points. Such a physiologically beneficial phenotype would be expected to be supportive of neuronal function. Important changes were also found in components of the extracellular matrix suggesting astrocytes should be amenable to manipulation in brain diseases to...
support the survival of neurones in brain pathologies. Thus the ROCK inhibitor Fasudil™ has potential uses through its panoply of beneficial outcomes in neurodegenerative and traumatic conditions involving reactive astrogliosis. A further strategy here is to use bioengineering and materials science to “engineer” astrogliosis into healthy brain pathologies. Dr Lau and Prof Bearthius thus built upon a long-term collaboration established by Prof Malcolm Horne with two specialists in this area, Drs John Forthsythe (Monash University) and David Nisbet (Australian National University). While bioengineering with nanoscaffolds has been applied to neurones and stem cells, there is negligible work in this area with astrogliosis. There are two key goals here: (1) to use the principles of bioengineering to generate healthy astrogliosis, and (2) to produce nanoscaffolds that induce astrogliosis to adopt in vivo a healthy biology. Exciting work undertaken by a BSc(Hons), Michelle Kovacevic, demonstrated that technically astrogliosis could be grown on biomatrices and maintained in healthy phenotypes. By employing random or aligned nanoscaffolds built from poly- caprolactone we found maintenance of mitochondrial activity; no elevation of the marker of astrogliosis GFAP and that G-actin showed huge increases indicative of disassembly of actin stress fibres. Moreover astrogliotic morphology was dependent on the type of nanoscaffold – tight clusters and elongated processes (>300 µm) were found on random and aligned biomatrices, respectively. Confocal microscopy revealed astrocytic fibres infiltrated random scaffolds, but were directed along nanofibres on aligned scaffolds. Both features are essential for brain repair viz. fibre penetration, extensive migration which may allow management of glial scarring in an injurious milieu in brain pathologies.

**MAJOR COLLABORATOR LINKS**

**National**

Profs Philip Nagley & Rod Devenish, Monash University.

Dr Leanne Bischof, CSIRO/Macquarie Univ, NSW.

Dr Steve Cheung, Deakin University.

Proff David Pow, University of Queensland.

**International**

Dr Marcus Rattray, University of Reading, UK.

Michelle Kovacevic, BBioMedSci (Melb)

Professor Niels Danbolt, University of Oslo, Norway.

Professor Ulf Nilsson, University of Reading, UK.

Dr Steve Cheung, Deakin University.

Dr Leanne Bischof, CSIRO/Macquarie University.

Profs Phillip Nagley & Rod Devenish, Monash University.

**EDITATIONAL POSITIONS**

Philip Beart

British Journal of Pharmacology Neurochemistry International

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**FIGURE 1**

Figure 1: Morphology of astrocytes maintained on aligned (left image) and random (right image) nanoscaffolds for 12 days in primary culture. Immunocytochemistry for the intermediate filament marker glial fibrillary acidic protein (GFAP) shows elongated processes following nanofibres (left panel) and tighter clusters where processes bounce into the biomatrix (right panel). Conventional fluorescence microscopy top panels (scale bar = 50 µm) and confocal-microscope-etch analyses bottom panels (scale bar = 10 µm).

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**CONFERENCES AND PRESENTATIONS**

**Philip Beart**

Australian Neuroscience Society.

Sydney 2010, Auckland 2011

American Society for Neurochemistry, Santa Fe, USA, 2010

Australasian Society for Clinical and Experimental Pharmacology and Therapeutics, Melbourne, 2010

Aza-Pacific Society for Neurochemistry, Phuket, Thailand, 2010

13th International Neuroscience Winter Conference, Sölden, Austria, 2011

International Society for Neurochemistry, Athens, Greece, 2011


IBRO School of Neuroscience, Beijing, China, 2011

**Ross O’Shea**

Australian Neuroscience Society, Sydney 2010

Linda Lau

International Symposium on ALS/MND, Sydney, 2011

Linda Mercer

Australian Neuroscience Society, Auckland 2011

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**PUBLICATIONS**


The Neurogenesis and Neural Transplantation laboratory, headed by Dr Lachlan Thompson, has a focus on regenerative approaches for brain and spinal cord repair. The research program encompasses two broad themes: 1) analysis and manipulation of the brain’s own limited capacity for ‘self-repair’ and 2) neural transplantation for re-construction of damaged brain circuitry. The aim of this research is to develop regenerative therapies that can restore function to patients with brain damage, for example as a result of acute trauma or stroke or through neurodegenerative diseases such as Parkinson’s disease or Motor Neuron.

Major Projects

Development of a stem cell based therapy for Parkinson’s disease (PD)

Cell therapy for PD is based on the idea that new dopamine neurons transplanted directly into the brain of the patient functionally replace those lost to the disease, thereby restoring motor function. Success with this approach in clinical trials has thus far relied on the use of human foetal tissue and there is now a great deal of international research dedicated to establishing a more sustainable cell source. We are working closely with the Stem cells and neural development laboratory at FNI to develop safe and effective procedures for the use of stem cells as a therapy for PD. This includes the use of stem cell lines engineered to express fluorescent proteins so that we can better understand their capacity for growth and integration following transplantation. We are also exploring a novel strategy for isolating the therapeutic dopamine progenitors from stem cell cultures prior to transplantation, thereby leaving behind other unwanted and potentially harmful cell types.

Understanding and promoting the brain’s own capacity for ‘self-repair’

Unlike other parts of the body, the brain has an extremely limited capacity for repair. This means that the loss of neurons as a result of damage to the brain is permanent and so is the functional consequence for the patient. Neurogenesis does persist, however, in two discrete locations in the brain throughout life. Recent studies show that these new neurons can be recruited to nearby sites of damage, although it is not clear that they become the right kinds of neurons or arrive in sufficient number to have a therapeutic impact. We are working to better understand the kinds of neurons that are attracted to the forebrain in response to damage, as occurs through stroke for example, and how if they can integrate appropriately into damaged brain circuitry. We are also using a gene-therapy approach to deliver growth factors to the brain in order to ‘boost’ the neurogenic response to injury.

Other projects

- Human pluripotent stem cells for replacement of cortical circuitry – in collaboration with Mirella Dottori and Mark Denham (Centre for Neuroscience, University of Melbourne)
- Transfer of α-synuclein between cells as a pathogenic mechanism in PD.

Staff

Lachlan Thompson BSc (Hons), PhD (Monash), NHMRC CDA Fellow
Chris Bye BSc (Hons) (UNSW)

Research and Technical Assistants

Mong Tien BSc

Students

Jordan Wright BSc (Hons) (Adelaide)

Conferences and Presentations

Lachlan Thompson

- Invited Symposium at the biennial meeting of the Asia Pacific Society for Neurochemistry (Phuket, Thailand).
- Invited presentation at the Centre for iPS Cell Research and Application (Kyoto, Japan).
- Presentation to patients and families as part of Motor Neuron Disease awareness week (Melbourne, Australia).
- Neuroscience Victoria Seminar Series (Melbourne, Australia).
The Stem Cells and Neural Development laboratory, headed by Dr Clare Parish, has a broad research interest relating to repairing the injured brain. There are a number of major research themes running within the laboratory, including: understanding development of dopamine neurons; improving cell-based therapy for Parkinson's disease; directed differentiation of pluripotent stem cells; molecular mechanisms underlying axonal targeting and synaptogenesis of midbrain dopamine neurons; and exploiting biomaterials to promote neural repair.

Understanding axon guidance in dopamine development
An ongoing challenge for biologists is to understand the intricate and precise wiring achieved during brain development. The same accurate wiring is also required to repair the injured brain. We have observed changes in dopamine (DA) plasticity in many pathologies in the adult brain including Parkinson's disease (PD), addiction and following treatment with antipsychotic drugs. The problems of DA axon guidance are exemplified by the attempts at repairing the degenerating pathways in PD through cell replacement therapy. Success of cell replacement therapy will depend on whether new DA cells integrate with the host circuitry and make appropriate connections.

Unfortunately, the molecular basis of dopamine (DA) axon guidance during development and repair of the diseased brain remains poorly understood. We are investigating new proteins in this context, with a particular focus on Wnts and their receptors. We have shown that several Wnts have a temporal and spatial expression that correlates with development of the DA pathways. Furthermore, Wnts can promote DA axon growth in vitro and in vivo and provide directional cues for developing axons. We are confirming many of these findings in various Wnt related transgenic mice as well as currently investigating the ability of Wnts to promote axon growth for transplanted DA neurons in PD mice. An understanding of the signalling in DA axon growth and guidance will markedly increase our understanding of DA neuronal connectivity. Such knowledge is not only important for developmental biology, but may provide new insights into a number of dopamine related diseases and present new avenues to improve cell replacement therapy for Parkinson's disease.

Biomaterials to support neurons and promote brain repair
In collaboration with the Materials Engineers at Monash University (John Forsythe and David Nisbet). Repair of the diseased or injured central nervous system will depend on the capacity to deploy replacement cells and providing appropriate signals for these new cells to differentiate and integrate and into the host circuitry. Promoting the survival and neuronal connectivity of new cells will rely on establishing appropriate chemical and physical environments to support neural cells and their processes. This will include structural elements reminiscent of the extracellular matrix, trophic proteins and suppression of inhibitory molecules. We are investigating the ability of 3-dimensional biomaterials to provide physical and chemical support for neural stem cells in vitro. We have shown that modified scaffolds can improve the survival, proliferation and differentiation of stem cells onto these scaffolds to enhance cell support. We are currently investigating the ability of these biomaterials to support transplanted stem cells in the brain and restore neural circuitry.

OTHER PROJECTS
• Restoring the nigrostriatal pathway in Parkinson’s Disease
• WNT signalling pathways in dopamine neuron differentiation

STAFF
Fellows, Senior Research Officers, and Research Officers
Clare Parish BBiomedSci (Hons), PhD (Monash), NHMRC CDA Fellow
Chris Bye BSc (Hons) (UNSW)
Chathurini Fernando BSc (Hons), MSc (Auckland) PhD (Melb)

Research and Technical Assistants
Doris Tomas BSc (Deakin)

Students
Brette Blakely BA (Hons) (Wellesley, USA), MA (Monash)
Jessica Kauhausen BSc
Jerani Pettikiriarachchi BSc BEng (Hons) (Monash)
CONFERENCEs AND PREsENTATIONS

Clare Parish
- Invited Symposium at the biennial meeting of the Asia Pacific Society for Neurochemistry (Phuket, Thailand).
- Invited presentation at the Centre for IPS Cell Research and Application (Kyoto, Japan).
- Neuroscience Victoria Seminar Series (Melbourne, Australia).

MAJOR COLLABORATIVE LINKS

National
- Dr Lachlan Thompson, Florey Neuroscience Institute
- Cell based repair for Parkinson’s disease.
- Dr John Forsythe and Dr David Nisbet, Department of Chemical Engineering, Monash University
- Biomaterials and scaffolds
- Prof Collin Pouton, Monash University
- Cell based therapies for Parkinson’s Disease
- Prof Stephen Stacker, Ludwig Institute for Cancer Research, Parkville, Australia
- Wnt/Ryk in dopamine development

International
- Prof Evan Snyder, Burnham Institute for Medical Research, USA
- Cell based therapies for Parkinson’s Disease
- Prof. Ernest Arenas, Dept Medical Biochemistry and Biophysics, Karolinska Institute, Sweden
- Wnt signalling in dopamine development
- Assoc Prof Jan Stenman, Ludwig Institute of Cancer Research, Stockholm, Sweden
- Wnt signalling in DA development

VISITING SCIENTISTS

Prof Ernest Arenas PhD
Karolinska Institute, Sweden

RESEARCH OVERVIEW

Generation of a doxycycline-inducible, tissue-specific aromatase-expressing transgenic mouse.

Our team successfully generated transgenic mice that express aromatase in a tissue-specific and doxycycline-inducible manner, the ArKin mouse. This mouse model could augment a huge breakthrough in understanding the paracrine and intracrine actions of oestrogens. Aromatase is expressed in many extra-gonadal tissues (including the brain, adipose tissues, mammary glands, prostates, bones) which also express oestrogen receptor. Local actions of oestrogens in these tissues must be important especially when circulating levels of oestrogens are low, for example in men and postmenopausal women. However, up till now, the understanding of the local functions of oestrogens in the brain during development has not been possible. In addition, this model can also aid research in other endocrinology areas such as osteoporosis, prostate cancer and breast cancer which have been shown to be under the influences of oestrogens.

Application of a revolutionary method to greatly enhance enzymatic reaction in microlitre ranges.

In collaboration with Dr Tim Aumann and Prof Malcolm Horne and CSIRO, we proved that the novel method acoustic microstreaming ("micromixing"), which mixes fluid at microlitre scales, could improve cDNA yields from reverse transcription (RT) reactions comprising single-cell quantities of RNA by 10- to 100-fold more than cDNA in the absence of micromixing. This method has application in microarray, high throughput Next Gen Technology. Provisional Patent of this Method has been filed, with 3 other inventors of the method and commercialisation is in progress. In applying this technology to my study I will be able to detect the changes in gene expression in single neurons associated with the observed phenotypes.

STAFF

Wah Chin Boon, PhD
Hui Kheng Chua, BSc Hons
We have now found a way to identify and separate the therapeutic from the dangerous cells in Parkinson’s disease. By identifying novel molecules on the therapeutic cells, we’ve been able to... pull them out and purify them – a significant advance towards clinical translation.

Lab-Head, Dr Lachlan Thompson speaking on ABC radio’s AM program, November 14, 2011

Publications


Multiple sclerosis (MS) is the most common neurological disorder causing neurological disability in young adults and affects approximately 2 million people worldwide. It causes disability due to loss of nerve conduction in myelinated axons. Our group is testing the hypothesis that drugs that selectively block sodium channels in damaged axons will reduce the neurological progression of this disorder. We have synthesised 30 novel compounds that block sodium channels in vitro as shown by patch clamping and will carry out in vivo testing in a mouse model of MS when funds are obtained.

MS is considered to be a chronic inflammatory disease of the central nervous system with intervals of remission followed by relapse in a majority of cases. Its pathology is characterized by multifocal inflammatory lesions in white matter due to the presence of infiltrated immune cells that migrate across the blood-brain barrier which then release a variety of proinflammatory mediators resulting in an attack on the myelin sheath. This results in axonal retraction and subsequent astroglosis and microgliosis. In the past, MS has been regarded as an autoimmune disease and the therapeutic strategy has been to reduce the inflammatory response to slow or block the autoimmune-induced destruction of the myelin sheath around axons by T-cells and macrophages which has resulted in some therapeutic benefit. However, it has become apparent that such drugs are not stopping or preventing disease progression. So research has now refocused on damage to the underlying axon in white matter that is causing irreversible neurological disability in MS patients. It also follows that drugs with a neuroprotective action as well as anti-inflammatory drugs could be a better strategy to limit the disability in MS subjects.

An accumulation of sodium ions in demyelinated axons through sodium channels is an important process in axonal degeneration and several studies in rodents with experimental autoimmune encephalomyelitis have shown that oral administration of sodium channel blockers such as phenytoin, carbamazepine and lamotrigine resulted in protection of the spinal cord corticospinal tract and dorsal column by anatomical and electrophysiological tests as well as reducing inflammatory cell infiltration into the spinal cord. Molecular biology techniques have shown that there are at least 9 subtypes of sodium channels in the body that have different pharmacological properties. Furthermore, demyelinated axons are expressing increased numbers of the sodium channel subtype, Nav 1.6 and that finding a compound which selectively inhibits this subtype but not other subtypes could lead to a better drug with fewer side-effects for treating MS. We therefore initiated a medicinal chemistry/neuropharmacology project to design and develop a Nav 1.6 selective sodium channel blocker.

Medicinal chemistry

We started with an orally active sodium channel blocking drug, mexiletine, that was developed to treat cardiac arrhythmias and have shown that by synthesising a related compound (HFI-1) that was more lipid soluble (Figure 1) then it was more potent than mexiletine in minimizing white matter injury after spinal cord injury in rats. HFI-1 was found to be 80 times more potent than mexiletine as a sodium channel blocker in a brain radioligand assay and thus it was appropriate to use HFI-1 as a template for designing and then synthesising related compounds. We have now identified structural analogues by modifications to the ring structures at both the left hand side and the right hand side of HFI-1 to give us a library of approximately 50 related compounds. Their synthesis and characterisation have been achieved by our collaborator, Associate Professor Spencer Williams in the School of Chemistry.
Neuropharmacology testing
All compounds have been tested using a radioligand assay for sodium channel blockers by measuring inhibition of binding of $^3$H-batrachotoxinin that binds to sodium channels in rat brain membranes as an initial screen. While this determines if a compound is a sodium channel blocker, it does not distinguish between the 9 subtypes of sodium channels that have been identified by molecular biology techniques.

Therefore we are screening our compounds by a patch clamping technique in stably transfected cell lines expressing either human Nav 1.6 or human Nav 1.2 ion channels in order to assess selectivity for Nav 1.6 channels with this electrophysiological technique. This is on going in 2012 and the most selective Nav 1.6 compound will then be evaluated in an established mouse model of MS (experimental autoimmune encephalomyelitis model) during chronic treatment orally for 21 days using behavioural tests before killing the mouse in order to quantitate neuroprotection against axonal pathology by imaging techniques.

STAFF
Honorary Professorial Fellow
Beyn Jarrott. PhD (Camb), BPharm (Hons) (UQ)

COLLABORATIVE LINKS
Assoc Prof Spencer Williams
School of Chemistry and Bio
21 Institute
University of Melbourne
Medicinal Chemistry

PUBLICATIONS
Our research focus on the relaxin family and their receptors continued in 2010 with important advances in understanding the peptide’s structure and function, the role of relaxin in fibrosis, receptor function and signalling, and the role of relaxin-3 in the modulation of arousal, sleep/wake patterns, mood and memory. Studies on the physiological role of the enzyme IRAP have opened the way for new treatments of memory disorders. Dr Siew-Yeen Chai and her colleagues discovered a series of small molecule IRAP inhibitors with memory enhancing properties that have exciting potential for improving memory loss.

The hormone relaxin mediates cardiovascular and kidney changes during pregnancy. These important functions have led to its current use in clinical trials for the treatment of acute heart failure, a condition affecting millions of patients worldwide. One of the world’s largest pharmaceutical companies, Novartis, is presently managing the final Phase III relaxin clinical trials for the treatment of acute heart failure. The Phase III clinical trial involves 1,000 patients worldwide and is expected to be completed in 2012. Our relaxin research program is an outstanding example of a basic Australian medical research discovery leading to the development of a clinically valuable therapeutic.

SNAPSHOT OF OUR STUDIES

Mental illness is an economic and health burden worldwide, with huge costs in medical spending, lost productivity, poor quality of life for sufferers and mortality. Relaxin-3 is a neuropeptide that acts widely within the brain to regulate key pathways and fundamental brain rhythms altered in conditions such as anxiety and mood/sleep disorders. Like the well known hormone insulin, relaxin-3 is complex and difficult to make commercially. Our 2010 studies have identified simpler forms of the peptide that either mimic or block the action of relaxin-3. These potential new drugs have been patented, and parallel studies have discovered that they may also be used for the treatment of obesity, depression and anxiety.

RESEARCH HIGHLIGHT

SNRIO STAFF

A/Professor Ross A. D. Bathgate
Dr. Siew Yeen Chai (until Jan 2011)
A/Professor Andrew Gundlach
Dr. Chrishan S. Samuel
Dr Daniel Scott (from October 2011)
Professor John D. Wade
Dr Stephanie Bissiere (from August 2011)
MAJOR RESEARCH PROJECTS

Functional and structural analysis of relaxin family peptide receptors
Ross Bathgate, Ray Kong, Natalie Witteveen, Daniel Scott, Jason Ling, Tania Ferraro, Sharon Layfield in collaboration with Emma Petrie, Patrick Shilling and Paul Gooley, Department of Biochemistry and Molecular Biology, University of Melbourne and Johan Rosengren, University of Queensland.

The Neuropeptides division has made great advances in understanding the biological functions of the various relaxin peptides. Relaxin is currently in a Phase III for the treatment of acute heart failure and the other peptides are showing great potential as therapeutics for numerous diseases (see other project details). However, to achieve our goals of designing smaller, more potent drugs targeting these peptide systems, a better understanding of ligand-receptor interactions is necessary for rational structure-based drug design.

The receptors for these peptides are all G-protein coupled receptors (GPCRs) which are the largest class of cell surface signalling molecules and major drug targets. The receptors for relaxin and INSL3, RXFP1 and RXFP2 are leucine rich-repeat containing GPCRs with large extracellular domains (see figure). Relaxin-3 and INSL5 interact with unrelated receptors RXFP3 and RXFP4 which are more like classic peptide GPCRs and lack a large ectodomain.

We are using various molecular and pharmacological techniques to determine the ligand binding specificities of the receptors, the mechanisms of receptor activation, and their cell signalling characteristics. These studies are performed on recombinant and native receptors expressed in cells, and provide a detailed understanding of the important functional domains of the receptor. However, other techniques are required to determine the precise interaction sites of the ligands with the receptors, so we are working with structural biologists A/Prof Paul Gooley and Dr Johan Rosengren in using various biochemical and NMR techniques to study aspects of the receptors structure and the functions of the individual receptor protein domains. A complete understanding of the binding sites is required to design drugs targeting these receptors, and this dual approach enables the precise mapping of ligand interaction sites in the receptors.

Role of brain relaxin-3 networks in control of complex behaviour revealed by studies with novel, minimised peptide analogues
Andrew Gundlach, Philip Ryan, Elena Büchler, Hanna Kastman, Shinie Ma, M Akhter Hassain, Fazel Shabanpoor, John Wade, Ross Bathgate, in collaboration with Andrew Lawrence, Florey Neuroscience Institutes, Johan Rosengren, University of Queensland.

A major challenge in the field of ‘systems neuroscience’ is to identify the neural circuits that underlie specific behaviours, and determine how they are perturbed in psychiatric disorders. This task has been aided in recent years by extensive neuroimaging studies of control and patient groups, better animal models of normal human behaviour and psychiatric illness and well integrated scientific investigations.

A primary experimental approach to understanding the physiological and therapeutic potential of a particular transmitter-neuronal membrane receptor combination is to determine the effect on brain chemistry and behaviour of direct injections into the brain of molecules that selectively activate (an agonist) or block (an antagonist) the receptor.

In our recent studies, we used newly designed, minimised relaxin-3 analogues to further our understanding of relaxin-3/RXFP3 signalling in rat brain. Initially, we determined the ability of acute central intra-cerebroventricular injection of a new RXFP3 agonist to replicate the stimulatory effect of native relaxin-3 on feeding in satiated rats. We then demonstrated that this RXFP3-A2 peptide reduced the level of anxiety-like behaviour in rats in the elevated plus maze and light/dark box tests. In separate studies we demonstrated that RXFP3 antagonists blocked the effect of RXFP3-A2 on feeding in naive rats and reduced levels of alcohol self-administration in a breed of alcohol-prefering rats. Studies are now being completed to assess the ability of antagonist peptides to block the agonist-induced effects on anxiety behaviour and determine whether the RXFP3 agonist can also alter motivated behaviours.

Similar acute and chronic administration studies are required to explore further putative physiological and pathological functions of this extensive neuropeptide-receptor network. This approach is being combined with local peptide injections into RXFP3-rich brain areas to determine more precisely which circuits are modified to generate specific behavioural effects. For example, activation of RXFP3 in the central amygdala reduces fear expression during the memory extinction training phase of a classic Pavlovian fear conditioning paradigm. Dysfunctional amygdala activity underlies anxiety disorders such as generalized anxiety and post-traumatic stress disorder; thus, our future studies will investigate the comparative role of relaxin-3 in modulating amygdala activity under neutral and stressful circumstances in rats, and its therapeutic potential as a treatment for anxiety. Our findings have attracted the interest of major pharmaceutical companies, and related studies will be expedited in 2011/2012 with access to the extensive behavioural facilities in the new Florey at the Melbourne Brain Centre.

Peptide chemistry and structure-function relationship studies on insulin superfamily peptides
M. Akhter Hassain, Suode Zhang, Fazel Shabanpoor, Ross Bathgate, Andrew Gundlach and John Wade in collaboration with K. Johan Rosengren, Institute for Molecular Bioscience, University of Queensland.

In the human, the insulin superfamily consists of ten members, eight of which possess a characteristic core structure comprising of two strands of peptides that are linked together by a pair of chemical bridges known as disulphide bonds. A third bridge exists within one of the strands. The resulting conformation of the molecules is very compact with only the termini of the
Relaxin (H2 relaxin), one of these insulin-like peptides, has long been a focus of study in our laboratory. It is a potent anti-fibrotic agent, also has pronounced actions on the cardiovascular system. It is currently undergoing Phase III clinical trials by our commercial partner, Novartis (Switzerland) for the treatment of congestive heart failure. The indications are that it is poised to enter the clinic in 2013. Despite this exciting development, it is evident from studies to date that H2 relaxin has far from ideal pharmacokinetic properties for effective therapeutic use. In particular, longer acting variants of H2 relaxin will be required if clinically manageable and cost-effective dosing regimens are to be developed. We are currently working to address this pressing issue in the anticipated clinical use of H2 relaxin. Building on our structure-function knowledge in 2010 and before, we will examine the metabolic stability and renal clearance properties that are responsible for the short half life exhibited by H2 relaxin. We will subsequently use this information to develop H2 relaxin analogues that are both more stable and less easily cleared by the kidneys than the parent, whilst still retaining potency as relaxin receptor agonists. A major outcome of our 2011 work is the preparation of the first ever relaxin receptor antagonist. We have demonstrated its effectiveness in native relaxin receptor expressing cells and, importantly, that it blocks H2 relaxin-induced cancer cell proliferation. We are currently working to improve the affinity and specificity of this compound and also seek to develop novel antagonists.

Relaxin-3 (H3 relaxin) is another insulin-like peptide that is primarily expressed within the brain. Studies in animals have shown that relaxin-3 is strongly responsive to stress, and that relaxin-3R3RPFP3 signaling influences spatial working memory and hippocampal activity, feeding and metabolism and related neuroendocrine function, and circadian activity and sleep patterns.

As such, H3 relaxin and its analogues have exciting therapeutic potential for the treatment of disorders such as obesity, depression and anxiety. Our 2010 studies on H3 relaxin structure and function relationships have led to the identification of a single, truncated B-chain peptide that acts as potent antagonist, and a minimized two-chain H3 relaxin that lacks an intra molecular disulide bond, that acts as an agonist. Our current focus is to utilize these newly generated analogues to design and chemically synthesize additional domain minimized and proteolytically-stable analogues of H3 relaxin. We are using state-of-the-art peptide synthesis technology and drug design tools in conjunction with NMR techniques, to prepare simpler H3 relaxin analogues in sufficient quantities to allow their subsequent detailed biological study, using molecular biology, pharmacology and neurobiology techniques.

Insulin-like peptide 5 (INSL5), another member of relaxin family, was recently identified by our commercial partner, Takeda Cambridge (UK), to be a gut peptide that is a potent regulator of feeding – a remarkable property that is very different to the actions of relaxin. Agonists and antagonists of this peptide may prove to be very useful for the treatment of eating disorders such as anorexia. Surprisingly, this peptide proved to be much more difficult to make than other insulin superfamily members, with each of its two peptide strands being very poorly soluble and obtained only in very low yields. However, using novel synthetic techniques developed in 2010 in our laboratory, we were able to produce sufficient quantities to not only confirm its potent receptor binding and activation potential but also to determine its tertiary structure by a technique known as solution NMR spectroscopy. This has allowed us to further identify important elements for its unique activity, information which we will now exploit for the production of potential therapeutics.

Role of relaxin-3 peptide-receptor networks in mammalian brain – insights from anatomical, regulatory and behavioural studies in normal and transgenic mice

Andrew Gundlach, Craig Smith, Ihaia Hosken, Berenice Chua, Qian Sang, Shene Ma, M Akhter Hossain, Sharon Layfield, Luke Chen, Weizmann Institute of Science, Rehovot, Israel

Functional studies looking at the effects of relaxin-3 knockout mice on the brain revealed likely species differences in the way that this is manifest behaviourally. The knockout control mice backcrossed onto a C57BL/6J genetic background display a behavioural phenotype similar in many ways to their littermates under basal physiological conditions. An important difference is the circadian hypothermia displayed by male and female relaxin-3 and receptor KO mice that is reflected by reduced voluntary wheel running during the normal active-dark phase. In studies employing viral vectors to restore relaxin-3 to the brain of relaxin-3 KO mice we are examining if we can restore the behaviour of these mice to that of their wildtype littersmates. Indeed, environmental changes or handling of KO mice have been observed to disrupt the ‘fragile’ hypothermia characteristic. KO mice are being tested for any differential responses to acute and chronic stressors; and we are using remote telemetry to follow these animals in arousal and motivated behaviour. The relaxation studies in normal and KO mice revealed likely species differences in the role of relaxin-3, or at least, in ways that this is manifest behaviourally.

Future studies are planned to explore the potential of relaxin-3 neuronal-receptor system in arousal and motivated behaviour and to take advantage of additional genetically-modified strains of relaxin-3 knockout mice that better understand the nature of target cells/circuits in the brain and the precise mechanisms of action involved.

OUR STAFF

Fellows, senior research officers, and research officers

Anthony Albiston
BSc (Hons) (Melb) (PhD) (Mon) (until Jan 2011)
Ross A. D. Bathgate
BSc (Hons) (PhD) (Eld)
Stephanie Bisserie (from August 2011)
BSc PhD
Siew Yean Chai
BSc (Hons) (Mon) (PhD) (until Jan 2011)
Shanti Divakarla
BSc (Hons) PhD (Melb) (until Jan 2011)
Andrew Gundlach
BSc (Hons) DipEd (Mon) (PhD) (Melb)
Mohammed Akhter Hossain
BSc (Hons) MSc (Dhaka) (PhD) (Tokyo)
Shene Ma
BSc (Hons) (Mon) (PhD) (Melb)
Tomin Mustafa
BSc (Hons) (VUT) PhD (Melb) (until Jan 2011)
Vi Pham
BSc (Hons) (Mon) (PhD) (Melb)
Fazel Shabanpoor
BSc (Hons), PhD (Melb)
Giang Sang
MD (Beijing) (PhD) (Melb)
Daniel Scott
BBiomedSc (Hons), PhD (Melb) (from October 2011)
Fazel Shabanpoor
BBiomedSc BSc (Hons) (PhD) (Melb) (from April 2010)
Craig Smith
BBiomedSc BSc (Hons) (PhD) (Melb)
John Waite
BSc (Hons) (PhD) (Mon)
CSci CChem FRACI FRSC
Melanie White
BSc (Hons) BA (Victoria University, NZ) PhD (University College, London) (from October 2011)
Shuode Zhang
BSc MSc PhD (Peking)
Mohammed Akhter Hossain
Neuro2010 Travel Award ($1000 plus Registration and Accommodation)
Millipore Postdoctoral Travel Award: $2000 (2011)
Harold Mitchell Foundation Travel Fellowship ($5000) (2011)
Best Oral Presentation Award, 3rd meeting of the Florey Postdoctoral Association, Austin Tower, Melbourne ($250 plus a certificate) (2011)
Australian Academy of Science Travel Award ($6300) (2011)

Ilaha Hosken
NHMRC (Australia) Dora Lush Scholarship

Fazel Shabanpour
David Hay post-graduate write-up award, University of Melbourne, Melbourne, Australia, Feb 2010

Sherie Ma
Australian Academy of Science and French Embassy, Visits to Europe Fellowship, 2010
Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) Denis Wade Johnson & Johnson New Investigator Award, 2010
Florey Neuroscience Institutes Postdoctoral Association and Scientific Travel Award, 2010
CASS Foundation Travel Grant, 2010
Gordon Research Conference Travel Grant, 2011
International Brain Research Organization Travel Grant, 2011

Philip Ryan
Aaye Berke and Liana Colvill Travel Award (best 2nd year student presentation) 2010

Roy Chze Khai Long
ASCEPT 2010 student poster prize finalist
Molecular Pharmacology of GPCRs 2010 student poster prize finalist

Craig Smith
International Society of Neurochemistry (ISN) Advanced School of Neurochemistry, Greece, 2011 (Invited Scholar)
International Society of Neurochemistry (ISN) Travel Grant, 2011
CASS Foundation Travel Grant, 2011

Holly Yeatman
Harold Mitchell Travel Award (Best 3rd year student presentation at HFI Annual Student Presentations)

CONFERENCEs AND PREsENTATIONS

Alessia Belgi
31st European Peptide Symposium, 5th-9th Sept, 2010, Copenhagen, Denmark
1st Italy-Australia (ITOZ) symposium, Prato, Italy 10th-12th Sept (2010)

Ross A D Bathgate
BIT’s 8th Annual Congress of International Drug Discovery Science and Technology (DDST), Beijing, China, October 2010 (Invited speaker)
2nd Annual GPCR Congress, Montreux, Switzerland, June 2010 (Invited Discussion Session Chair)
Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists, ASCEPT, Melbourne, November 2010 (Invited speaker)

Linda J Chan
5th International Peptide Symposium, Dec 4-9, Kyoto, Japan

Gabrielle Callander
FENS 7th Forum of European Neuroscience, Amsterdam, The Netherlands

Despina Ganella
INF 7th International Congress on Neuroendocrinology, Rouen, France

Andrew Gundlach
FENS 7th Forum of European Neuroscience, Amsterdam, The Netherlands
INF 7th International Congress on Neuroendocrinology, Rouen, France

Society for Neuroscience Annual Meeting, San Diego, CA, USA, 2010
5th Australian Health & Medical Research Congress, Melbourne, Victoria, Australia (Invited Symposium Chair)
Centre for Brain Research, The University of Auckland, New Zealand (invited seminar)
5th CINP Pacific-Asia Regional Meeting, Kuala Lumpur, Malaysia (Invited Chair , Symposium) 2011

Mohammed Akhter Hossain
Neuro2010 symposium, Sept 2-4, Kobe, Japan
17th Diabetic & Endocrine Conference, Dhaka, Bangladesh 13-15 December 2011 (invited speaker)

Christian Samuel
International Symposium on Dupuytren’s Disease, Miami, Florida, USA (invited speaker)
20th World Congress of the International Society for Heart Research, Kyoto, Japan
5th Australian Health & Medical Research Congress, Melbourne, Victoria, Australia

John D Wade
31st European Peptide Symposium, Sept 5-9, Copenhagen, Denmark
1st Italy-Australia (ITOZ) symposium, Sept 10-12, Prato, Italy
5th International Peptide Symposium, Dec 4-9, Kyoto, Japan

Sherie Ma
Society for Neuroscience Annual Meeting, San Diego, CA, USA, 2010
Australian Neuroscience Society Satellite - Neuroscience of Fear and Anxiety, Sydney, 2010 (Invited speaker)
Gordon Research Conference Amygdala in Health and Disease, New Hampshire, 2011

Philip Ryan
Society for Neuroscience Annual Meeting, Washington DC, USA, 2011
21st Neuropharmacology Conference - Anxiety and Depression, 2011
Biological Psychiatry Australia, 2011

Craig Smith
International Society of Neurochemistry (ISN) Advanced School of Neurochemistry, Greece, 2011
5th Australian Health & Medical Research Congress, Melbourne, Victoria, Australia (Invited Symposium Chair)

MAJOR COLLABORATIVE LINKS

National
Prof Michael Conlon
University of UAE, UAE
Antimicrobial peptides

Dr Xiao-Jun Du
Baker Heart Research Institute, Melbourne
Role of relaxin in the heart and potential of relaxin as a treatment for cardiac fibrosis

A/Prof Paul Gooley
Department of Biochemistry and Molecular Biology, University of Melbourne
Structural studies on the relaxin family peptide receptors

Dr Tim Hewson
Department of Nephrology, Royal Melbourne Hospital, Melbourne
Investigating the therapeutic potential of relaxin in intestinal renal fibrosis

Dr Tony Hughes
Department of Pharmacology, University of Melbourne
Design and synthesis of conformationally constrained mimetics of relaxin and INS3

Prof John Hutson
Douglas Stephens Surgical Laboratory, Murdoch Children’s Research Institute, Melbourne
The role of RLF (INS3) in gynecological development

Prof Richard Iwell
School of Molecular and Biomedical Sciences, University of Queensland
Role of IRAP in antigen cross presentation

Dr Anthony White,
Department of Pathology, University of Melbourne
Role of IRAP in Alzheimer’s disease

A/Prof Tony Verberne
Department of Medicine - Austin and Northern Health, University of Melbourne
Relaxin-3 systems in the brain: Neuropsychology and behaviour

Dr's Robert Widdop and Tracey Gaspar
Department of Pharmacology, Monash University
Investigations into the cardiovascular function of the IRAP knockout mouse

A/Prof Ray Rogers
Reproductive Medicine Unit, Department of Obstetrics and Gynecology, University of Adelaide
The function of ovarian follicular INS3

A/Prof Andrea J Robinson
School of Chemistry, Monash University
Chemistry of alcalba relaxin peptides

Dr Johan Rosengren
University of Queensland
Tertiary structure determination of relaxin-2, relaxin-3, INS3, R3-I5, and INS5 peptides

Prof Roger Summers
Department of Pharmacology, Monash University
Relaxin receptor signalling

A/Prof Mimi Tang
Department of Allergy and Immunology, Murdoch Children’s Research Institute, Melbourne
Investigating the therapeutic effects of relaxin on airway remodeling and function

Prof Walter Thomas
School of Biomedical Sciences, University of Queensland
Viral strategies for studying relaxin peptide function

Dr Philip Thompson,
Department of Medieval Chemistry, Monash University
Design of small molecular IRAP inhibitors

Dr Jose Villadangos,
Walter and Eliza Hall Institute
Role of IRAP in antigen cross presentation

Vinod Nair
BBSc (Hons) (Melb) (from Feb 2011)

Philip Ryan
MBBS (Hons) BMedSc (Melb)

Fazel Shabanpour
BBSc (Hons) (Melb) (to March 2010)
Natalie Anne Witteveen
BBSc (Hons) (Melb)
Andrew Walker (from July 2011)
BBSc (Nebraska, Lincoln)
Su Ee Wong
BBSc (Hons) (Melb)

Kelvin Junwei Yong
BBSc (Melb)
Holly Yeatman
BBSc (Melb) (to March 2010)

(2011)
Holly Yeatman
BBSc (Melb) (Murdoch University) (until Jan 2011)

Visiting scholars
Elena Buchler, University of Applied Sciences Gelsenkirchen, Recklinghausen, Germany, Undergraduate Research Project
Jia Mei Hong, National University of Singapore, Singapore, Endeavour Australia Cheung Kong Research Fellowship, 2010
Anna Blasiak, Jagiellonian University, Krakow, Poland, Endeavour Australia Research Fellowship, 2011
Ana-Maria Sanchez-Perez, University of Valencia, Valencia, Spain, Koplowitz Foundation Training Fellowship

AWARDS

Linda J Chan
Young Investigators’ Award (Good Stone Award for oral presentation at the 5th International Peptide Symposium, Kyoto, Japan)
Melbourne Abroad Travelling Scholarship (MATIS), The University of Melbourne
Special Postgraduate Studenthips, School of Chemistry, The University of Melbourne

Despina Electra Ganella
Florey 2010 second year student talk prize

Mohammed Akhter Hossain
Neuro2010 Travel Award ($1000 plus Registration and Accommodation)
Millipore Postdoctoral Travel Award: $2000 (2011)
Harold Mitchell Foundation Travel Fellowship ($5000) (2011)
Best Oral Presentation Award, 3rd meeting of the Florey Postdoctoral Association, Austin Tower, Melbourne ($250 plus a certificate) (2011)
Australian Academy of Science Travel Award ($6300) (2011)
Dr Edward Amento
Molecular Medicine Research Institute, San Francisco, USA
Relaxin and extracellular matrix biology

Dr Anna Blasiak
Department of Neurophysiology & Chronobiology, Jagiellonian University, Krakow, Poland
Relaxin-3 systems and biorhythms

Dr Marco Capagno
MRC Anatomical Neuropharmacology Unit, Oxford, UK
Cellular physiology of relaxin-3 in amygdala

Prof Alon Chen
Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel (and Prof Andrew Lawrence, Florey Neuroscience Institute)
Interaction of relaxin-3 and CRF systems

A/Prof Gavin Dawe
Department of Pharmacology, National University of Singapore, Singapore
Relaxin-3 systems in adult neurogenesis and hippocampal function

Prof Pierre De Meyts
Receptor Systems Biology Laboratory, Hagedorn Research Institute, Denmark
Dimerization of relaxin family peptide receptors

Prof Aaron Hsueh
Division of Reproductive Biology, Department of Gynecology and Obstetrics, Stanford University Medical Centre, Stanford University, CA, USA
Defining the structural domains of the LGR7 and LGR8 receptors and determining the functions of INSL3 in the gonads

Dr Patrick Kehoe
Department of Clinical Science at North Bristol, University of Bristol, Frenchay Hospital, Bristol, UK
IRAP gene polymorphism and Alzheimer’s disease

Dr Jose Lanciego
Neurosciences Division, CIMA, University of Navarra, Pamplona, Spain
Comparative studies of relaxin-family peptides and receptors in non-human primate

Prof Tim Lovenberg
Pharmaceutical Research and Development, Johnson and Johnson, San Diego, USA
Distribution, regulation and function of relaxin-3 and GPCR135 in the brain

Prof Thomas McCown
Department of Psychiatry, University of North Carolina, Chapel Hill, NC, USA
Viral strategies for studying relaxin-3 function

Dr Ewan McNay
Section of Endocrinology, Yale University School of Medicine, New Haven, CT, USA
Role of IRAP in modulating brain glucose uptake

Professor Yvette Michotte, Professor Ilse Smolders and Dr Patrick Vanderheyden, Vrije Universiteit Brussel, Belgium
Characterisation of the global and tissue specific IRAP Knockout mouse

Dr Sietse Mosselman and Dr Anne Riesewijk
Department of Pharmacology, NV Organon, The Netherlands
Phenotyping the LGR7 KO mouse and cloning and characterising rodent LGR7 genes

A/Prof Angel Nunez
Department of Anatomy, Histology & Neuroscience, University Autonoma Madrid, Madrid, Spain
Nucleus incertus and relaxin-3 in theta rhythm generation

A/Prof Francisco Olucha-Bordonau
Department of Human Anatomy and Embryology, University of Valencia, Valencia, Spain
Anatomy and function of nucleus incertus and relaxin-3 pathways

Prof Lazlo Otvos Jr
The Wistar Institute, Philadelphia, USA
Structural analyses of relaxin and INSL3

Prof David Sherwood
Department of Molecular and Integrative Physiology, University of Illinois, USA
Studies on rodent relaxin receptors

Dr Dennis Stewart and Dr Elaine Unemori
Corthera, San Mateo, USA
Development of relaxin as an antibiotic drug

Tas Task concluded: 125
PUBLICATIONS


The ischaemic penumbra continues to be an important focus of our research. This is brain tissue which, while damaged, continues to live after the onset of the stroke process. The duration of this is uncertain in humans, although we now have the ability to create images of its behaviour using positron emission tomography (PET) and magnetic resonance imaging (MRI).

Using MRI, we have shown that salvageable penumbral brain tissue may last up to 48 hours post stroke onset, and that the distribution of this salvageable tissue becomes more variable with time. Further, improved computer co-registration techniques may identify more patients than previously realised who have penumbral tissue. This may expand the number of patients eligible for treatment with thrombolytic agents such as tPA.

Another initiative of significance is the use of PET, using 18F-PIB to image amyloid plaque in patients with recent onset ischaemic and haemorrhagic stroke. There are interesting links between vascular dementia, stroke and Alzheimer’s disease which may be, in part, unravelled using this technique.

We are completing an investigation concerning the clinical significance of a newly appreciated ultrasound sign, referred to as “small vessel knock”. The Florey is collaborating with Compumedics DWL, a Melbourne-based transcranial Doppler ultrasound company, to determine whether knock is useful in the diagnosis of stroke. Dr Chesda Udommongkol, a stroke research fellow from Bangkok, is completing his PhD study of knock, due for completion in 2012. Dr Udommongkol’s work has found that there are other causes of knock-like signals including wall-motion artefacts. In collaboration with CSIRO and a group in Leicester UK, he created a bench-top scaled-up model of a vessel bifurcation perfused by blood-mimicking fluid and a pump simulating normal pulsatile flow.
In vivo 11C-PiB binding is increased in patients with cerebral amyloid angiopathy related haemorrhage

John V. Ly, Geoffrey A. Donnan, Victor L. Villermagne, Jorge A. Zavala, Henry Ma, Graeme O’Keefe, Sylvia J Gong, Rico M Gunawan, Tim Saunders, Uwe Ackerman, Henri Tochon-Dangoy, Christopher C Rowe

The in vivo-diagnosis of cerebral amyloid angiopathy (CAA) is inferred from clinical and structural imaging features. 11C-PiB is a PET ligand that binds to beta-amyloid (Ab) both in extracellular plaques and vessel walls. We hypothesized that patients with a clinical diagnosis of CAA-related ICH (CAA-IH) have increased 11C-PiB uptake and that the pattern differs from that seen in AD. 11C-PiB PET may assist the in-vivo diagnosis of CAA and serve as a surrogate marker for future therapeutic studies.

CAA detected by PiB PET predisposes to ITPA related haemorrhage

John V Ly, Christopher C Rowe, Victor L Villermagne, Jorge A Zavala, Henry Ma, Graeme O’Keefe, Sylvia J Gong, Rico M Gunawan, Leandil Churilov, Tim Saunders, John Sachinidis, Uwe Ackerman, Henri Tochon-Dangoy, Geoffrey A Donnan

Increasing evidence suggests that cerebral amyloid angiopathy (CAA) may be an important predisposing factor for the haemorrhagic complications of rtPA therapy. N-Methyl-11C-2-(4’-methylaminophenyl)-6-hydroxybenzothiazole (11C-PiB) is a PET amyloid ligand which also binds to beta-amyloid in cerebral arteriolar walls. We hypothesized that patients who developed parenchymal haemorrhage (PH) after ITPA may have increased 11C-PiB PET retention compared to those with no haemorrhagic complications. Patients treated within 3 hours of onset of ischaemic stroke with ITPA were studied using PET to compare PiB retention in those with and without parenchymal haematomata formation and normal age matched controls. Ischaemic stroke patients treated with ITPA who developed PH were shown to have higher neocortical PiB retention compared to those without PH, suggesting underlying CAA as a predisposing factor for ITPA related haemorrhage. The high sensitivity and specificity for this finding may provide an impetus for the development of a more practical rapid pre-treatment screening technique.

Other research projects

Preconditioning stroke fMRI
Geoff Donnan, Amy Brodmann

The human brain is a remarkably plastic organ, capable of reorganisation following injury even until the ninth decade. This reorganisation – or remodelling – has been well described following stroke, but the drivers and determinants of this phenomenon remain poorly understood. It is understood that a lack of blood supply, such as in ischaemic stroke, leads to utilisation of other areas of the brain, both adjacent to the injury and in other distant but functionally connected cortex. However, it is not known whether reduced blood supply, such as occurs in patients with arterial narrowing (stenoses), resulting in chronic hypoperfusion, may also be driving remodelling. In this study, I am examining the effects of this reduced blood supply on cortical organisation. The study involves magnetic resonance imaging in a group of patients with arterial narrowing not causing stroke, or asymptomatic carotid stenosis. Perfusion scanning is performed to assess the arterial supply, MR spectroscopy to look for neuronal markers of chronic ischaemia, and functional MRI to create maps of motor and visual activation. These maps are compared both with those obtained from a healthy control group and the unaffected side in the patient population. If shifts to these activation maps are demonstrated and correlated with perfusion imaging, these results may provide evidence of how the brain “prepares” itself for further injury, by shifting cortical function to other, well perfused regions of the functional cortical network.

The non-uniform topographic evolution of the ischaemic penumbra with time.
Ma H, Zavala JA, Teoh HJ, Churilov L, Gunawan M, Ly J, Wright P, Phan T, Arakawa S, Davis S M, Donnan G A

The classical pattern of the ischaemic penumbra is defined by a central infarct core surrounded by a uniform annulus of salvageable tissue. With time, numerous factors may influence the rate and extent of penumbral salvage and, hence, alter this pattern. We hypothesized that with co-registration of magnetic resonance diffusion and perfusion weighted images (MR DWI/PWI), we could identify a series of differing penumbral patterns which evolved with time. Patients were recruited with MR studies performed within 48 hours of ischaemic stroke onset. Mismatch pattern was demonstrated by co-registration of DWI/PWI images and categorized as classical (majority of the DWI within the PWI lesion) or non – classical patterns. The proportion of the two patterns was assessed with reference to time. With 48 hours of stroke onset both classical and non-classical mismatch patterns were identified. The former occurred earlier with larger mismatch volumes. The reduction of classical pattern with time reflected a non-uniform evolution of the ischaemic penumbra. The recognition of these patterns highlights the need to individualize patients when considering the potential for tissue salvage.

Detection of small vessel knock signals by Doppler ultrasound in a laboratory model simulating a penetrating branch occlusion.
Chesda Udommongkol, Brian Chambers, Richard Maraness, Ilja Sutala, Ben Aldham, Emma Chung, Geoffrey Donnan

Small vessel knock (SVK) signals were recorded using Doppler ultrasound in a laboratory model simulating a penetrating branch occlusion. Aim: To demonstrate SVK in a laboratory model simulating a cerebral branch occlusion. Materials and method: In a series of in vitro studies in a new transcranial Doppler (TCD) model of penetrating artery occlusion. However, knock-like signals may be caused by wall motion of normal basal cerebral arteries. Preliminary in vivo studies in Leicester suggest that SVK can be recorded using TCD ionisation of a side-branch lumen independent from the vessel wall.

The circuit was filled with blood mimicking fluid (BMF) that contained physical and acoustic properties similar to human blood. Flow and viscosity were controlled to conform to normal human physiology. A scaled-up synthetic bifurcation was formed from silicone tubing with internal diameter 9.5 mm for the main artery and 2.38 mm for the side branch. It was submerged in the water tank and insonated by a Doppler probe and Sonix RP Ultrasound with 7.5 MHz probe. Results: BMF recirculation in the blocked branch was detected by video recording after dye injection and colour Doppler. Spectral Doppler demonstrated corresponding continuous flow with no low-frequency Doppler signals. However, SVK was not observed. Conclusion: In our in-vitro model, SVK was not detected by TCD and duplex ultrasound. Factors that might explain...
These negative results include angle of vessel bifurcation, flow pressure and tube material.

**Biomarkers in stroke (see also stroke basic science)**

Mane Dragonnier, David Howells, Victoria O’Collins, Jenny Favaloro, Bill Wilson, Helen Dewey, Geoffrey Donnan

There has always been a great need to develop blood based biomarkers to aid in the diagnosis of stroke, much the same way in which CPK and Troponin are very useful biomarkers to increase the sensitivity and specificity for the diagnosis of myocardial infarction.

A number of inflammatory and other biomarkers have been known to be elevated in the blood for many years, but several technological advances now make identification of a much larger number of compounds simpler and feasible at the point of care. These are being studied in animal models in Prof. David Howells laboratory in relation to factors such as time of stroke onset, the presence of haemorrhagic transformation and the proportion of infarct core vs penumbra among others.

**Aim:** to validate biomarker changes found in animal models of ischaemic stroke in the human stroke paradigm.

**Methods:** this will be undertaken in a number of different clinical cohorts ranging from denovo patients with recent onset stroke and within the context of the CSIRO funded START-EXTEND program. In the latter, longer time windows up to 9 hours are examined. Pilot programs will commence during 2010.

**Pooled analysis of EPITHET and DEFUSE datasets**


Data from the double blinded, randomized, controlled EPITHET trial co-chaired by Stephen Davis and Geoffrey Donnan has been pooled with the open label non-randomized DEFUSE trial of tPA 3-6 hours post stroke onset. A series of hypotheses are being tested on the combined dataset to increase our understanding of the role of imaging in relation to patient selection for clinical trials and the behaviour of the ischaemic penumbra.

**STAFF AND STUDENTS**

Geoffrey Donnan, MBBS, MD, FRACP, FRCP (Edin)
Associate Professor Brian Chambers, MBBS, MD, FRACP
Amy Brodtmann, MBBS FRACP PhD
Yoshinari Nagakane, MD
John Ly, MBBS, FRACP
Henry Ma, MBBS FRACP
Ramesh Sahathevan, MD (UKM), MRCP(ire), M Med(UKM)
Toshiyasu Ogata, MD, PhD
Naoshi Sasaki, MD, PhD
Dr. Chesda Udombmongkol, MD
Dr. Anne Abbott, Honorary Research Fellow
Renee Lichter, PhD Candidate
Daniel Liu, AMS Student

**CONFERENCES AND PRESENTATIONS**

GA Donnan
Stroke: antiplatelets, anticoagulants & the new medical strategies, Atherosclerosis, 21 Century Epidemic symposium, The Vatican, Rome
New insights in the pathophysiology of stroke, 1st Congress Swiss Federation of Clinical Neuro-Societies, Basel, Switzerland
Combination therapy trials for acute ischaemic stroke to extend therapeutic time window, World Stroke Congress, Seoul, Korea
Thrombolysis and Acute Stroke Therapies, New York

**MAJOR COLLABORATIVE LINKS**

International
Professor Marc Hommel, University of Grenoble, France
Professor Greg Albers, Stanford Stroke Centre, Stanford University Medical Centre, USA
Imaging parameters for the ischaemic penumbra.
Dr. Emma Chung, Leicester, UK
Small vessel knock
Dr Paul Syme, Scotland, UK
Small vessel knock

**National**

Dr. Richard Mannaseh, CSIRO, Australia
Small vessel knock

**EDITORIAL POSITIONS**

Prof Geoffrey Donnan
Stroke
Journal of Neuroimaging
Journal of Internal Medicine
Lancet Neuroimaging
International Journal of Stroke (Editor in Chief)
Journal of the CardioMetabolic Syndrome
Annals of Indian Academy of Neurology

Associate Professor Brian Chambers
International Journal of Stroke


SNAPSHOT OF OUR RESEARCH

The aim of our research is to prevent neuronal death after central nervous system injury, promote regeneration within the pathways that are damaged, and translate our understanding of these processes into effective treatments for human stroke. Our aim is to provide a streamlined pathway for stroke therapy development. Systematic review and meta-analysis of the world literature are being used to identify treatments with real potential for use in patients while our stem cell-derived human neuronal culture systems are used to filter out those that won’t work in humans and provide the first assessment of truly novel compounds. Those that pass this stage move on to our animal modeling program for testing in the range of circumstances likely to be encountered in the clinic. If successful, they will move onto clinical trial. Our biomarker projects and functional assessment program tie the human and animal testing together by providing the same tools to assess outcome in both species. We are working with international collaborators to establish a multi-centre network able to focus expertise and permit rapid evaluation of new drug candidates.

RESEARCH HIGHLIGHT

We have made two significant breakthroughs. In the first we have developed a new method that allows us to screen new stroke drugs for activity in human tissues to avoid the risk of clinical trials of drugs that might work well in animals but not in humans. These methods, which use cultured neurons derived from human stem cells, were developed by Ana Antonic. In the second we have used our animal models of stroke in collaboration with CSIRO to identify new blood biomarkers which allow us to follow the time course of injury after stroke. We believe this “Stroke Clock” can be used help us to identify the stroke patients likely to benefit most from our best treatment, thrombolysis, and to avoid the risks of this treatment in those with little to gain.

SENIOR STAFF LISTING

Assoc Prof David Howells
Dr Peter Batchelor
Dr Jenny Favoloro
Dr Victoria O’Collins
Dr Emily Lam
Dr Liam Vo

OVERVIEW

2011 saw the occupation of our new Melbourne Brain Centre laboratories at the Austin campus. The new tissue culture facilities in particular have rapidly advanced our aim of providing a human stem cell derived neuronal culture system for novel drug screening for stroke and have facilitated new international collaborations evaluating the therapeutic potential of novel NADPH oxidase and GABA receptor inhibitors. Our bench to bedside philosophy has seen the start of the $10.5 million EuroHyp-1 clinical trial of hypothermia in stroke and the award of a $1 million NHMRC-EU collaborative grant for biomarker discovery within this program. New initiatives with CSIRO partners include a hyper-acute biomarker detection program called EXTEND-TIMES in patients with ischaemic and haemorrhagic stroke at the Austin and Royal Melbourne Hospitals. At the bench we are developing models of haemorrhagic stroke, a minimally invasive model of ischaemic stroke and photo-acoustic imaging of cerebral blood flow and brain tissue oxygenation. A major recent success has been the recruitment of Dr Emily Sena to the team.

MAJOR RESEARCH PROJECTS

Translation to the clinic

Hypothermia as a treatment for neural injury
David Howells, Peter Batchelor, Emily Sena, Sarah Rewell, Victoria O’Collins, Charlotte Krennus and Malcolm Macleod

Past systematic review and meta-analysis identified moderate hypothermia as perhaps the most robustly tested candidate neuroprotective strategy. Fully randomised, blinded and appropriately powered experiments confirmed this in the laboratory both for ischaemic stroke and spinal cord injury when combined with reperfusion and early physical decompression respectively. Both are now moving into the clinic with direct involvement of the stroke team in the EU sponsored multinational EuroHyp-1 randomised control trial in stroke and the ICED (Immediate Cooling and Emergency Decompression) multicentre trial in Australian spinal cord injury patients. The latter is being run by Dr Peter Batchelor who now runs an independent laboratory within the division. Since hypothermia is now routinely used to protect against the cerebral consequences of heart attack we believe it is likely to also work in stroke and spinal cord injury. The basic science laboratories biomarker discovery program in EuroHyp1 has been awarded a $1m NHMRC-European Union Collaborative Research Grant.
Our biomarker discovery program is linked to a technology development program in collaboration with Steve Petrou and Stan Skafidas (NICTA, University of Melbourne) to manufacture high specificity and sensitivity nanowire sensors for a high speed point-of-care biomarkers detector.

**CAMARADES:** Systematic review and meta-analysis of treatments for stroke

David W. Howells, Malcolm Macleod, Emily Sena, Victoria O’Collins, Ulrich Dimgi, Philip Bath. Dr Emily Sena’s appointment to FNRI is a major advance for the CAMARADES collaboration (http://www.camrades.info), cementing the already strong relationship and bringing an exceptionally dynamic young researcher to Melbourne. We have joined forces with European, Canadian and American researchers from NASA/LSR, SYRCL and NIH to establish an umbrella organisation to disseminate information on evidence based translation from animal experiment protocols, standardisation of animal housing and animal experimentation. The inaugural meeting in 2012 (Nijmegen, Holland) will be followed in 2013 and 2014 by meetings in Edinburgh and Melbourne. Our current major focus is establishing a framework for international multicentre animal stroke drug evaluation which builds upon the lessons learned over the last 20 years in human clinical trials. Critical elements of this venture will be registration of study protocols, standardisation of methodologies and introduction of centralised randomisation, blinding and data validation.

**Mapping the long term plastic changes that occur after stroke**

David Howells, Sarah Rewell, Emily Sena, Malcolm Mcleod, Mikael Jerndal, Kelie Fonsberg, Jennifer Lees, Simon Koblic, Neil Spratt, Heide Jensen, Thomas Linden, Julie Bernhardt, Leanne Carey and Michael Nilsson. Sarah Rewell has completed her evaluation of the long term anatomic and behavioural changes that occur after stroke in rats. This study has allowed us to evaluate the utility of animal behavioural endpoints measured at the same time after stroke as is most common in man (>3 months in clinical trials) and understand how acute and very focal stroke lesions lead to development of very distant cell death; most noticeable so far has been significant contralateral hippocampal injury. Engagement of our human rehabilitation teams is helping refine the behavioural studies available to us and leading to greater understanding of the functional impact of distal neural injury. A new study with Julie Bernhardt and Karen Borschmann is examining the impact of stroke on bone structure and function. Victoria O’Collins has completed an analysis of the impact of hypertension on stroke outcome in experimental animals and has introduced the renovascular (2K/1C) model of hypertension to the laboratory.Charlotte Krenus and Atte Meretoja are establishing an angiotensin II mediated model of hypertension. Mikael Jerndal from Gotteborg has started a PhD examining the impact of growth factor treatment on long term outcome after stroke. This PhD will involve blocks of experimentation in Sweden and Australia.

Human stem cells as a test bed for evaluating stroke therapeutics. David Howells, Ana Antonic Ana Antonic has established human embryonic stem cells as a renewable and constant source of human neurons, and has developed models of injury that mimic the key features of stroke. She is testing the hypothesis that failure to get drugs to work in animals to work in man is because the drugs simply do not work in man. She has found that NXY059, (a free radical scavenger) does not protect animal neurons in her assays. This may explain the failure of the clinical trials of this drug. However, she has also found that drugs of another class, the NADPH oxidase inhibitors, do work on human cells. This has led to an exciting new collaboration with Dr Dave Lambeth from Emory University in Atlanta to screen his library of novel NADPH oxidase inhibitors for activity in human cells. This is the first stage in finding effective enzyme isoform specific inhibitors for in vivo testing before consideration for the clinic.

The availability of the system has also led to a collaboration (Drs. Clarkson and Collins, Dunedin and Sydney respectively) to examine the potential of GABAA receptor mediated tonic inhibition in stroke.

**OTHER CONTINUING RESEARCH PROJECTS**

**Data mining to identify the best candidate stroke therapeutics**

David Howells, Victoria O’Collins, Malcolm Macleod, Emily Sena and Stan Skafidas. This study is testing the hypothesis that drugs with high activity in one of the NADPH oxidase inhibitors for activity in human cells. This is the first stage in finding effective enzyme isoform specific inhibitors for in vivo testing before consideration for the clinic.

**Amelioration of macrophage cytotoxicity after spinal cord injury: impact on secondary injury and axonal regeneration**

Batchelor, Loy, Howells. The potential to allow us to treat the 20% of patients who don’t qualify for thrombolysis because they wake with stroke and to “individualise” treatment of patients who still have the potential for benefit but currently miss out because high risk of bleeding demands a consensus approach. The human blood collection within the START-EXTEND (http://www.strokecenter.org/trials/TrialP3C1/who/1005) randomised, multicentre, double blinded, placebo controlled Phase III trial of thrombolyisis between 3-9 and 9 hours in patients selected with significant penumbral mismatch is progressing well, and has been joined by EXTEND-TIMES, an observational study run by Marie Dagonnier (Belgium) at Austin Health and Atte Meretoja (Finland) Royal Melbourne Hospital. The aim of both these studies is to collect samples frequently during the early stages of stroke to determine accurately how biomarker expression changes with time. Emily Sena and his supervisor Steve Davis bring an interest in haemorrhagic stroke and haemorrhagic transformation in ischaemic stroke to the project. Messenger RNA and protein profiling after stroke in rats has led to a full international patent application. Follow-on experiments have now examined expression after sham surgery, in animals with genetic and renovascular hypertension and in animals with a clinical course similar to TIA (transient ischaemic attack) where ischaemia produces acute symptomatic deficits but does not produce frank infarction.
PUBLICATIONS


MAKING SENSE OF OUR DATA

The Florey is home to an invaluable service in the Victorian research sector – dedicated statistics and informatics research expertise. Here, the leader of the team, Dr Leonid Charlov, describes the range of initiatives offered by this platform.

The Statistics and Informatics Research Platform provides expertise in data, quantitative, and statistical aspects of research projects. It serves the Florey’s neuroscientists as well as some other external clients seeking sophisticated number crunching.

The Florey has developed some unique modelling methodology and promotes the use of high-standard, rigorous quantitative methods by scientists. Advanced statistical and modelling methodology is of little use for real applications without the availability of appropriate computational and modelling tools. Therefore, adapting, extending and validating complex statistical and decision modelling software is another basic task for the platform. It serves as a hub for collaboration within the Florey and with other Australian and international research institutions in the areas of statistical, data, and decision modelling.

The platform is an important resource of statistical and data management support to a number of large international clinical trials including the Florey’s AVERT and EXTEND programs. It also provides statistical support for SCIIPA: a unique, multi-disciplinary, multi-centre program of research to promote neurological recovery, maintain health and wellness, and optimise independence following spinal cord injury.

Consulting services are offered to Neuroscience Trials Australia and to a variety of clients both in government and in the health industry.

In addition to consulting and research collaboration activities, the team has developed and delivered experimental design and analysis training for the neuroscience component of a course offered to higher degree research students and the special certificate in clinical neurosciences course.
STROKE

NEUROREHABILITATION & RECOVERY

SNAPSHOT OF OUR STUDIES
The neurorehabilitation and recovery research program investigates neural plastic mechanisms underlying post-stroke rehabilitation and recovery, identifies and measures factors that impact on recovery, and develops new treatment approaches to facilitate neural plastic changes and better outcomes.

Our clinical research focuses on the assessment and treatment of touch, body sensations and motor functions following stroke, and has developed new science-founded approaches to sensory rehabilitation. This research is enhanced using functional neuroimaging techniques and biomarker discovery.

In addition to improving rehabilitation, knowledge of underlying mechanisms will guide more effective selection of rehabilitation after stroke while increased use of brain imaging and biomarkers will also identify patients ‘at risk’ of depression.

Research outcomes have included developing more effective approaches to stroke rehabilitation that are founded on theories of neuroplasticity and learning, and empirically tested for both clinical and neuroanatomical outcomes.

PROF LEEANNE CAREY’S TRAILBLAZING WORK HAS SEEN HUNDREDS OF PEOPLE WHO HAD LOST SENSATION, RETRAINING THEIR BRAINS TO REDISCOVER THE SENSE OF TOUCH. THE BRAIN HAS AN EXTRAORDINARY CAPACITY TO LEARN AND TO COMPENSATE WHEN AN AREA IS DAMAGED.

PROF GEOFF DONNAN, DIRECTOR OF THE FLOREY

A HIGHLIGHT OF OUR YEAR
Research highlights have included publication of the first controlled trial demonstrating an effective approach to sensory rehabilitation based on principles of neural plastic changes in the brain and learning, and novel findings on the relationship between touch impairment and brain activation in stroke patients with lesions of subcortical and cortical somatosensory regions. Prof Carey edited the book Stroke Rehabilitation: Insights from Neuroscience and Imaging, and we were successful in obtaining an NHMRC project grant titled “Effective sensory rehabilitation after stroke: Targeting viable brain networks”.

SENIOR STAFF
Professor Leanne Carey
Dr Susan Palmer

PROF LEEANNE CAREY’S TRAILBLAZING WORK HAS SEEN HUNDREDS OF PEOPLE WHO HAD LOST SENSATION, RETRAINING THEIR BRAINS TO REDISCOVER THE SENSE OF TOUCH. THE BRAIN HAS AN EXTRAORDINARY CAPACITY TO LEARN AND TO COMPENSATE WHEN AN AREA IS DAMAGED.
OVERVIEW
The Neurorehabilitation and Recovery research program focuses on investigating of neural plastic mechanisms underlying post-stroke rehabilitation and recovery, identification and measurement of factors that impact on recovery, and development of treatment approaches to facilitate neural plastic changes and better outcomes. Research outcomes have included development of effective approaches to stroke rehabilitation that are founded on theories of neuralplasticity and have been empirically tested for both clinical and neuroanatomical outcomes. Our clinical research focuses on the assessment and treatment of touch sensation and motor function following stroke, and has involved development of novel assessments and science-founded approaches to sensory rehabilitation. This research is enhanced by investigation of the neurobiological mechanisms of recovery post-stroke using neuroimaging techniques and biomarkers. Outcomes from our studies have led to development of restorative approaches to rehabilitation, and will advance knowledge of underlying mechanism to guide more optimal selection of rehabilitation interventions for individuals after stroke and identification of patients ‘at risk’ of depression through novel brain imaging and biomarkers. Research highlights for the Neurorehabilitation and Recovery group in 2011 have included publication of the first controlled trial demonstrating an effective approach to rehabilitation based on principles of neural plastic changes in the brain and learning: the SENSe training approach. We are now facilitating dissemination of this training approach into clinical settings through development of multimedia resources to train therapists. During 2011, Prof Carey edited the book Stroke Rehabilitation: Insights from Neuroscience and Imaging. This book challenges clinicians to adopt more restorative and scientifically based approaches to stroke rehabilitation. We have also published novel findings on the relationship between touch impairment after stroke and brain activation in stroke patients with lesions of subcortical and cortical somatosensory regions. Positive findings from these studies highlight the importance of investigating functional and structural changes in brain networks associated with SCI and TET sensory rehabilitation in stroke patients with cortical or subcortical somatosensory lesions. (i) predict ability to benefit from sensory rehabilitation based on structural connections of interhemiarchic and thalamo-cortical tracts between key somatosensory regions. (ii) develop a data-driven model of somatosensory recovery after cortical and subcortical lesions, based on functional and structural connections in the brain. We will use established brain imaging methods to quantify functional and structural connections between regions of the somatosensory network hypothesized to be involved in sensory recovery. START PrePARE – Prediction and Prevention to Achieve Optimal Recovery Endpoints after stroke: PrePARE is part of a larger stroke cohort study known as START: StrokeImaging pRevention and Treatment. Leanne Carey (NSRI, FNI), Sheila Crowther (LaTrobe), Thomas Lindan (Sweden), Oliver Salvado (CSIRO), Georgia Fox (FNI), Stephen Davis (RMI), David Hawell (FNI), Bill Wilson (CSIRO), Lance Macaulay (CSIRO), Leanne Carey (NSRI, FNI), Alan Connelly (FNI), Henry Ma (FNI). START: Stroke imaging, pRevention and Treatment is a large, multicentre clinical trial that comprises the EXTEND study (EXTEND the time for Thrombolysis in Emergency Neurological Deficits) and PrePARE study (Prediction and Prevention to Achieve Optimal Recovery Endpoints after stroke). Our previous work on the Neurorehabilitation and Recovery research group is on identifying predictors of post-stroke depression and functional outcome and on putative biochemical, genetic and imaging biomarkers in the START cohort. Our PrePARE subgroup will investigate the relationship between novel imaging markers, depression and functional outcome as well as the association of post-stroke depression, cognition, and participation in daily activities and life roles. Advanced clinical and imaging tools will be used to analyze the relationship between novel imaging markers (resting state activity, cortical thickness and fibre track integrity) and functional outcome and depression. We will also identify the association between post-stroke depression and functional outcomes including cognition, sensorimotor function, and participation in daily activities and life roles. In 2011 the PrePARE study was also approved as a standalone study with an increase in numbers from 75,000 to 100,000. Clinical and imaging outcomes. Sixty-four patients have been recruited to the START cohort and 27 to the PrePARE cohort. Outcomes from this study will target early identification of stroke survivors “at risk” of depression and recurrent stroke through novel brain imaging and biomarkers. It will also provide evidence of late morphological changes in the brain and their association with post-stroke depression and functional outcome. The three main aims of this study were to investigate: i) the psychophysical function of proprioception; ii) how brain activation during proprioceptive perception; and iii) how brain activation changes in both stroke subjects with and without subjective proprioceptive impairment. Functional magnetic resonance imaging scans were performed during a cognitive proprioceptive perception task. Between-group comparisons revealed a significantly greater improvement in sensory capacity following sensory discrimination training. Improvements were maintained at 6-weeks and 6-months. Patients with varying side of lesion, severity of sensory impairment, age and time post stroke were able to benefit from training. The program is clinically-oriented and advanced training to target effective novel stimuli. Therapists can use this training approach when working with people who experience sensory loss after stroke. We have also published novel findings on the analysis of individual patient factors that may impact on the ability to benefit from this rehabilitation approach. CoNNECT: Connecting New Networks for Everyday Contact through Touch. Leanne Carey (NSRI, FNI), Rudiger Seitze (Germany), Donald Toureen and Alan Connelly (BRI, FNI), Chris Levi, Mark Parsons and Isabel Hubball (Newcastle). New therapies have been developed to help the brain recover after stroke. We are developing new therapies that can help people acquire new skills: Stimulus Specific Adaptation. The program includes training for individuals who have experienced a stroke to perform a range of daily activities and life roles. In 2011 the program was also approved as a standalone study with an increase in numbers from 75,000 to 100,000. Clinical and imaging outcomes. Sixty-four patients have been recruited to the START cohort and 27 to the PrePARE cohort. Outcomes from this study will target early identification of stroke survivors “at risk” of depression and recurrent stroke through novel brain imaging and biomarkers. It will also provide evidence of late morphological changes in the brain and their association with post-stroke depression and functional outcome. The three main aims of this study were to investigate: i) the psychophysical function of proprioception; ii) how brain activation during proprioceptive perception; and iii) how brain activation changes in both stroke subjects with and without subjective proprioceptive impairment. Functional magnetic resonance imaging scans were performed during a cognitive proprioceptive perception task. Between-group comparisons revealed a significantly greater improvement in sensory capacity following sensory discrimination training. Improvements were maintained at 6-weeks and 6-months. Patients with varying side of lesion, severity of sensory impairment, age and time post stroke were able to benefit from training. The program is clinically-oriented and advanced training to target effective novel stimuli. Therapists can use this training approach when working with people who experience sensory loss after stroke. We have also published novel findings on the analysis of individual patient factors that may impact on the ability to benefit from this rehabilitation approach.
primary sensory motor areas and the premotor cortex. Three case studies of stroke subjects with proprioceptive impairment and common lesion site in postero-lateral thalamus, were conducted. Patterns of brain activation lacked the extent of right supramarginal cortex activity typical of healthy participants. Insights gained can assist in choosing and interpreting proprioceptive tests. Combined with brain activation studies, knowledge gained can improve diagnosis of subjects with proprioceptive deficits, and aid in future development of proprioceptive treatments, together with the assessment of their effectiveness. This study was conducted as a PhD research thesis by Ettie Ben-Shabat.

**OTHER RESEARCH PROJECTS**

- **IN Touch - late stages of recovery**
  - Leanne Carey (FNI)

Is the Hand Function Survey reliable and responsive to change during stroke rehabilitation? Rebecca Avery (La Trobe), Jannette Blennerhassett (Austin Health, NSRI), Leanne Carey (FNI, La Trobe)

- **Of touch sensation after stroke: Attentuation, functional connectivity, and neuroplasticity**
  - Leanne Carey (FNI), Sheila Crewher (La Trobe University), Louise Bannister (La Trobe University)

A functional MRI study of upper limb recovery in community dwelling stroke survivors. Isobel Hubbard (Newcastle), Leanne Carey (FNI), Mark Parsons (Newcastle, NSW)

- **Occupation-based outcomes associated with sensory retraining post stroke**
  - Mary Mastos (FNI, La Trobe), Leanne Carey (FNI, La Trobe)

The contribution of somatosensory impairment to pinch grip ability after stroke. Jannette Blennerhassett (Austin Health), Leanne Carey (NSRI, FNI), Thomas Matyas (NSRI, La Trobe)

- **An evaluation of occupational therapy and modified constraint induced movement therapy following Botulinum toxin-A injection in the upper limb in children with spastic hemiplegic cerebral palsy.**
  - Bran Hoare (La Trobe), Christine Imms (La Trobe), Leanne Carey (FNI, La Trobe)

- **Modification and investigation of the construct validity of the Melbourne Assessment of Unilateral Upper Limb Function**
  - Melinda Randall (La Trobe, Children’s Hospital), Christine Imms (La Trobe), Leanne Carey

Factors impacting on neural plasticity and recovery after stroke. Kate Noonan (La Trobe), Sheila Crewher (La Trobe), Leanne Carey (FNI, La Trobe)

**CONFERENCES AND PRESENTATIONS**


**MEDIA INTERACTIONS (2011)**

Multiple media interactions ‘Lookin’ good: battling cerebral palsy with Botox’ – based on doctoral work of Brian Hoare. (Carey primary supervisor)

Multiple interactions linked with story of stroke survivor involved in our studies as follows: stroke-safe ambassadort_ WinterEditionJuly2011, pp 4-5. ABC 7.30 The latest drug therapy that could prevent a lifetime of disability (linked with START program and contribution of brain images and story of patient involved in our studies. (May 2011)
NATIONAL AND INTERNATIONAL COLLABORATIVE LINKS

Professor Rudiger Seitz, Germany
IN Touch: Imaging Neuroplasticity of Touch

Professor Aina Puce, USA
IN Touch: Imaging Neuroplasticity of Touch

Professor Thomas Matyas, La Trobe University, Australia
SENSe: Study of Effectiveness of Neurorehabilitation on Sensation

Professor Sheila Crewther, La Trobe University, Australia
IN_Touch: Imaging Neuroplasticity of Touch

Professor Thomas Linden, University of Toronto, Canada.
Chair Department of Occupational Therapy and Human Impairment (special issue)

Professor Leeanne Carey
Neurorehabilitation and Neural Repair

VISITING SCIENTISTS

Prof Helene Polatajko: Professor and Chair Department of Occupational Rehabilitation

ANE Marie Tan, Janet Sloan Stroke Rehabilitation Research Award

BOOK AND BOOK CHAPTERS


PUBLICATIONS


The main research activities in the Epidemiology Division during 2011 have included BAT24, a study of BP patterns and outcomes in patients with TIA, compared to age and sex matched controls.

Transient Ischaemic Attack (TIA) is a well-recognized risk factor for stroke and hypertension is a major risk factor for TIA and stroke. Furthermore, different BP patterns have been described using Ambulatory Blood Pressure Monitoring (ABPM) and a "non-dipping" pattern i.e. an absence of the normal reduction in nocturnal BP is shown to be associated with stroke and a worse prognosis for target organ damage. On the background of insufficient data about the common BP pattern after TIA and minor stroke and the relationship between the different BP patterns and TIA/minor stroke outcomes, this natural history case control study is being conducted.

The purpose of this project is to provide new information about the association between BP variability and autonomic nervous system function in TIA or minor stroke patients and the subsequent risks of recurrent TIA or stroke by assessing Heart Rate Variability and 24-hour ABPM for TIA/minor stroke patients within 7 days after symptoms onset as well as for control participants and assessing outcomes at 3 months.

OVERVIEW: PUBLIC HEALTH

The research activities of the Public Health Division for 2011 have included a range of projects related to improving clinical management of stroke and disease prevention. Research quantifying the quality of stroke care in public hospitals continues to be an important area of research.

The Victorian Stroke Telemedicine Project:
Chis Bladin, Dominique Cadilhac, Sonia Denisenko, Helen Dewey, Peter Disler, Tony Walker, Bruce Winzar; Ian Mosley, Natasha Moloczij.

The overall objectives of this project are to investigate the potential benefits and value of telemedicine for enhancing capacity in rural and regional hospitals to provide increased access to acute stroke treatments. The Victorian Stroke Telemedicine (VST) program has been designed to demonstrate the use of a formalised telemedicine protocol in a regional Victorian health service (Bendigo Health). The VST program includes access to a pool of external (Melbourne-based) physicians with expertise in stroke using video-conferencing technology (telemedicine). Clinical decision-making is enhanced by having the ability to perform joint clinical consultations with the view to building sustainable, long term capacity and capability. The program also includes use of community, ambulance staff and clinician education to raise awareness and knowledge about stroke. This project has been co-funded with a Victorian Science Investment Fund Department of Innovation, Industry and Regional Development (DIIIRD) grant (now the Victorian Department of Business and Innovation).

In 2011, the VST project included the development of a pilot telemedicine protocol that was implemented for 15 patients. Several changes were then implemented and the finalised protocol is now in place for a year (October 2011 to October 2012) as part of the evaluation process.
Australain Stroke Clinical Registry (AuSCR):
Dominique Cadilhac, Natasha Lannin, Craig Anderson, Joyce Lim, Chris Price, Steven Faux, Chris Levi and Geoffrey Donnan.
The main aim of the Registry is to ensure a systematic way of monitoring and providing evidence for improvements in the quality of hospital stroke care throughout Australia. The Registry includes a minimum data-spine on acute hospital care and a 90 to 180 day patient outcome survey.
The Registry is governed by a nationally representative Steering Committee chaired by Professor Sandy Middleton. In 2011, 20 hospitals were participating in the registry. A four-year NHMRC partnership grant (APP1034445) was awarded in 2011 to expand use of the registry in Queensland, explore the potential for harmonisation with the National Stroke Foundation Audit Program as well as data linkage with other health datasets, and evaluate the impact of a quality improvement program underpinned by AuSCR and National Stroke Foundation audit data.

PUBLIC HEALTH OTHER RESEARCH PROJECTS

Victorian Stroke Clinical Network (VSCN) Phase II evaluation:
The aims of this evaluation are to describe current clinical practice for stroke as perceived by health professionals working within a range of nominated hospital settings and compare these to the baseline period (2007-2008). Clinical audit and other objective data are also used. These data will provide evidence for improved access to evidence-based practice following the implementation VSCN plans and initiatives since 2007.
Dominique Cadilhac, Karen Moss, Tara Purvis, Monique Kilkenny, Karen Borschmann.

Australain Stroke Survivor Survey
Dominique Cadilhac, Nadine Andrew, Monique Kilkeny, Rebecca Naylor, Jacqui McKenzie, Natasha Moloczi. In collaboration with the National Stroke Foundation and Monash University.
New South Wales Health stroke program evaluation:
Time series clinical audits of hospitals in both metropolitan and rural NSW since 2003. Aggregated data have been used to provide evidence that stroke care improvement initiatives have significantly reducing disability following stroke.

Know Your Numbers program evaluation:
Dominique Cadilhac, Monique Kilkeny, Raylin Johnson, Belinda Wilkinson, Alison Hicks. In 2011, this program was expanded to include the piloting of a diabetes risk assessment.

National Stroke Services Audit:
Dominique Cadilhac, Monique Kilkeny, Chris Price, Elizabeth Ritchie, Linton Harris.

New South Wales stroke audit program
Dominique Cadilhac, Monique Kilkeny, Mark Longworth, Chris Levi

Stand Firm (APPS86605) in collaboration with Monash University

Quality in Acute Stroke Care Project

ICARUSS
In collaboration with the National Ageing Research Institute. Contributors include Dominique Cadilhac, Helen Dewey, Geoff Donnan, Jacques Joubert. Patient recruitment for this randomised controlled clinical trial is now complete.

Evaluation of the National Stroke Foundation Counsellor Education Program
In collaboration with the National Stroke Foundation and BeyondBlue.

NSW Rural Stroke Project
Dominique Cadilhac, Tara Purvis, Monique Kilkeny, Chris Levi, M Pollock, M Gill, L Custer, M Longworth

STAFF AND STUDENTS

Epidemiology:
Helen M. Dewey, MB BS, PhD, FRACP, FAFMR (RACP)
Wenwen Zhang, MD

Public Health:
Dominique Cadilhac, PhD, MPubHth, B Nurs (post-reg), RN
Monique Kilkeny, B Appl Sc (MRA), G Dip (Epideimiol & Biostats), MPH (Research)
Karen Moss, BBSc (Hons)
Natasha Moloczi, MSc (Hons)
Karen Borschmann, B Physio
Kate Pace Grad cert IT, Grad Dip HSc, B Med Rec Admin
Nicole Wallis PhD BSc(David Carey

CONFERENCES AND PRESENTATIONS

Public Health:
Dominique Cadilhac
How many strokes are there each year in Australia? The issue of imperfect data – an example from the North East Melbourne Stroke Incidence Study (NEMESIS). Stroke Society of Australasia 2011 Annual Scientific Meeting, Adelaide, September 2011


“What is the potential economic impact of a national blood pressure awareness program for the prevention of stroke”?
International Health Economic Association Conference, Toronto Canada, July 2011
What is it worth to reduce physical inactivity and increase consumption of fruit and vegetables in Australia?
International Society of Behavioural Nutrition and Physical Activity Conference, Melbourne, July 2011

Know your numbers”: checking your blood pressure is easy, living with a stroke isn’t. European Stroke Conference, Hamburg, May 2011

Know your numbers (KYN) 2007-2009 Program: Is there evidence the KYN programs improves knowledge and behaviour of participants? National Heart Foundation National Conference 2011, Melbourne, March 2011

The Australian Stroke Clinical Registry: the first year of implementation.
National Heart Foundation National Conference 2011, Melbourne, March 2011


Self-management programs for stroke survivors: results of a phase II, single blind randomised controlled trial.
Stroke Society of Australasia 2010 Annual Scientific Meeting, Melbourne, September 2010

The Australian Stroke Clinical Registry: formative evaluation. Stroke Society of Australasia 2010 Annual Scientific Meeting, Melbourne, September 2010

The Australian Stroke Clinical Registry (AuSCR): Coffee-Dig information collection about hospital stroke care. Smart Strokes 6th Australasian Nursing and Allied Health Congress, Terrigal, NSW, August 2010

Target setting for risk factor reduction in Australia: European Conference on Health Economics, Helsinki Finland, July 2010

The metropolitan-rural divide for stroke outcomes and the impact of stroke units. European Stroke Conference Barcelona, Spain, May 2010

Monique Kilkeny
Outcomes of stroke in young indigenous Australians. Society of Australasia 2010 Annual Scientific Meeting, Melbourne, August 2010

Natasha Moloczi

Dominique Cadilhac
Interactive session with Prof Doris Young (University of Melbourne) on how to translate research findings into practice. Translational Research - Strategies for Engagement. Melbourne Health Centre for Clinical Research Excellence 2011 Research Retreat, Lancemore Hill, Victoria, November 2011


The Australian Stroke Clinical Registry: harmonisation potential and data linkage. National Stroke and Quality Improvement meeting, Adelaide Convention Centre, September 2011

The Australian Stroke Clinical Registry: progress and future directions. Presentation to Heart Foundation staff, Melbourne University, September 2011


Co-presenter on “TIs: The challenge of measurement rapid management and prognosis”. National Stroke Research Institute and collaborative centers annual scientific meeting, Pokolbin, NSW, August 2010

Surviving stroke in Australia (plenary session), Smart Strokes 6th Australasian Nursing and Allied Health Congress, Terrigal, NSW, August 2010

Preventing most strokes: Myth or reality? Workshop co-facilitated with A/Prof Jon Stumm, August 2010

Overview and progress of the Australian Stroke Clinical registry. Registries Special Interest Group meeting, Monash CCRE in Patient Safety, Alfred Hospital, Prahran, February 2010

Epidemiology:
Wen Wen Zhang
The disagreement between clinic BP and 24 hour ambulatory BP in patients with Transient Ischaemic Stroke or mild stroke. Platform presentation at the Stroke Society of Australasia, Adelaide, September 2011

Circadian blood pressure variation and heart rate variability in patients with TIA or minor stroke compared with controls. Poster presentation at the Stroke Society of Australasia, Adelaide, September 2011

Circadian blood pressure patterns in patients with TIA or minor stroke. Poster presentation at the European Stroke Conference, Hamburg, May 2011

Rehabilitation in China: A Systematic Review of Randomized Controlled Trials. Poster Presentation at World Stroke Congress (WSC), Seoul Korea, October, 2010

Helen Dewey
Prevention work shop: Overview of Transient Ischaemic Attack. Annual Scientific Meeting Stroke Society of Australasia, September 2011

Debate: ‘Endovascular treatment for acute ischaemic stroke is the way of the future: NO!’ Stroke Heads Meeting (Boehringer-Ingelheim), 21 October 2011, October 2011

NATIONAL AND INTERNATIONAL COLLABORATIVE LINKS

Public Health - National
Prof Craig Anderson, George Institute, Australia
AuSCR
Dr Natasha Lanin, University of NSW Rehabilitation Studies Unit, Australia
AuSCR
A/Prof Amanda Thrist, Monash University, Australia
NEMESIS, AuSCR and STAND-FIRM for stroke patients (RCT)
A/Prof Theo Vos, University of Queensland, Australia
A/Prof Helen Dewey, International Journal of Stroke

AWARDS

Dominique Cadilhac
2010
NHMRC Australian Based Public Health Fellowship (2010-2013: total $285,000)
2011
Department of Medicine Southern Clinical School, Monash University, Certificate of Ment
2011
NSW Premier’s Awards, Finalist – Excellence in delivery as a contributor to the Quality in Acute Stroke Care Project

Prof Chris Bladin, Eastern Health and Monash University – Victorian Stroke Telemedicine Project
Prof Stephen Davis as part of the Centre for Translational Excellence in Neuroscience

PUBLICATIONS

9. Nurney, Physiotherapists, Speech and Occupational Therapists and Study/Monitoring Assistants. European Stroke Conference Barcelona, Spain 2010

FLOREY NEUROSCIENCE INSTITUTES ANNUAL REPORT 2011

CONTENTS


SNAPSHOT OF OUR STUDIES
We aim to develop, test and implement new models of rehabilitation that promote increased physical activity in people who have suffered a stroke. Physical activity is a powerful intervention with many health benefits. In the past, the barriers to physical activity in people affected by stroke have contributed to extremely low levels of activity in these individuals both early and late post stroke. In our lab, we undertake research that test ways to measure activity in the population (using both observation and devices). This helps us understand how activity influences brain and body health. We develop and run large clinical trials testing the efficacy and cost effectiveness of high intensity physical activity.

RESEARCH HIGHLIGHT
In 2011 we reached a major milestone in the AVERT trial when our 1000th participant was recruited. This was an outstanding achievement for the collaboration and is testament to the hard work of over 500 clinicians and researchers around the world, over the course of the project. The trial also expanded rapidly throughout the United Kingdom in 2011 in response to funding from the Stroke Association of the UK. This is the first time a clinical trial planned, coordinated and led from outside the UK has received support from this organisation. In 2011 we held our second ‘exercise forum’ with representatives from clinical research organisations across Australia. This was an opportunity to share insights and experiences of working in this rapidly changing field of research.

SENIOR STAFF
Assoc Prof. Julie Bernhardt
Dr Janice Collier
Dr Toby Cumming
Fiona Ellery
Jan Chamberlain

OVERVIEW
Starting active rehabilitation very early after stroke is a relatively new concept with enormous potential to reduce death and chronic disability. While there is some evidence that exercise and purposeful, task specific activity aids recovery after stroke, currently there are no specific recommendations about post stroke exercise. Pilot work by our group suggests that commencing activity within 24 hours of stroke may lead to faster recovery of function, less disability and better long term quality of life at a lower cost than current care. To test these hypotheses, we are conducting the first randomised controlled trial of very early rehabilitation (AVERT - A Very Early Rehabilitation Trial). Over 2000 patients with stroke will be recruited to this international, multicentre, Phase III efficacy and cost effectiveness clinical trial. The findings of this study have the potential to change clinical practice around the world. Our group also aim to identify key mechanisms by which early exercise may improve the outcome of people affected by stroke.

STROKE
AVERT EARLY INTERVENTION RESEARCH PROGRAM

Left: Associate Professor Julie Bernhardt, co-head in our Stroke Division and an ARC Future Fellow
RESEARCH HIGHLIGHTS

AVERT is an international, multicentre (35), randomised controlled trial. This is a single blind, Phase III trial with planned recruitment of 2104 patients (1052 each arm) and intention to treat analysis. Eligible patients are those admitted to hospitals with a stroke unit within 24 hours of stroke symptom onset. Patients over 18 years with confirmed stroke, who meet physiological entry criteria for safety are eligible. Patients who are treated with thrombolysis (tPA) are also eligible. Those admitted directly to intensive care, or with pre-morbid disability (mRS>2) or other life threatening illness are excluded. Randomisation is completed online, and patients are stratified by stroke severity on admission (NIHSS) and by site. Those randomised to very early rehabilitation commence out of bed training within 24 hours of stroke, delivered by nurses and physiotherapists. This continues until 14 days or discharge (whichever comes first). Primary outcome is mRS at 3 months post stroke. Secondary outcomes include cost effectiveness, walking recovery, ADL, mood, cognitive function and quality of life. Follow up is completed at 12 months post stroke. Blinded assessors gather all follow up data. Recruitment at December 2011 was 1112 patients from 5 countries (Australia, New Zealand, Malaysia, Singapore, Northern Ireland, Wales, England, Scotland). This trial meets international clinical trials standards and is overseen by an international Steering Committee, Data Safety and Monitoring Committee, with adverse events independently adjudicated by an Outcomes Committee. Further information about the trial can be found at the ANZCRT trials register:

OTHER RESEARCH PROJECTS

Factors influencing the decision to mobilise following thrombolysis in Australia

This was a case cross-over study that employed a web-based questionnaire to probe clinician decision-making. At present, we know very little about how thrombolysis and other factors may influence the decision to delay mobilisation of a patient after stroke. This hypothesis generating study found factors that may influence the decision to delay or promote out of bed activity. We plan to follow this work with a prospective study in the clinic.

Change in physical activity in the first 6 months after stroke

In this collaborative study with Trondheim in Norway, we used accelerometer-based electronic monitors to measure changes in physical activity over multiple time periods following stroke. This was the first study to examine changes in activity over time using this technology.

Changes in bone, glucose and muscle in the first 6 months after stroke

Having a stroke often leads to physical inactivity, and the combination of these two things can have a range of negative physiological effects. This study tracks changes in bone density, glucose metabolism and lean muscle mass after stroke and investigates the effect of physical activity on these physiological outcomes.

Finding a valid screening tool for cognitive impairment after stroke

Cognitive impairment is a common and important consequence of stroke, yet cognitive outcomes are often ignored in stroke research trials. In this study, we are aiming to establish whether a recently developed short screening tool (the Montreal Cognitive Assessment) is a valid measure of cognitive impairment after stroke.

Exercise preferences after stroke

This study found that stroke survivors had different and more variable exercise preferences than a group of age and sex-matched controls. Understanding an individual’s preferred mode of exercise should help the design of rehabilitation programs that promote long term adhered to exercise.

Individual patient factors that predict amount of therapy received in the first 2 weeks after stroke

Using the large pool of therapy data from many countries that has been built up in the AVERT Early Intervention Research Program, the aim of this study is to determine whether certain patient factors (e.g. age, gender) influence the amount of therapy received in acute stroke.

STAFF

Ass Prof Julie Bernhardt
Dr Janice Collier
Dr Toby Cumming
Fiona Ellery
Sally Speare
Karen Borschmann
tara purvis
Luke Cosgrave
Jan Chamberlain

HONORARY ASSOCIATES

Dr Coralie English, University of South Australia
Prof Thomas Linden, University of Gothenberg, Sweden
Dr Torunn Askim, St Olav's University Hospital, Norway

REFERENCES AND PRESENTATIONS

In 2010 a number of our group were invited to present at national and international conferences such as: World Stroke Conference, Seoul, Korea, UK Stroke Forum, Australian Physiotherapy Association Conference, Australian Neuroscience Nursing Association Education day.

National and international collaborative links

Prof P Langhome, Glasgow Royal Infirmary, Glasgow, UK.
Prof B Indredavik, St Olav’s Hospital, Trondheim, Norway.

VISITING SCIENTISTS

Dr Torunn Askim, St Olav’s University Hospital, Trondheim, Norway (6 months visit)
Prof Bent Indredavik, St Olav’s University Hospital
Prof Thomas Linden

Students

PhD/Prof Doc:
La Trobe University
Luker J. (2009) Ageism in stroke care
University of South Australia
Craig L. (2009) Early rehabilitation after stroke
Glasgow University, UK
University of Newcastle
La Trobe University
Borchmann K. (2009) Bone and muscle loss after stroke
La Trobe University

Masters by research:
West T. (2009) Difference in activity between acute and comprehensive stroke unit care. La Trobe University

Honours students:
Cosgrove L. (2010-2011) What factors predict amount of therapy after stroke
La Trobe University
Banks G. (2009-2010) Exercise preference and self efficacy after stroke
La Trobe University

CONFERENCES AND PRESENTATIONS

In 2010 a number of our group were invited to present at national and international conferences such as:


EDITORIAL POSITIONS

Julie Bernhardt is on the Editorial Boards of:
International Journal of Stroke
International Journal of Therapy and Rehabilitation
Topics in Stroke Rehabilitation
Journal of Neurological Physical Therapy

FLOREY NEUROSCIENCE INSTITUTES ANNUAL REPORT 2011

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DESCRIPTION

Prof Robin McAllen heads the Systems Neurophysiology Group at the Howard Florey Institute and Prof Richard Macdonell heads the clinical arm at the Austin Hospital. Our research area is brain function in health and disease. One particular focus is on how the brain controls basic bodily functions such as blood pressure, body temperature, body fluids and breathing. A second focus is on the ways that disease processes change the excitability of neurons. A third focus is on the heart, and its complex interactions with the nervous system in health and disease. The final focus is on neurorehabilitation and neural repair.
Changes in motor cortex excitability in patients with epilepsy and response to anticonvulsant treatment. Badawy RA, Macdonnell RA, Jackson GD, Benkovcic SF.

The electrical changes in the brain which give rise to epilepsy remain poorly understood. Using transcranial magnetic stimulation (TMS) we have shown that increased excitability of the motor cortex occurs in both sides of the brain in generalised epilepsies whereas this hyperexcitability is confined to the side containing the seizure focus in partial epilepsies. Using this technique we have also shown that environmental factors such as sleep deprivation and timing of the menstrual cycle, which are known to increase seizure risk, do so by increasing cortical excitability. By exploiting this finding we were interested in exploring whether seizure control using antiepileptic drugs (AED) could be predicted using this technique. To this end we enrolled patients who successfully responded to drugs returned to normal within a few weeks but in patients where AEDs failed the motor cortex remained hyperexcitable, suggesting that this is a tool which could be used to test for likely individual responsiveness to an AED or to predict the development of new drugs for epilepsy.

We also explored the possible clinical role of TMS in assessing patients after a 1st seizure with respect to the risk of further seizures (epilepsy) and future risk of seizures. In addition between increased cortical excitability and risk of further seizures (epilepsy) and after a 1st seizure with respect to the role of TMS in assessing patients.

Working model: Independent cold-defence pathways

Ganglionic transmission: a neglected locus of autonomic control

Sympathetic control of the heart and blood vessels and parasympathetic control of the heart are matters of great interest because of their important roles in health and disease. Nearly all cardiovascular and many other diseases are associated with increased sympathetic and reduced parasympathetic activity, both of which adversely affect outcome. It is often assumed, without good evidence, that this imbalance is due to altered sympathetic and parasympathetic drives from the brain. But another possibility, supported by some evidence, is that there is altered transmission through the synapses in their respective autonomic ganglia. It turns out that the mechanisms determining ganglionic transmission in vivo, whether in health or disease, are still poorly understood. We have addressed the issue of ganglionic transmission in vivo in two major studies published recently in the Journal of Physiology.

First, in collaboration with Prof. Wilfried Jang from the University of Bristol, we made intracellular recordings from sympathetic vasomotor ganglion cells whilst they were receiving their normal extracellular stimulation on their respective (usually two or three) preganglionic neurons in anaesthetised rats. Depending on the strength of each sympathetic efferent, it either triggered an action potential in the preganglionic cell to be transmitted on to the blood vessel, or failed because the preganglionic cell remained at or below threshold. Calculations showed that most vasomotor ganglion cells are more responsive to cold skin; those sensitive to brain temperature, while those that do shiver are more sensitive to cold skin; those regulating brown fat are intermediate. The motor output pathways for these actions have a synaptic relay stage in a brainstem nucleus called the medullary raphe, but despite this, they do not appear to communicate directly with each other.
Heart failure continues to be a major health problem as evidenced by a rise in hospital admissions for heart failure, the number of deaths attributed to heart failure, and the increasing costs associated with care. It is established that the increase in activity of the nerves that stimulate the heart to beat harder and faster (cardiac sympathetic nerves) are activated very strongly in heart failure. This oversensitivity exacerbates the disease process and can trigger sudden death. The activity of these nerves is controlled by the brain and our aim is to understand why they are overactive in heart failure.

We have developed an animal model of heart failure (the sheep) in which we have shown by direct electrical recordings that there is a large increase in activity of the cardiac sympathetic nerves. We have shown that in the healthy state, activity in these nerves is decreased by an increase in blood volume. It is thought that this results from increased pressures inside the heart stimulating neural signals to the brain, which reduces nerve activity to the heart.

Understanding this is important because one of the consequences of heart failure is an increase in blood volume. We have recently discovered that in heart failure this system is dramatically desensitised and that increases in blood volume do not reduce cardiac sympathetic nerve activity. This indicates that in heart failure, the inhibitory mechanisms that should inhibit cardiac sympathetic nerve activity in response to the increased blood volume are absent, thus allowing other stimuli to maintain activity at a high level.

This graph shows how the activity in the sympathetic nerves to the heart (cardiac sympathetic nerves) respond to changes in blood volume. In normal sheep (closed circle) volume receptors in the area of the heart stimulate a reflex that inhibits the activity of cardiac sympathetic nerves. This reflex stops working in sheep that have heart failure (open symbols).
Raphé tauopathy alters serotonin metabolism and breathing activity in terminal Tau.P301L mice: possible implications for


OVERVIEW
Neuroscience Trials Australia is a not-for-profit, contract research organisation within Florey Neuroscience Institutes. The business is co-chaired by Professors Geoffrey Donnan and Stephen Davis. In mid 2011 Dr Tina Soulis was appointed General Manager to re-launch the business so that it primarily services our academic investigators in all areas of neuroscience clinical research. Our areas of expertise include stroke and stroke-related conditions, multiple sclerosis, epilepsy, Parkinson’s disease, spinal cord injuries, Huntington’s disease, neuromuscular disease and pain. We have strategic alliances with many therapeutic disease groups and we can provide access to key opinion leaders, sites and clinical trial associates.

Service focus is the contract management of all aspects of clinical trials, from study feasibility, project planning and implementation to the conduct of clinical trials and project and data management. We provide a range of clinical research services, including study design and implementation, and optimising independence. Strategies that compensate for loss of function rather than exercising the paralysed limbs. This paradigm of rehabilitation is being challenged by evidence from basic and applied science for activity-dependent plasticity of neural circuits below the level of injury. The importance of maintaining target systems below the level of injury (e.g. muscle, bone, circulation) essential for improvement of health outcomes, and for future cures to be realized, is also being recognized.

SCIPA is a five year program of research funded by the Victorian Neurotrauma Initiative and the Lifetime Care and Support Authority in NSW. It comprises four projects investigating novel rehabilitation strategies for people with spinal cord injuries:
- SCIPA Hands-On (Clinical Trial)
- SCIPA Full-On (Clinical Trial)
- SCIPA Switch-On (Clinical Trial)
- SCIPA Com (Community Educational Program)

The multi-centre randomised controlled clinical trials involving all spinal units in Australia and New Zealand are examining the effectiveness of very early exercise for the lower limbs, task-specific training for the arm and hand, and an intensive activity-based therapy program for the whole body including the paralysed limbs. In addition, an on-line educational program to improve the knowledge and confidence of fitness instructors in the community regarding exercise for people with spinal cord injury has been developed and is being evaluated. The focus of these projects is on promoting neurological recovery, maintaining health and wellness, and optimising independence. The program will be evaluated using a comprehensive suite of outcome measures, including neurophysiological and functional assessments to examine the effects on multiple systems (neurological, musculoskeletal, cardiovascular), as well as quality of life, and measures of community participation. Economic analyses will be conducted to evaluate cost-effectiveness.

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MAJOR RESEARCH PROJECTS
START Collaboration
The S1r0ke imAging pRevention and Treatment (START) program is a research consortium funded by the CSIRO. The program targets stroke, the most widespread and economically costly of central neurodegenerative diseases. The START collaborators include: Florey Neuroscience Institute, University of Melbourne, Neurosciences Victoria, Melbourne Health and of course CSIRO. The Flagship Cluster Leaders for the project are Prof Geoffrey Donnan (Florey) and Prof Stephen Davis (Melbourne Health).

This study is one of the CSIRO Flagship projects that is targeted at national goals which are closely aligned to the Australian Government’s National Research Priorities. The Flagship involves collaboration between leading Australian scientists, research institutions, commercial companies and CSIRO. Their scale, longer time-frames and clear focus on delivery and adoption of research outputs are designed to maximise their impact in key areas of economic and community need.

The program incorporates the three key strategies nominated as the focus of the Flagship:
- To obtain early stage measures of the key changes in the central nervous system in brain disease of significance to Australia.
- To identify strategies to delay or retard the onset of those changes and disease.
- To provide functional platforms from which to test evidence based approaches designed for the prevention of those unwanted outcomes.

The main vehicle used to achieve these objectives is a Clinical Trial called EXTEND (Extending the time of the Flagship: Key strategies nominated as the focus of the Flagship). This is a large national/international clinical trial of stroke patients involving data and tissue collection with an intervention/treatment arm, using the registered thrombolyis treatment

Tissue Plasminogen Activator (tPA®), Boehringer-Ingelheim) beyond the usual registered time window ie up to 9 hours post-stroke, which is double the usual time window for treatment. The trial is being conducted at approximately 20 hospitals throughout Australia and New Zealand and aims to recruit 200 patients. To date, the majority of sites are approved by their local ethics committees and are in the process of recruiting subjects into the study.

The information that will be collected includes:
- 1. Health information covering the following: outcomes (recovery, recurrence, complications, depression, quality of life, participation), risk factors, medications, diet and lifestyle and,
- 2. Blood for biomarkers: Blood samples will be collected at 6 timepoints on all 200 patients, and aliquoted and stored in a central tissue bank for later biomarker analysis (proteins, DNA, RNA).

A sub-set of patients participating in the EXTEND trial will be recruited into a specialised sub-study called PrePARE (Prediction and Prevention to Achieve Optimal Recovery Endpoints after Stroke).

This study will perform highly specialized brain imaging techniques and advanced assessments for stroke outcomes such as depression, cognition and participation. All assessments will be conducted at a central facility in Melbourne.

Neuroscience Trials Australia acts as the lead agency for research funded by the Victorian Neurotrauma Initiative and the Lifetime Care and Support Authority. SCIPA Program (Spinal Cord Injury and Physical Activity: Intensive Exercise from Acute Care to the Community Program).

Prof Mary Galea (University of Melbourne)

Rehabilitation after spinal cord injury has traditionally been based on expectations regarding functional outcomes predicted by initial level of injury and severity of impairment. It has been characterized by teaching strategies that compensate for loss of function rather than exercising the paralysed limbs. This paradigm of rehabilitation is being challenged by evidence from basic and applied science for activity-dependent plasticity of neural circuits below the level of injury. The importance of maintaining target systems below the level of injury (e.g. muscle, bone, circulation) essential for improvement of health outcomes, and for future cures to be realized, is also being recognized.

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The focus of these projects is on promoting neurological recovery, maintaining health and wellness, and optimising independence. The program will be evaluated using a comprehensive suite of outcome measures, including neurophysiological and functional assessments to examine the effects on multiple systems (neurological, musculoskeletal, cardiovascular), as well as quality of life, and measures of community participation. Economic analyses will be conducted to evaluate cost-effectiveness.

Figure 1: Key services provided and Stakeholders for Neuroscience Trials Australia.
Neuroscience Trials Australia are program managers for SCIPA, and are responsible for setting up the program studies and data management. In addition, our staff are responsible for monitoring all site issues including recruitment, study progress and preparation for data and safety review meetings.

OTHER RESEARCH PROJECTS

AVERT Study
A Phase III, multicentre, randomised controlled trial of very early rehabilitation after stroke (AVERT) is a large (n = 2104) trial of very early rehabilitation compared to standard care in stroke patients. It is an investigator-initiated clinical trial, which was funded by an NHMRC grant in 2006. At that point, Neuroscience Trials Australia contracted a part time project manager to the team who brought skills from both a clinical and pharmaceutical industry background. The trial progressed quickly from concept to reality with a rapid development of all trial documents and processes enabling the first patient to be recruited in early July 2006. The trial attracted subsequent funding from international sources which has led to the successful expansion of the trial into 5 countries.

NEUROSCIENCE TRIALS AUSTRALIA STAFF:
Co-chair, Geoffrey Donnan, MBBS, MD, FRACP, FRCP (Edin)
Co-chair, Stephen Davis
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Su Cox, BSc, MSc
Elise Cowley, BSc (Hons)
Melanie Hurley, BSc, MBA
Lisa Brauman, BA (Social Science)

SCIENTISTS NEED TO TELL THEIR STORIES TO NON-SCIENTISTS, BECAUSE SCIENCE STORIES HAVE TO COMPETE WITH OTHER STORIES ABOUT HOW THE UNIVERSE WORKS, AND HOW IT CAME TO BE. SOME OF THOSE STORIES – BIBLE STORIES, MOVIE STORIES, MYTHS – CAN BE VERY BEAUTIFUL AND VERY COMPELLING. BUT TO PROTECT SCIENCE AND SCIENTISTS – AND THIS IS NOT A GENTLE COMPETITION – YOU’VE GOT TO GET IN THERE AND TELL YOUR VERSION OF HOW THINGS ARE, AND WHY THINGS CAME TO BE.

ROBERT KRULWICH, RENOWNED AMERICAN CORRESPONDENT ON THE PBS INVESTIGATIVE SERIES FRONTLINE
Front cover: Professor Andrew Lawrence is an Associate Director and head of the Florey’s Behavioural Neuroscience Division, where he leads the Addiction Neuroscience laboratory. His group is studying the ways the brain is affected by drug-taking and how adaptations result in persistent drug-seeking. His team is also chasing therapeutic targets to curb drug and alcohol abuse disorders. He has published 175 original articles and reviews and, over the last 24 months, his group has won $3 million in competitive grant funding.

The Florey Neuroscience Institutes acknowledges the traditional owners of this land, the Wurundjeri people and the Kulin nations. We pay our respects to their Elders, past and present. We would like to acknowledge that our building rest on this precious land.