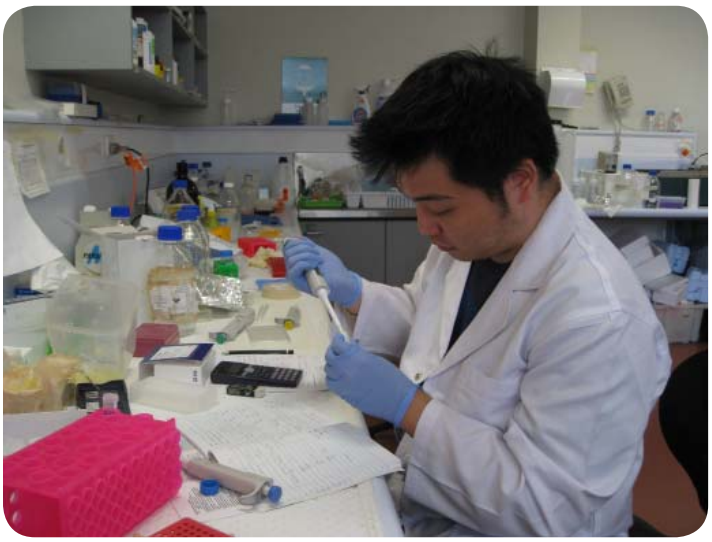
2012



PHARMACOLOGY

BSc HONOURS & MSc (RT)

PROJECT BOOKLET

PROSPECTIVE STUDENTS

It is a great pleasure to introduce you to the projects that are on offer by the Department of Pharmacology for 2012. Most projects offered will be in our spacious, high quality research laboratories on the 8th and 9th floors of the Medical Building. The remainder will be conducted in affiliated Research Institutes with external supervisors and co-supervision by Department staff.

It is not a simple task to select a project, laboratory and supervisor. We suggest you talk to several potential supervisors, as well as to their current Honours or RHD students, to gain some appreciation of the research problems being addressed and the related techniques.

The Department of Pharmacology Honours Course is directed at students with above average scientific ability. The year is a transition year from formal lectures and teaching, to self-directed learning and exploration of your own scientific problem. We will introduce you to skills in communication, data analysis and assessment of scientific papers. Your supervisor and laboratory staff will guide you through the challenges, strengthen your technical skills and introduce you to the excitement of research – its rewards and its disappointments. You will have the opportunity to use the latest in equipment and to share with PhD scholars their recent research experiences in the laboratory. The "Honours Experience" will require self-motivation and discipline, and you will learn a lot about your own problem-solving ability.

We hope you will join us in Pharmacology for the 2012 Honours year. We aim to give you the best opportunity to 'have a go' at solving a research problem, teach you important skills for future employment in various biomedical vocations and provide a solid basis for those who want to go further in a research career.

Very best wishes for the next step in your journey!

Associate Professor Christine Wright
Honours & MSc (RT) Co-ordinator

Associate Professor James Ziogas
Head of Department

'Research is to see what others have seen - but to think what no-one has thought!'

Szent-Gyorgi

CONTENTS

PROJECTS
for
BSc HONOURS
or
MSc (RT)
in PHARMACOLOGY

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INVESTIGATING PARKINSON'S & ALZHEIMER'S DISEASE

Supervisor: **Prof Kevin J Barnham**
Email: **kbarnham@unimelb.edu.au**
Telephone: **8344 2555**
Location: **Bio21 Institute, The University of Melbourne**
Pharmacology Supervisor: A/Prof James Ziogas

The following projects are offered as BSc Honours or MSc(RT)

TERNARY COMPLEX FORMATION OF METAL IONS WITH ALZHEIMER'S AMYLOID-β PEPTIDE, 8-HYDROXYQUINOLINE THERAPEUTICS AND ENDOGENOUS SMALL MOLECULES – IMPLICATIONS FOR ALZHEIMER'S DISEASE AND THERAPY

Co-Supervisors:

Dr Simon Drew (Centre for Neuroscience)
Email: sdrew@unimelb.edu.au,
Dr Cathryn Haigh (Department of Pathology)
Email: chaigh@unimelb.edu.au

The amyloid-β (Aβ) peptide is the believed to be the causative agent in Alzheimer's disease (AD) and is the primary component of the insoluble aggregates found in the extracellular senile plaques of AD patients. The metals hypothesis implicates Cu and Zn ions in the pathogenesis of AD and subsequently a number of therapies targeting metal/Aβ interactions have been pursued. One class of therapeutics based upon the 8-hydroxyquinolines has shown promising results, with one such compound known as PBT2 showing good efficacy in a phase IIa clinical trial. The mode of action of substituted 8-hydroxyquinolines remains to be fully elucidated, although they are known to be multifunctional molecules capable of disaggregating Aβ plaques, metal chelation and/or chaperoning. This project will use a combination of spectroscopic and cell culture techniques to characterise the structure of a range of Cu2+(Aβ)X and Cu2+(8-hydoxyquinoline) X ternary complexes are formed with number of endogenous biomolecules (X) present at the synapse. The relevance of ternary complexes to the neurotoxic effects of Aβ and the cognitive-enhancing properties of 8-hydroxyquinolines will be explored.

PARKINSON'S DISEASE: INVESTIGATING THE EFFECTS OF NITRATED A-SYNUCLEIN IN THE PATHOGENESIS OF PARKINSON'S DISEASE

Co-Supervisor: Dr Lin Wai Hung

Parkinson's disease (PD) is a common motor neurodegenerative disorder, affecting up to 5 million people worldwide. It is a progressive and chronic disease characterized by dyskinesia, rigidity, instability and tremors. While the pathogenesis of PD has not been elucidated, evidence points to the presence of aggregated α-synuclein as pathogenic. Aggregation of α-synuclein is influenced by a number of factors, one of which is peroxynitrite-induced nitration. Nitrated forms of α-synuclein is found abundantly in the brains of PD patients and injection of these forms of α-synuclein into animals causes neurodegeneration to specific populations of neurons involved in the coordination of motor functions. Recently, it was shown an inhibitor of nitration, CuII(atsm), effectively reversed Parkinsonian-like symptoms in mouse models and improved motor function.

This project would, therefore, aim to investigate the biophysical effects of α-synuclein upon nitration and whether CuII(atsm) would be able to reverse these effects. A number of techniques will be utilized including bacterial cultures to synthesis recombinant forms of α-synuclein. Biophysical characterisation would be undertaken to investigate the effects of nitration on the secondary and tertiary structure of α-synuclein. This would be correlated with the proteins ability to bind lipid membranes and induce toxicity in cell culture models. These experiments would provide a better insight into the role nitration plays in manifesting the toxicity of α-synuclein.

PARKINSON'S DISEASE: INVESTIGATING NON MOTOR SYMPTOMS IN PD MICE MODEL

Co-Supervisor: Dr Lin Wai Hung

Parkinson's disease (PD) is a common motor neurodegenerative disorder, affecting up to 5 million people worldwide. It is a progressive and chronic disease characterized by dyskinesia, rigidity, instability and tremors. Current therapies that are available target these symptoms; however, they do not halt or reverse pathology. However, non-motor symptoms are also associated with this disease, at times even before the manifestation of motor symptoms. Non motor symptoms include, insomnia, gastrointestinal, olfactory and autonomic dysfunction. There are no therapies currently available that could target these non-motor symptoms

While there are numerous behavioural tests that could evaluate motor symptoms in animal models of PD, there are very few tests that investigate the non-motor aspects. Hence, this project would look to develop effective non-motor behavioural tests that could be used to predict disease status. Once developed these behavioural tests would be used in a variety of animal models, including transgenic animals expressing mutated forms to protein found in familial PD patients, to delineate the pathogenesis of PD. These animal models would also be treated with drugs to investigate whether they could restore normal non-motor functions.

Therefore, this project would help to correlate non-motor symptoms of PD to motor impairment and further our understanding PD pathogenesis.

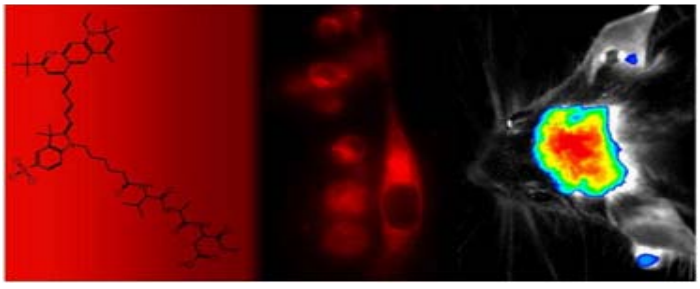
NON-INVASIVE NEAR-INFRARED IMAGING OF DISEASE PROGRESSION AND RESPONSE TO THERAPY IN A LIVE ANIMAL MODEL OF ALZHEIMER'S DISEASE

Co-Supervisor: Dr Simon Drew (Centre for Neuroscience)

Dementia currently affects over 240,000 Australians with estimates predicting that there will be over 1 million sufferers by 2050, with an increasing public health burden. The most common cause of dementia is neurodegeneration arising from the aggregation of normal cellular proteins and their deposition, often as amyloid. These neurodegenerative conditions include Alzheimer's disease, Parkinson's disease, Huntington's disease, and transmissible spongiform encephalopathies (prion diseases). Current medical imaging paradigms focus strongly on the detection of amyloid, but deposition of protein aggregates most likely happens after significant neuronal dysfunction has occurred and in some instances amyloid can be present even in the absence of disease. Therefore, imaging agents capable of identifying molecular events independent of protein deposition and prior to the onset of clinical symptoms are most desirable to enable intervention before quality of life has deteriorated beyond meaningful recovery.

In vivo fluorescence imaging that uses near-infrared (NIR) wavelengths (650-900 nm) to harmlessly penetrate mammalian tissue is an emerging imaging modality. Optical imaging of amyloid plaques in mouse models of neurodegenerative disease has been demonstrated using various NIR amyloid binding agents [1]; however, the correlation between amyloid deposition and disease severity remains unclear. To monitor disease progression and response to treatment, NIR contrast agents that are sensitive to the toxic signaling cascade that drives the neuronal dysfunction must be sought. A key player in this biochemical cascade is the caspase family of enzymes and we have recently shown that NIR imaging agents targeting these enzymes can provide a more suitable in vivo marker of disease development prior to onset of clinical features [2].

This project will use two NIR contrast agents to correlate amyloid burden (THK-265 agent) [1] and caspase activation (NIRD agent) [2] during disease progression and in response to therapy (such as the 8-hydroxyquinoline clioquinol) in transgenic mice expressing human amyloid precursor protein (APP).



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1. N. Okamura, M. Mori, S. Furumoto, T. Yoshikawa, R. Harada, S. Ito, Y. Fujikawa, H. Arai, K. Yanai, Y. Kudo, In vivo Detection of Amyloid Plaques in the Mouse Brain using the Near-Infrared Fluorescence Probe THK-265, J. Alzheimer's Dis. 23 (2011) 37–48.
2. V.A. Lawson, C.L. Haigh, B. Roberts, H.M.J. Klemm, V.B. Kenche, C.L. Masters, S.J. Collins, K.J. Barnham, S.C. Drew, Near Infra-Red Fluorescence Imaging of Apoptotic Neuronal Cell Death in a Live Animal Model of Prion Disease, ACS Chem. Neurosci. 2010, 1, 720–727.

INVESTIGATING THE EFFECTS OF NITRATED TAU IN THE PATHOGENESIS OF PARKINSON'S DISEASE

Co-Supervisor: Dr Lin Wai Hung

Parkinson's disease (PD) is a common motor neurodegenerative disorder, with an average age of onset of 55 years and disease duration of approximately 20 years. It is a progressive and chronic disease characterized by dyskinesia, rigidity, instability and tremors. The pathognomonic indicator of disease is the presence of Lewy bodies, consisting primarily of aggregated α-synuclein protein. Recently, it was discovered that the MAPT gene, that encodes the tau protein, is associated with increased risk of PD. Tau is an important physiological cyto-skeletonal protein; however, aberrant and mutated forms of the protein is involved in a range of tauopathies, including Alzheimer's and now, Parkinson's diseases.

This project will investigate how post-translational modifications of the tau protein could be involved in the pathogenesis of PD, in particular, nitration of tyrosine residues. Tau can be nitrated by peroxynitrite (ONOO-), a reactive nitrogen species that is also involved in PD pathology; nitrated α-synuclein is found in the Lewy bodies of PD.

In this project, the tau protein synthesized using bacterial cultures would be nitrated with ONOO- and its toxicity measured in cell cultures. To further evaluate the role of nitrated tau, neuronal cell cultures would be used whereby cells will be treated with an ONOO- releasing compound, SIN-1. SIN-1 toxicity would be compared in cells expressing normal forms of tau with tau knockout or tau mutant over-expressing cells in order to delineate tau toxicity during ONOO- insult. In addition, levels of nitrated tau would be investigated in animal models of tauopathy as well as in PD human patients. Therefore, this project would encompass a wide range of experimental techniques ranging from in vitro testing to analysing human samples.

NEUROPEPTIDE RECEPTOR LABORATORY

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Facsimile: 9348 1707
Location: Florey Neuroscience Institutes
Pharmacology Supervisor: A/Prof Tony Hughes

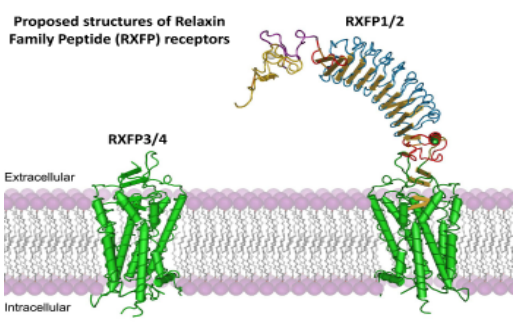
The following projects are offered as BSc Honours or MSc(RT)

STUDIES ON NOVEL NEUROPEPTIDE G-PROTEIN COUPLED RECEPTORS; RELAXIN FAMILY PEPTIDE RECEPTORS, EVOLUTION, STRUCTURE, FUNCTION AND DRUG DEVELOPMENT

My research focuses on the relaxin peptide family and their G-protein coupled receptors RXFP1-4. The peptides relaxin, relaxin-3, insulin-like peptide 3 (INSL3) and INSL5 have numerous essential biological roles. Relaxin induces its effects by regulating collagen turnover, stimulating tissue growth and angiogenesis and inducing blood vessel dilatation. It is currently being used in a Phase III clinical trial for acute heart failure being performed by Novartis. INSL3 is essential for germ cell maturation and drugs targeting its receptor RXFP2 have considerable potential as fertility regulators in both males and females. INSL5 is a gut hormone that has potential roles in fat and glucose metabolism and we are working with Takeda Cambridge to develop compounds targeting its receptor RXFP4 which may be useful for treating obesity and/or diabetes. Relaxin-3 is a specific neuropeptide which our laboratory recently discovered (8) and has potential roles in regulating behaviours which are perturbed in mental illnesses including anxiety, depression, sleep disorders, and memory deficits. Hence drugs targeting the relaxin-3 receptor RXFP3 may be potential therapeutics to treat these mental illnesses. We are working with pharmaceutical industry partners (Johnson and Johnson, Takeda and Novartis) to determine the biological roles of the peptides and to develop drugs targeting their receptors.

Receptor projects:

The receptors for these peptides are all G-protein coupled receptors (GPCRs) which are the largest class of cell surface signaling 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 as well their cell signaling characteristics. Furthermore we are working with A/Prof Paul Gooley 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 mechanism of ligand binding and activation is required to design drugs targeting these receptors.



Furthermore, viral strategies are being utilized in rodents to study receptor function in vivo via over expression or RNA-interference induced knockdown of receptor and/or ligand expression (see also specific viral project below).

Honours and PhD projects are available to study the pharmacology of these novel receptors and their splice variants. Candidates will undergo training in various techniques including molecular cloning, cell biology, protein chemistry, site-directed mutagenesis, confocal microscopy, viral expression and animal behavioural phenotyping.

Recent Publications:

1. Bathgate RAD, et al., (2006) International union of pharmacology (IUPHAR); Recommendations for the nomenclature of receptors for relaxin family peptides. Pharmacological Reviews 58: 7-31.
2. Callander GE, Thomas WG and Bathgate RAD (2009) Prolonged RXFP1 and RXFP2 signaling can be explained by poor internalization and a lack of β-arrestin recruitment. American Journal of Physiology, Cell Physiology 296: C1058-66
3. Hossain MA, et al., and Bathgate RAD (2008) The A-chain of human relaxin family peptides has distinct roles in the binding and activation of the different relaxin family peptide receptors. Journal of Biological Chemistry 283: 17287 - 17297.
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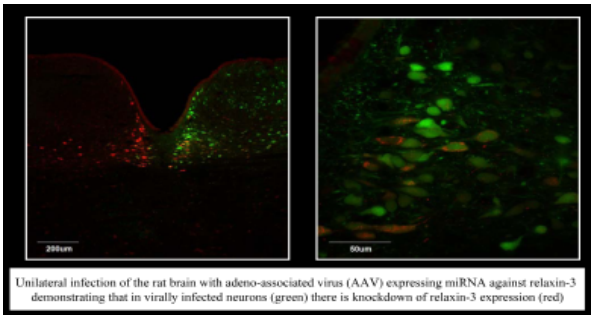
7. Bathgate RAD, et al., (2002) Human relaxin gene 3 (H3) and the equivalent mouse relaxin (M3) gene: Novel members of the relaxin peptide family. Journal of Biological Chemistry 277: 1148-1157.
8. Callander GE, Thomas WG, Bathgate RAD (2009) Development and Optimization of miRNA against relaxin-3. Annals of the New York Academy of Sciences 1160: 261-264.

ADENO-ASSOCIATED VIRUS MEDIATED MODULATION OF NEUROPEPTIDE FUNCTION IN BRAIN

Mental illness is a large and increasing health and economic burden in Australia and worldwide and more research is urgently required to identify new and innovative therapies. In this regard, GPCR neuropeptide receptors may be better therapeutic targets than receptors for the 'primary' transmitters (amino acids and monoamines), as they offer reduced side-effects, due to their modulatory rather than primary excitatory or inhibitory actions. Studies by our group have shown that relaxin-3 has potential roles in regulating behaviours which are perturbed in mental illnesses including anxiety, depression, sleep disorders and memory deficits. The study of neuropeptide systems is complicated by their modulatory actions such that gene knockout animals are potentially susceptible to developing functional compensation. We utilize viral gene transfer to transduce specific neuronal populations allowing the chronic modulation of neuropeptide or neuropeptide receptor function by either gene silencing or by over expression of peptide agonists or antagonists in adult animals thus avoiding compensation.

We have successfully employed gene silencing to modulate the expression of the neuropeptide relaxin-3. We have produced adeno-associated viral (AAV) particles expressing microRNA targeting relaxin-3 which when infused at the site of relaxin-3 production, the nucleus incertus resulted in ablation of relaxin-3 expression. Co-expression of EmGFP simultaneously confirmed injection sites and labelled tranduced neurons (see figure above). We are using this technology to elucidate the role of relaxin-3 in behaviours disrupted in mental illnesses. Additionally, we have utilized AAV and lentiviral particles over expressing a relaxin-3 agonist to modulate feeding and arousal in rat models. This research will identify influences of relaxin-3 signaling on parallel animal behaviours to those disrupted in mental illnesses such as anxiety, depression, sleep disorders, and memory deficits; and support future studies to identify effects of altered relaxin-3 activity in experimental models of human disease to uncover new therapies for these disorders. We have intellectual property (IP) and commercial links in the area that will facilitate therapeutic opportunities.

Honours and PhD projects are available to study the modulation of neuropeptide and neuropeptide receptor (GPCR) expression using recombinant adeno-associated viruses. Candidates will undergo training in various techniques including molecular cloning, cell biology, confocal microscopy, viral expression and animal behavioural phenotyping.



Unilateral infection of the rat brain with adeno-associated virus (AAV) expressing miRNA against relaxin-3 demonstrating that in virally infected neurons (green) there is knockdown of relaxin-3 expression (red)

LUNG REGENERATION LABORATORY

The broad interest of the Lung Regeneration Laboratory is to characterize epithelial and mesenchymal stem cells in the normal and diseased lung, including chronic obstructive pulmonary disease, asthma, pulmonary fibrosis and cancer. Our long-term goal is to identify factors regulating lung stem cells as a prerequisite to the development of therapeutic strategies to attenuate lung disease and regenerate the injured lung.

This work addresses an area of utmost importance in human health with more than half a billion people worldwide struggling each year for life and breath due to chronic respiratory diseases. Successful completion of these projects will help us to understand the mechanisms involved in regulating lung epithelial stem cells which will ultimately inform the development of stem cell targeted therapies to enhance the regenerative capacity of the lung. Equally, this research will provide critical insight into the role of perturbed epithelial stem cell regulation in lung epithelial remodeling, a major clinical manifestation of chronic asthma and COPD, and in the initiation, propagation and metastasis of lung cancers.

This year we will be offering up to three honours projects aimed at determining the biological and pathophysiological behaviour of endogenous lung stem cells in respiratory diseases, including asthma, chronic obstructive pulmonary disease and lung cancer. These projects will involve cutting edge research using flow cytometry-based cell separative strategies, novel three-dimensional cell culture assays, in vivo transplantation models and molecular biology techniques.

The following projects are offered as BSc Honours or MSc(RT)

THE ROLE OF ADULT LUNG STEM CELLS IN LUNG INJURY AND REPAIR

Supervisor: **A/Prof Ivan Bertoncello**
Email: **ivanb@unimelb.edu.au**
Telephone: **8344 6992**
Facsimile: **8344 0241**
Location: **Department of Pharmacology, Level 8 Room N808 Medical Building**
Co-Supervisor: **Dr Jonathan McQualter (jlmcq@unimelb.edu.au)**

The central hypothesis to be tested in this proposal is that disruption of the epithelial-mesenchymal trophic unit during chronic respiratory disease and lung cancer results in unbalanced signalling in lung stem cells leading to a disturbed (pathologic) regenerative process. This project will analyse the temporal pattern of depletion and recovery of lung epithelial stem cells following lung injury in transgenic mouse models of lung disease. Cell culture analysis of the proliferation, self-renewal and lineage specificity of lung stem cells at various stages of injury and repair will provide valuable insights into the role in endogenous epithelial stem cells in injury and repair of the adult lung.

PROFILING INTRACELLULAR SIGNALLING PATHWAYS INVOLVED IN REGULATING ADULT LUNG STEM CELLS

Supervisor: **Dr Jonathan McQualter**
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Facsimile: **8344 0241**
Location: **Department of Pharmacology, Level 8 Room N808 Medical Building**
Co-Supervisor: **A/Prof Ivan Bertoncello**

Recently we have identified a population of multipotent adult lung stem cells and developed new culture systems to assess their proliferative and differentiative potential. Using this assay, we have shown that hepatocyte growth factor (HGF) in co-operation with fibroblast growth factor (FGF-10) is essential for lung stem cell growth. This project will characterize the intracellular signalling pathways downstream of the HGF receptor tyrosine kinase (Met) that are fundamental to the survival, proliferation, and differentiation of lung epithelial stem cells in health and disease. Completion of this project will provide fresh insight into the previously unexplored role of HGF/Met signalling in regulating distinct cellular processes of adult lung stem cells in health and disease, ultimately leading to the identification of novel therapeutic targets for preventing aberrant stem cell growth (i.e. Cancer and epithelial remodelling) and promoting epithelial regeneration.

THE ROLE OF CELL-CELL AND CELL-MATRIX INTERACTIONS IN LUNG EPITHELIAL CELL REGENERATION AND REPAIR

Supervisor: **A/Prof Ivan Bertoncello**
Email: **ivanb@unimelb.edu.au**
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Location: **Department of Pharmacology, Level 8 Room N808 Medical Building**
Co-Supervisor: **Dr Jane Bourke (jane.bourke@unimelb.edu.au)**

The regenerative capacity of lung stem cells is not only determined by their intrinsic developmental potential, but also by their interaction with extracellular matrix, cytokines, and stromal, endothelial and immunomodulatory cell elements which comprise their niche. This project will analyse the role of cell-matrix and cell-cell interactions in the engraftment, proliferation and differentiation of candidate lung stem cell populations utilizing de-cellularized and cellularized whole mouse lung slice cultures seeded with defined fluorescent donor lung stem/progenitor cells at clonal cell densities. Preservation of the structural organisation of the airways and distal lung in lung slice culture will allow us to identify key spatial cues which specify lung stem cell fate and regulate the temporal pattern of regeneration and repair of lung epithelial cell lineages. Immunophenotypic and molecular cell lineage tracing techniques, and clonal stem/progenitor cell assays, will be used to measure the regenerative potential of repopulating cells and analyse their ability to give rise to descendent cell lineages. Successful completion of this project will provide data informing translational studies aimed at developing cellular therapies for intractable lung diseases.

RESPIRATORY PHARMACOLOGY LABORATORY

Supervisor:	**Dr Jane Bourke**
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Co-Supervisor:	**Dr Ken Snibson (Veterinary Science, 8344 8716)**

The following project is offered as BSc Honours or MSc(RT)

OVERCOMING BRONCHODILATOR RESISTANCE BY TARGETING SMALL AIRWAYS IN ASTHMA

Despite the clear importance of inducing airway relaxation to oppose airway hyperresponsiveness (AHR) in asthma, recent research has focused on targeting inflammation and airway wall remodelling. However, excessive contraction of airway smooth muscle (ASM) in severe asthma, particularly in small airways, may be relatively insensitive to treatment with β_2-adrenoceptor agonists. This highlights pressing needs to validate appropriate animal models to assess altered small airway reactivity and to characterize mechanisms underlying resistance to bronchodilator therapy.

Our sheep model of chronic allergic airways disease (AAD) mimics key features of human persistent asthma including AHR (Snibson et al., 2005). The goal of this project is to use this model to identify novel agents targeting small airways to limit contraction or elicit relaxation when AHR is present and β_2-mediated relaxation is limited.

To achieve this goal, you will examine the functional pharmacology and physiology of small airways using the lung slice technique to measure regulation of both airway calibre and calcium signalling in vitro. You will also use wedged bronchoscopy in lung segments in conscious sheep to assess small airway reactivity in vivo. Comparisons will be made between novel and currently used bronchodilators (e.g. PPARγ agonists, bitter taste receptor agonists, short- and long-acting β_2 agonists).

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and

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4. Deshpande DA, Wang WC, McIlmoyle EL, Robinett KS, Schillinger RM, An SS, Sham JS, Liggett SB. Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. Nat Med. 2010 16:1299-304

CELL SIGNALLING AND LUNG DISEASE

Supervisor: **Dr Steven Bozinovski**
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Facsimile: **8344 0241**
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Level 8 North Medical Building

The following projects are offered as BSc Honours only

IDENTIFYING MOLECULAR LINKS IN COPD (EMPHYSEMA) AND LUNG CANCER

Co-Supervisors: **Prof Gary Anderson**
Prof Louis Irving (Royal Melbourne Hospital)

Lung cancer is the #1 cause of cancer death worldwide killing more people each year than breast, colon, ovarian and skin cancer combined. Current treatments extend life by only a few months. COPD (Chronic Obstructive Pulmonary Disease / Emphysema) is the #4 cause of cause of all death worldwide and both diseases will continue to be a major health burden for decades to come. Intriguingly, COPD is also recognised to be major risk factor for lung cancer, and interestingly this can occur independently of smoking status, which implicates shared molecular pathways. This project will investigate novel molecular pathways in COPD very recently discovered in our laboratory and explore their significance in lung cancer susceptibility. A range of molecular and cell biology methods will be implemented including phenotyping of macrophage and myeloid lineages that may be important in tumour evasion and progression.

You will be trained in a wide suite of techniques including Quantitative PCR, cell and tissue culture, histology, FACS analysis of cell populations; ELISA and Western blotting, In vivo disease models and viral culture.

An important part of this project is that your basic research will be translational i.e. linked in with the Lung cancer research effort at Royal Melbourne Hospital.

HOW DOES SMOKE EXPOSURE WORSEN VIRAL AND BACTERIAL INFECTIONS

Co-Supervisors: **Dr Desiree Anthony**
Prof Gary Anderson

Viral lung infections are known to predispose susceptible people to secondary bacterial infections that can be very serious or fatal but the underlying mechanism are obscure. Cigarette smoke exposure is known to alter immune responses to viral infections and is a major epidemiological risk factor for severe chest infection. Chronic smokers with a disease termed COPD are particularly susceptible to aggressive infectious events known as exacerbations.

Understanding why the immune system of COPD patients does not respond appropriately to infection is a central theme of our laboratory, as this will lead to better ways of treating this debilitating disease. Using immunological and molecular techniques, this project will investigate how smoke exposure alters viral responses and how this in turn promotes susceptibility to secondary bacterial transmission.

You will be trained in a wide suite of techniques including Quantitative PCR, cell and tissue culture, FACS analysis of cell populations; ELISA and Western blotting, In vivo disease models and viral culture.

ANAESTHESIA & PAIN MANAGEMENT UNIT

Supervisor: **Dr Jenny Callaway**
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Facsimile: **8344 0241**
Location: **Department of Pharmacology,**
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Medical Building
Co-Supervisor: **Prof Colin Royse**

The following project is offered as BSc Honours only

WHAT CAUSES POST-OPERATIVE COGNITIVE DECLINE?

It is remarkably common to experience loss of memory and concentration following surgery and anaesthesia. While this is more likely to be long-lasting and cause significant problems in people over the age of 65 year, it can and does happen in young patients. This phenomenon is now recognised and is referred to as Post-Operative Cognitive Dysfunction (POCD) and the cause is currently unknown. We are currently investigating the possibility that type of anaesthesia, age, and inflammation or infection may interact to cause POCD.

This project will involve considerable hands-on work in anaesthetised and conscious rats. The techniques involved will include surgery, blood sampling, memory testing methods as well as investigation of brain pathology and assessment of inflammatory markers using immunohistochemistry and biochemical assays. This exciting project will give the right student the opportunity to work within the pharmacology department in a research science laboratory as well as to interact with anaesthetists and surgeons with direct interest in POCD research.

NEUROPHARMACOLOGY LABORATORY

Supervisor: **Dr Peter Crack**
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Telephone: **8344 8417**
Facsimile: **8344 0241**
Location: **Department of Pharmacology**
Level 8, Medical Building
Co-Supervisor: **Dr Juliet Taylor**

The following projects are offered as BSc Honours or MSc(RT)

INNATE IMMUNITY AND NEURAL INJURY

A major new area of research in our laboratory is the role that the innate immune system plays in the progression of neural injury. It is now appreciated that the central nervous system (CNS) does exhibit features of inflammation, and in response to injury, infection or disease, resident CNS cells generate inflammatory mediators, including proinflammatory cytokines, prostaglandins, free radicals and complement, which in turn induce chemokines and adhesion molecules, recruit immune cells, and activate glial cells. Cerebral ischemia triggers acute inflammation, which exacerbates primary brain damage. Activation of the innate immune system is an important component of this inflammatory response. The innate immune system uses a newly discovered family of receptors to transducer its' signal called the Toll-like receptors (TLRs). The roll that the TLR's play in the progression and response to neural injury is an exciting and emerging field of research. The molecular mechanisms that are influenced by the TLRs comprise new targets for therapeutic intervention into acute neurological conditions such as stroke and neurotrauma and chronic neurological diseases such as Multiple Sclerosis and Parkinsons disease.

Skill acquisition: In vivo disease models, histology, immunohistochemistry, morphometry, quantitative PCR, FACS analysis of cell populations, cell and tissue culture, ELISA, molecular biology and Western blotting.

OXIDATIVE STRESS AND NEURAL INJURY

The major focus of our laboratory are the mechanisms that underpin the progression of neural injury. The causes of neural injury are multifactorial so our laboratory's research is focused on the role that oxidative stress and reactive oxygen species (ROS) play in the predisposition and/or progression of neural injury. Rather than serving solely as harmful by-products of aerobic metabolism, it has become apparent that ROS have a much broader role in the regulation and co-ordination of cellular homeostasis. ROS are used to fine-tune cellular signaling and play an important role in the transduction of message along specific signal transduction pathways. In the event of oxidative stress, which is associated with varied human diseases including neurological disorders, the persistent inactivation of signal transduction pathways by ROS may lead to reduced or ablated, sustained or elevated cellular signaling and predispose or otherwise contribute to disease pathology. In understanding how signal transduction systems are regulated by oxidative stress and ROS we can gain a better understanding how new generation therapeutics can target these pathways in the hope to reduce and or prevent neuronal pathology.

Skill acquisition: In vivo disease models, histology, immunohistochemistry, morphometry, quantitative PCR, FACS analysis of cell populations, cell and tissue culture, ELISA, molecular biology and Western blotting.

THE ROLE OF NEUROINFLAMMATION IN PARKINSON'S DISEASE.

Parkinson's Disease (PD) is a progressive neurological disease that is characterized by the loss of dopaminergic neurons, primarily in the substantia nigra. The loss of these neurons leads to a motor handicap, associated depression, pain and general decreased quality of life. The mechanism for the loss of the dopaminergeric neurons is unknown although it is hypothesised that protein mis-folding, oxidative stress and neuro-inflammation may contribute to the cell death. We hypothesise that the neuroinflammatory response triggers deleterious events (eg, oxidative stress and cytokine-receptor-mediated apoptosis), potentiating dopaminergic cell death and contributing to disease progression. This project proposes to study the molecular and cellular events associated with neuro-inflammation in an animal model of PD. A multi disciplinary approach using an in vivo mouse model of PD coupled with in vitro studies to investigate the specific molecular pathways involved will investigate the role that neuro-inflammation plays in the progression of PD.

Skill acquisition: The techniques involved in this project entail a mouse model of PD, immunohistochemistry, primary neural cell culture, ELISA, DNA cloning, siRNA and Western analysis and data analysis.

UNDERSTANDING TRAUMATIC BRAIN INJURY

Traumatic brain injury (TBI) represents the major cause of death in young individuals in industrialised countries. Despite the improvement of neurosurgical procedures as well as critical care management, morbidity and mortality are still high and approximately 25% of these patients remain with permanent disabilities becoming a familiar, social and economic burden for society. A better understanding of events occurring in the brain after traumatic brain injury is essential to identify ways to limit the damage and ultimately improve the outcome. The advent of microarray technology has given the researcher the ability to potentially identify the regulation of thousands of genes and enables a broad assessment of gene changes after traumatic brain injury. With the backing of the Victorian Trauma Foundation we have undertaken a microarray study to determine a temporal profile of gene changes in the brain after TBI. This data is being used to understand the molecular pathways that are changed in the brain after TBI.

Skill acquisition: In vivo disease models, histology, immunohistochemistry, morphometry, quantitative PCR, FACS analysis of cell populations, cell and tissue culture, ELISA, molecular biology and Western blotting.

NEUROPEPTIDE BIOLOGY/SYSTEMS NEUROSCIENCE

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Location: **Howard Florey Institute/Florey Neuroscience Institutes**
Co-Supervisors: **Dr Sherie Ma, Dr Craig Smith, Dr Melanie White**
Pharmacology Supervisor: A/Prof Tony Hughes

The following projects are offered as BSc Honours or MSc(RT)

ROLE OF RELAXIN-3/RXFP3 SIGNALLING IN BEHAVIOURAL STATE CONTROL – IMPLICATIONS FOR HEALTH AND DISEASE

Our laboratory is in the Neuropeptides and Behavioural Neuroscience Divisions of the Florey Neuroscience Institutes and we conduct systems neuroscience research. Our primary focus is the role of neuropeptide signalling in the control of complex behaviours such as arousal, stress and mood, and associated memory processes under normal and pathological conditions. Senior scientists in the laboratory offer a range of distinct projects that involve studies in experimental models of psychiatric disease and that are suitable for Honours and PhD students. We use a range of unique experimental tools including RXFP3-selective agonist/antagonist peptides, ligand/receptor knockout mice, viral vectors, and selective neurotoxins. Project examples are listed below and more information can be obtained via the contact details above.

Project 1: Nucleus incertus and relaxin 3/RXFP3 signalling in arousal, stress, mood and cognition – selective lesion and mRNA knockdown studies

Project 2: Role of relaxin-3 neurons and RXFP3 signalling in the amygdala in fear memory and stress-related cognitive dysfunction in rat brain

Project 3: Behavioural and neurochemical responses to relaxin-3/RXFP3 transmission in mice – Implications for mood regulation and mental illness

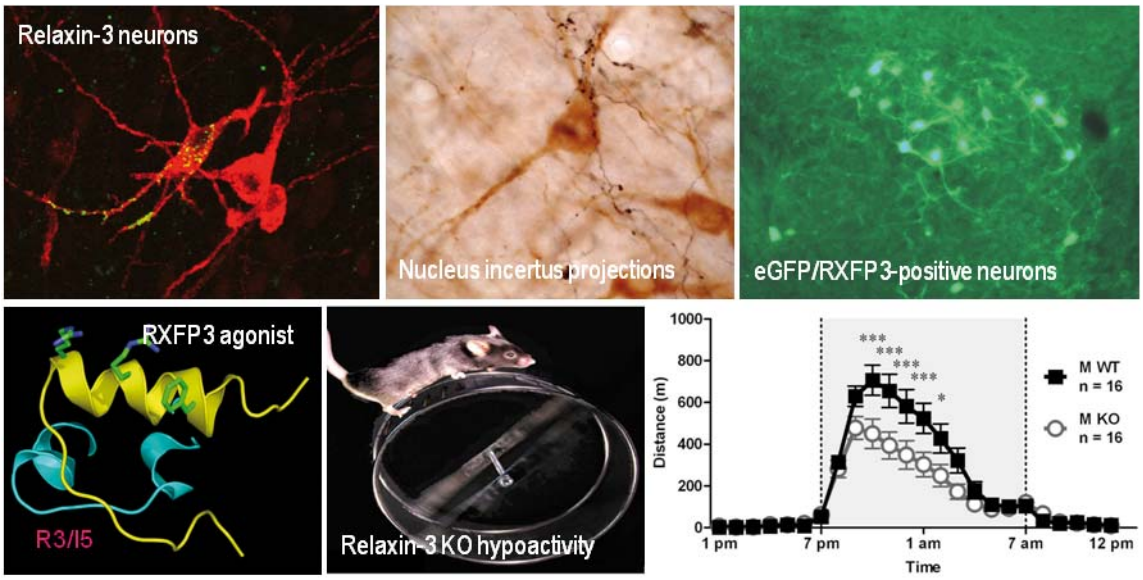
Project 4: Peptide control of 'behavioural state' – effect of chronic changes in relaxin-3/RXFP3 signalling on arousal and related behaviours in transgenic mice

Project 5: Relaxin-3/RXFP3 signalling in limbic networks involved in social interaction – studies in mouse models of autism spectrum disorders (ASD)

[NB: With multiple projects available, we can offer up to 3 Honours places].

Recent Publications:

1. Ma S et al. Neuroscience 144 (2007) 165-190
2. Ma S et al. J Comp Neurol 517 (2009) 856-872
3. Ma S et al. Learn Mem 16 (2009) 730-742
4. Gundlach AL et al. Ann NY Acad Sci 1160 (2009) 226-235
5. Smith CM et al. Ann NY Acad Sci 1160 (2009) 236-241
6. Banerjee A et al. Neuropharmacology 58 (2010) 145-155
7. Smith CM et al. J Comp Neurol 518 (2010) 4016-4045
8. Ryan PJ et al. Neurosci Biobehav Rev 35 (2011) 1326-1341
9. White MD et al. Front Mol Neurosci 4 (2011) 8
10. Smith CM et al. J Chem Neuroanat (in press)



LIMITING TISSUE DAMAGE AFTER SPINAL CORD INJURY

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Co-Supervisors: **A/Prof KM Dziegielewska &**
Prof NR Saunders

The following project is offered as BSc Honours or MSc(RT)

HYPEROXIA AS A TREATMENT TO LIMIT SECONDARY TISSUE DAMAGE AFTER SPINAL CORD INJURY.

Spinal cord injuries are devastating for patients because they result in the permanent loss of motor, sensory and autonomic functions below the level of the injury. The extent of disability is largely determined by the spinal level of the injury and the amount of tissue damage. Hyperoxia (100% inspired oxygen) has been shown in animal models to reduce the extent of tissue damage and reduce the loss of functional abilities after injury. However trials of hyperoxia in human patients have so far been negative but this could be due to the significant time delays prior to treatment.

This project will investigate the effectiveness of hyperoxia as a treatment to limit the amount of tissue damage after spinal injury and the extent to which a delay prior to treatment might compromise the efficacy of hyperoxia.

In this project you will learn how to conduct controlled experiments involving whole animal studies. Techniques applied range from physiological experiments to detailed morphological analysis of spinal cord tissue.

LINKING DEVELOPMENTAL PATHWAYS & CANCER

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Pharmacology Supervisor: Prof Alastair Stewart

The following project is offered as BSc Honours or MSc(RT)

EXAMINING THE CONNECTION BETWEEN U12-TYPE mRNA SPLICING, DEVELOPMENT AND CANCER

As a result of the detailed genetic and morphological characterisation of a zebrafish mutant, we have identified a gene, known as RNA-binding region containing protein 3 (Rnpc3) that is indispensable for a stage in development when intestinal epithelial cells are highly proliferative. Previous studies have established that this gene encodes a protein, Rnpc3/65K involved in a specialised form of mRNA splicing. Specifically, Rnpc3 is a component of the minor class or U12-type spliceosome that catalyses the removal of a minor class of introns, called U12-type introns, from pre-mRNA molecules. U12-type introns are rare but are highly conserved in the plant and animal kingdoms. There are approximately 700 U12-type introns in the human genome (out of a total of >20,000 introns). Interestingly, these introns are not randomly distributed throughout the genome, but are found in "information processing genes". Intriguingly, they are a feature of some tumour suppressor genes and oncogenes.

Because many of the behaviours of developing cells and tissues (eg. proliferation, cell migration and angiogenesis) are recapitulated by cancer cells, we believe that genes that play a role in intestinal development may also contribute to the development of cancer. To explore the possibility that Rnpc3 is required for the correct expression of tumour suppressor genes, we recently generated conditional and global Rnpc3 knockout mice. Using these mice, we aim to determine whether impaired U12-type splicing contributes to colon tumourigenesis.

This Honours project will entail analysis of Rnpc3 expression and function in our new mouse models. The specific aims are: (i) to describe the spatio-temporal patterns of Rnpc3 expression during normal mouse embryonic development and in adulthood and (ii) to determine whether impaired Rnpc3 activity increases cancer susceptibility in tumour-prone mutant mice.

Skills Acquisition: The project will provide opportunities to become skilled in a variety of molecular and cell biology techniques, including in-situ hybridisation, immunohistochemistry, real-time PCR and histology.

This research topic could potentially fulfil 2 Honours students.

PROTEOMICS & CALCIUM BIOLOGY LABORATORY

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The following project is offered as BSc Honours only

PHARMACOPROTEOMIC INTERROGATION OF DEVELOPMENTAL DENTAL DEFECTS

This project will be part of an ongoing programme that employs proteomic analysis of mouse models to better understand (1) the molecular pathogenesis of developmental dental disorders; and (2) the safety of paediatric drugs in a dental context.

Proteomics provides a suite of useful discovery tools – allowing the expression profile of hundreds of proteins to be screened in parallel, and enabling altered expression profiles in disease states to be characterised comprehensively. The use of proteomic technologies in pharmacology (pharmacoproteomics) enables the pharmacologist to identify the key proteins involved during disease progression (through changes in their expression, localisation, post-translation modification state, etc.), thus identifying novel targets. Such analyses have proven valuable in a variety of disease contexts, and have led to development of novel diagnostic and therapeutic approaches that improve health outcomes.

Dental health is an important sector that is yet to benefit fully from pharmacoproteomics. A key problem that dentistry faces is developmental dental defects (DDD) – which affect about half the population to some degree and inflict substantial burdens upon sufferers and the society that foots the treatment-bill (e.g. in Australia more money is spent on treating DDD than major cancers such as breast and prostate). Most DDD seem to be caused by environmental factors – however, the identity of these factors and the way in which they act upon dental development are not well understood, which reduces our ability to inform parents about what they can do to ensure their children have healthy teeth. Because adult teeth develop postnatally, the developing tooth is a potential target for drug side effects. Experimentally, the developing tooth in animal models can be used as a toxicological test bed for screening potential developmental toxicants (including paediatric therapeutics).

The present project seeks to use novel mouse models of DDD in a proteomic study that will characterise molecular pathogenesis and dental safety of a common childhood medication. You will learn about clinically relevant mouse models of disease, and how they may be exploited using cutting-edge proteomic methods. We anticipate that the results will contribute to our understanding of what causes DDD, and perhaps shed light on ways to improve dental health through reducing the incidence of DDD.

Further reading:

1. Mangum JE et al. (2010a) Surface integrity governs the proteome of hypomineralized enamel J Dent Res 89:1160-1165
2. Mangum JE et al. (2010b) Proteomic analysis of dental tissue microsamples Methods Mol Biol 666:309-325

The following projects are offered as BSc Honours or MSc(RT)

LOW MOLECULAR WEIGHT MIMETICS OF BRAIN-DERIVED NEUROTROPHIC FACTOR AS PROMOTERS OF CNS AND PNS MYELINATION

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Co-Supervisor: Dr Simon Murray (Anatomy and Cell Biology, and Florey Neuroscience Institutes)

Demyelinating diseases, such as multiple sclerosis, are characterised by the loss of myelin that ensheathes nerves in both the central and peripheral nervous system. Initially nerve conduction is affected, and this can ultimately result in neuronal death. To date, there are no effective therapies that can ameliorate or reverse the loss of myelin that underpins the pathology of these diseases. The precise mechanisms of myelination during development and of remyelination following pathological insult are yet to be fully elucidated. However, we and others have shown that the protein brain-derived neurotrophic factor (BDNF) can regulate myelination during development, and promote remyelination following disease (1). Furthermore, we have previously designed and synthesised small, highly conformationally constrained peptides that act as structural and functional mimetics of BDNF (2,3), and very recently determined that one of these can promote PNS myelination in vitro and in vivo. This novel and exciting finding raises the possibility of using these novel small peptides that target BDNF signalling as agents to delineate demyelination/remyelination mechanisms, as well as being potential lead compounds for the treatment of demyelinating disorders of the PNS and CNS.

In this project, you will learn a variety of modern experimental techniques in pharmacology and neuroscience and use them to examine the effects of these novel peptides in models of PNS and CNS myelination. The project is envisaged to be largely biology-based, although if you were interested, the project could also include some design and chemical synthesis of new analogues of the BDNF mimetic peptides.

References:

1. Xiao, J., et al. (2010) Brain-derived neurotrophic factor promotes central nervous system myelination via a direct effect upon oligodendrocytes. Neurosignals, 18: 186-202.
2. O'Leary, P.D. and R.A. Hughes (2003) Design of potent peptide mimetics of brain-derived neurotrophic factor. J Biol Chem, 278: 25738-44.
3. Fletcher et al. (2008) Design of a conformationally defined and proteolytically stable circular mimetic of brain-derived neurotrophic factor. J Biol Chem, 283: 33375-83.

SIMULATING DRUGS BINDING TO RECEPTORS – WHAT CAN YOU DO WITH MOLECULAR DYNAMICS AND HIGH-END COMPUTING?

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Molecular dynamics (MD) is a computational technique that allows the simulation of the dynamic behaviour or molecules. Although molecular dynamics approaches have been available for over twenty years, recent advances in computing speed have increased the potential utility of the approach. Indeed, it is now possible to run molecular dynamics simulations over a long enough time period to allow drug-receptor interactions to be observed. A challenge in the field now is how to relate the dynamic behaviour of drugs and receptors in these simulations (effectively movies of drugs interacting with receptors) to parameters such as KD i.e. the sorts of measures that pharmacologists like to use to quantify drug-receptor binding. Being able to do this could revolutionise in silico approaches to drug screening, for which predictions of binding are currently of limited quality, and thus be of enormous interest to scientists working in the field of drug discovery.

In this project, you will use high-end supercomputing resources available through VPAC to carry out MD simulations on well-characterised drug-receptor systems. You will then explore novel means of deriving standard pharmacology-type measurements of affinity from the MD movies that you generate. This project sits at the cutting edge of computational biology, and we envisage it to be largely a theoretical study. Although you will get training in the computational techniques that you are going to use, you will need to be comfortably with exploring unknown scientific territory, and not averse to spending considerable time in front of a computer screen!

CANCER THERAPEUTICS

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Location: **Peter MacCallum Cancer Centre**
Pharmacology Supervisor: A/Prof James Ziogas

The following project is offered as BSc Honours only

DEVELOPMENT OF GENETICALLY ENGINEERED MOUSE MODELS OF AML TO STUDY TUMORIGENESIS AND RESPONSE TO NOVEL THERAPEUTICS.

The genetic heterogeneity of cancer influences the trajectory of tumor progression and may underlie clinical variation in therapy response. To model such heterogeneity, we aim to produce genetically and pathologically accurate mouse models of common forms of human acute myeloid leukemia (AML) expressing oncogenic fusion proteins involving the mixed lineage leukemia gene (MLL). MLL is fused with one of over 60 distinct partner genes through chromosomal translocations in various human acute leukemias, resulting in the formation of multiple MLL fusion proteins (MLL-FPs). MLL-FPs are capable of leukemic transformation and dysregulation of multiple genes, often through the aberrant recruitment of epigenetic modifying enzymes such as histone deacetylases and methyltransferases. Using retoviral gene transduction of hemopoietic stem cells to express diverse MLL-FPs we will produce mice that develop AML driven by different ongogenic fusion proteins. These mice will be utilized to study disease onset and progression and determine the oncogenic potential of different MLL-FPs. Moreover, these mouse models will be used to test the efficacy of epigenetic modifying agents such as histone dacetylase inhibitors and histone methyltransferase inhibitors as well as conventional chemotherapeutic drugs. These studies will assess the importance of genetic information in guiding the treatment of human AML and determine if genetically engineered mouse models of human cancer can accurately predict therapy response in patients.

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The following projects are offered as BSc Honours only

EXOSOMES: NOVEL REGULATORS OF AIRWAY FUNCTION IN ASTHMA

Co-Supervisors: **Prof Alastair Stewart,**
Prof Mike Hubbard,
Mr John Mangum
Dr Mark Hulett (La Trobe University)

Recent studies have identified that small cellular vesicles termed exosomes can be released from many cell types. Exosomes contain a number of membrane proteins, as well as mRNA and micro RNAs, and have been shown to regulate the function of other cells. There is evidence to suggest that the protein and RNA profiles of exosomes varies between different cell types and in disease states. This highlights exosomes as serving specific cell/cell communication roles and perhaps also contributing to disease pathology.

In this project, you will prepare exosomes from activated and resting human mast cells, airway epithelial cells and from non-asthmatic and asthmatic airway smooth muscle cells and characterise their unique protein expression profiles using flow cytometry and cutting-edge proteomics techniques. Exosome RNA content will also be profiled. You will also examine the ability of exosome preparations from different airway cell types to regulate the actions of other airway cells. This project will provide new knowledge about the composition and regulatory roles of exosomes and, more specifically, how these novel vectors control airway cell function.

The project will utilise a variety of methodologies including cell culture, flow cytometry, 1D and 2D gel electrophoresis, mass spectrometry, immunoassays and quantitative PCR.

References:

1. Simpson RJ, Lim JW, Moritz RL, Mathivanan S. Exosomes: proteomic insights and diagnostic potential. Expert Rev Proteomics. 2009;6:267-83.
2. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9:654-9.

GPR35: IS IT THE FUNCTIONALLY IMPORTANT RECEPTOR FOR THE ANTI-ALLERGIC DRUG CROMOGLYCATE?

Co-Supervisor: Prof Alastair Stewart

The anti-allergic drug cromoglycate was at one stage commonly used to treat asthma. However, the superior efficacy of the glucocorticoids has meant that cromoglycate is now little used. The demise of cromoglycate as a clinically used drug was aided by a lack of understanding of its mechanism of action and thus an inability to generate perhaps more potent versions that might share cromoglycate's excellent safety profile. In the past year new information about how cromoglycate might be producing its anti-allergic effects has emerged. An 'orphan' GPCR called GPR35 has been shown, in transfected cell systems, to be activated by cromoglycate. However, it is unclear if this receptor does in fact mediate the anti-allergic actions of cromoglycate.

We have identified expression of GPR35 on mast cell and macrophage cell lines. In this study, you will use a number of known GPR35 agonists and antagonists to examine the signalling mechanisms and function of GPR35 in these cells. Results will determine if GPR35 offers an exciting new target for allergic diseases such as asthma.

Techniques to be used in the study will include cell culture, flow cytometry, immunohistochemistry, Western blotting, quantitative immunoassays for cytokines and second messengers and quantitative PCR.

References:

1. Yang Y, Lu JY, Wu X, Summer S, Whoriskey J, Saris C, Reagan JD. G-protein-coupled receptor 35 is a target of the asthma drugs cromolyn disodium and nedocromil sodium. Pharmacology. 2010;86:1-5.
2. Milligan G. Orthologue selectivity and ligand bias: translating the pharmacology of GPR35. Trends Pharmacol Sci. 2011;32:317-25.

STRESS NEUROBIOLOGY

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The following projects are offered as BSc Honours or MSc(RT)

ROLE OF BRAIN NADPH OXIDASE IN MODULATING ANXIETY AND CARDIOVASCULAR REACTIVITY

Neuropharmacological evidence suggests that a free radical, superoxide, may modulate the cardiovascular response to emotional stress. One of the major sources of superoxide production is NADPH oxidase. The current project aims to determine whether genetic inactivation of NADPH oxidase in specific forebrain regions alters anxiety-related behaviour and cardiovascular reactivity in mice. Several subunits of the NADPH oxidase complex will be targeted, including gp91phox, p67phox and Rac1. As a first step, mice will be microinjected with lentiviral vectors to overexpress a dominant negative form of Rac1 (Rac1N17) in selected brain regions. Mice will then be implanted with telemetry devices and a state-of-the-art blood pressure telemetry and video recording system will be used to remotely monitor behavioural and cardiovascular responses to anxiogenic stimuli (elevated plus-maze test, contextual fear conditioning, and social interaction with a new partner). In addition, the effects of NADPH oxidase inactivation on anxiety-like behaviour will be validated pharmacologically using anxiolytic and anxiogenic compounds.

Techniques: blood pressure telemetry recording and analysis, behavioural testing and analysis using a video tracking software, immunohistochemistry, brain stereotaxic (microinjection) and telemetry implantation surgeries in mice.

REDOX MODULATION OF THE BRAIN NORADRENERGIC SYSTEM: ROLE IN ANXIETY AND PAROXYSMAL HYPERTENSION

Altered activity of the brain noradrenergic system is an important element of many aspects of pathological anxiety, including panic disorder, and may also play a role in the development of paroxysmal hypertension (episodic high blood pressure). The current project examines the effect of inhibiting free radical production in brainstem noradrenergic neurons on cardiovascular and behavioural responses to emotional stressors in laboratory rats. The cell-type specific viral gene transfer will be used to overexpress a key scavenger of superoxide anion, superoxide dismutase (SOD), in noradrenergic A1 and A6 cell groups. The project will involve behavioural and cardiovascular experiments in conscious rats chronically implanted with blood pressure telemetry devices. Behavioural and cardiovascular reactivity to anxiogenic stimuli (contextual fear conditioning, elevated T-maze and social interaction tests) will be examined before and after microinjection of lentiviral vectors into selected noradrenergic nuclei. The influence of SOD overexpression on anxiety-like behaviour will also be validated pharmacologically using anxiolytic and anxiogenic drugs.

Techniques: blood pressure telemetry recording and analysis, behavioural testing and analysis, immunohistochemistry, brain stereotaxic (microinjection) and telemetry implantation surgeries in rats.

MOLECULAR PHARMACOLOGY LABORATORY

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The following projects are offered as BSc Honours or MSc(RT)

Our laboratory is currently focussing on the modulation of TRP ion channels by post-translational modification. We are particularly interested in how intracellular signalling events such as those resulting from GPCR stimulation, leads to protein phosphorylation which can act to modulate (or even activate) some of these channels. We think that these modifications alter the ion channel conformation and affect protein-protein associations to change channel function. These projects are also available as the research component of a MSc. (RT) Degree.

We are offering four projects next year on the thermally responsive TRP channels TRPA1, TRPV3 and TRPV4.

TRPV3

TRPV3 was first cloned by a team including Professor McIntyre in 2003 [1] and is found in keratinocytes and sensory neurones, but its function is not yet well understood. In collaboration with Prof Bruce Kemp (St Vincent's Institute of Medical Research), we have used mass spectrometry to identify several novel sites within TRPV3 that are phosphorylated in response to agonist activation. This project will investigate the effects of mutation of these sites on the function of the ion channel. We will express the normal and mutated versions of TRPV3 in HEK293 cells and assay their responses to activating stimuli using a FlexStation calcium fluorescence assays and by western blotting

TRPV4 PROJECT 1

Project 1: TRPV4 is activated by warm temperatures, hypo-osmolarity and shear stress and may be a mechanoreceptor. It has recently been shown by our group to be activated by the inflammation-sensing GPCR, Protease Activated Receptor 2 (PAR2). It was initially thought that this channel might be directly gated by mechanical stretch but it is now becoming clear that it is actually activated by intracellular signalling. We are using site-directed mutagenesis and mammalian cell expression studies to understand the signalling pathways that result in activation by a number of stimuli. This project will involve using normal and mutated versions of TRPV4 and src-family kinases expressed in HEK293 cells and comparing responses to hypo-osmolarity and PAR2 activation using FlexStation calcium fluorescence assays.

TRPV4 PROJECT 2

TRPV4 has been associated with a number of inherited human diseases of the skeleton and nervous system. Working with the group of Dr Shireen Lamande at the Murdoch Children's Research Institute, we have recently identified mutations in the N-terminus of the human TRPV4 protein as the cause of an inherited recessive form of arthropathy termed Familial Digital Arthropathy Brachydactyly (FDAB). This condition causes arthritis in later life in the joints of the hands and feet of affected individuals. We have shown the mutated form of TRPV4 does not respond normally to hypo-osmotic stimulus whereas it appears nearly normal in other respects. This work has very recently been accepted for publication in Nature Genetics. The mutation occurs in a region of the N-terminus that is known to be involved in protein-protein interaction. We have identified interesting interacting partners that we wish to characterise further and we are currently investigating what proteins other interact with TRPV4 and how the mutations affect the interactions.

TRPA1

Several TRP ion channels are able to be activated by receptor-activated signalling pathways. We believe that many TRP ion channels are normally coupled to GPCR activation, for example in sensing pain or itch. TRPA1 was first cloned by a team including Professor McIntyre in 2002 [2] and is expressed in pain and itch-sensing sensory nerves. It is likely to be an important transducer of pain signals. We are investigating if it is able to couple to GPCRs that sense substances that evoke itch or pain. ATP is one such pain-evoking substance and we have new evidence that TRPA1 is activated as a result of P2Y purinoreceptor activation in HEK293 cells. We will use site-directed mutagenesis and mammalian cell expression studies to understand the signalling pathways that result in activation ATP and other stimuli. This project will involve using normal and mutated versions of TRPA1 expressed in HEK293 cells and comparing their responses to activating stimuli using FlexStation calcium fluorescence assays.

References:

1. Peier AM, Reeve AJ, Andersson DA, Moqrich A, Earley TJ, et al. (2002) A heat-sensitive TRP channel expressed in keratinocytes. Science 296: 2046-2049.
2. Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, et al. (2003) ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 112: 819-829.

HEART FAILURE PHARMACOLOGY

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Location: **Baker IDI & Diabetes Institute**
Pharmacology Supervisor: Dr Jane Bourke

The following projects are offered as BSc Honours or MSc(RT)

NEW STRATEGIES TO RESCUE DIABETES-INDUCED CARDIAC DYSFUNCTION

Diabetes is Australia's fastest growing chronic disease; one million Australians have been diagnosed, with close to one million more yet to be identified. Most of these patients will eventually die from cardiovascular causes. As diabetes induces left ventricular (LV) dysfunction, this increases the risk of death from heart failure in affected patients. Patients with diabetes are 2.4-fold more likely to develop heart failure, even when adjusted for age and coronary artery disease. Onset of heart failure occurs at a younger age in diabetic patients, with heart failure prevalence increased five- to eight-fold in middle-aged patients. New therapies for restoring cardiac function in the diabetic heart are thus highly desirable. In most forms of non-diabetic heart failure, systolic (contractile) dysfunction is the first and predominant functional abnormality. The aetiology of diabetic heart disease is distinct from other causes of LV dysfunction, as it is characterised initially by diastolic dysfunction, where relaxation of the cardiac muscle following contraction is prolonged. Diabetes-induced cardiac dysfunction is often exacerbated by underlying LV fibrosis (increased extracellular matrix deposition), hypertrophy (abnormal pathological growth) of cardiac myocytes, and excess generation of reactive oxygen species (ROS) such superoxide.

Our laboratory has demonstrated that antioxidant and/or ROS-suppressing approaches, as well as activation of cardioprotective signalling and negative regulators of LV hypertrophy, are beneficial for treating the cardiac complications of type 1 and type 2 diabetes in the intact heart. We are now offering an exciting student research project in 2012, exploring a novel potential therapeutic strategy for rescuing cardiac function and structure in the diabetic heart. This project will determine whether post-translational protein modifications induced by high glucose and implicated in insulin resistance play a causal role in the development of diabetic cardiomyopathy, and investigate whether pharmacological and/or gene-based strategies targeted at limiting these modifications can prevent diabetes-induced LV dysfunction and remodelling. The scope of this project will be tailored depending on the student's abilities and interests, and will provide the opportunity for learning a range of techniques, including physiological (e.g. isolated rodent hearts ex vivo or in vivo models of diabetic cardiac disease, for assessing cardiac function and blood pressure) biochemical (Westerns, ROS detection, ELISA), molecular (real-time PCR, Northerns) and/or histological techniques. This project will be performed in A/Prof Ritchie's laboratory at the Baker IDI Heart and Diabetes Institute in Prahran. Ultimately, treatment strategies that may emerge from these studies may provide significant benefits alone or in combination with current standard care, to ultimately reduce progression to heart failure and death in diabetic patients.

NITROXYL, A RELATIVE OF NO, IS A NATURALLY-OCCURRING CARDIOPROTECTIVE MOLECULE

The nitric oxide (NO•)/cGMP signalling system is as a powerful cardiac antihypertrophic mechanism. Nitroxyl (HNO), a novel redox sibling of NO•, has several therapeutic advantages for the treatment of cardiovascular diseases. We have shown that HNO prevents hypertrophy (abnormal pathological growth) and generation of superoxide in isolated cardiomyocytes. Excitingly, HNO also potentiates cardiac function, in contrast to NO•, via the cardiac calcium handling proteins, SERCA2a (sarcoplasmic reticulum Ca^{2+} ATPase) and the ryanodine receptor RyR2. The activity and expression of these enzymes is abnormally affected in cardiac pathologies (LV hypertrophy, heart failure, diabetes), and together with the upregulation of ROS is recognised for as playing a causal role in the development of LV dysfunction. HNO thus is likely to be favourable for treating these cardiac pathologies.

We are now offering an exciting student research project in 2012, exploring whether HNO or related strategies represent novel pharmacotherapy for the prevention and treatment of myocardial dysfunction, induced by chronic LV hypertrophy, heart failure or diabetes. The project will examine whether the mechanisms by which HNO acutely enhances cardiac function in intact heart are different to those that prevent hypertrophy and elicit ROS suppression, and determine if acute or chronic HNO treatment is cardioprotective in isolated cardiomyocytes and the intact myocardium in vivo in settings of chronic cardiac impairment.

The scope of this project will be tailored depending on the student's abilities and interests. It will provide the opportunity for learning a range of techniques, including cell culture (cardiomyocytes and/or cardiac fibroblasts), physiological/pharmacological (e.g. isolated rodent hearts ex vivo or in vivo models of cardiac disease, for assessing cardiac function and blood pressure) biochemical (Westerns, ROS detection, ELISA, real-time PCR) and/or histological techniques. The outcome of this project will be

definitive information regarding the mechanism(s) and effectiveness of HNO-mediated rescue of myocardial dysfunction. This project will be performed in A/Prof Ritchie's laboratory at the Baker IDI Heart and Diabetes Institute in Prahran. Ultimately, HNO-based strategies may offer new treatment options for cardiac disease, either alone or on top of standard care.

Targeting the anti-inflammatory protein Annexin-A1 for protection from myocardial infarction (heart attack)

Myocardial ischaemia, in which coronary blood flow is reduced, causes anginal chest pain, myocardial infarction (MI, also known as heart attack), and death. Myocardial infarction represents the major cause of death in Western societies, and in the next decade, this will expand to all corners of the world. The primary determinant of outcome from MI is the extent of cell death during and after ischaemia, from necrosis, apoptosis and/or autophagy. Restoration of blood flow (reperfusion) however is associated with the development of further cell death and impaired recovery of cardiac function, referred to as "reperfusion injury". Myocardial ischaemia-reperfusion induces an inflammatory response, with damage resulting from both infiltration of circulating inflammatory cells, as well as neutrophil-independent direct actions on myocardium and endothelium (including Ca^{2+} overload, ROS generation and impaired mitochondrial regulation all contributing mechanisms to cell death. In addition, there is incomplete recovery of LV function. Together these phenomena contribute to increased risk of ischaemic cardiomyopathy, heart failure and death. Novel treatment strategies that protect against multiple mechanisms of MI injury will have major clinical impact.

The therapeutic potential of the anti-inflammatory mediator annexin-A1 (ANX-A1) has been recognized in a range of systemic inflammatory disorders. Importantly, we have shown that ANX-A1 has powerful protective actions against cardiac injury and loss of LV contractile function. We are now offering an exciting student research project in 2012, exploring the potential for mimetics of ANX-A1 to represent potential new pharmacotherapy for treating cardiac inflammatory disorders such as ischaemia-reperfusion (I-R) injury. The project will test the hypothesis that ANX-A1 represents a novel modulator of myocardial viability and LV contractile function following ischaemia-reperfusion, and will seek to investigate the cardioprotective function of endogenous ANX-A1 in I-R injury, the receptors responsible for cardioprotection elicited by ANX-A1 and its mimetics, and examine the potential therapeutic opportunities offered by exogenous ANX-A1 mimetics after I-R injury in the intact heart.

The scope of this project will be tailored depending on the student's abilities and interests. It will provide the opportunity for learning a range of techniques, including cell culture (cardiomyocytes), physiological/pharmacological (e.g. isolated rodent hearts ex vivo or in vivo models of ischaemic cardiac disease, for studying impact on cardiac function and structure) biochemical (Westerns, ROS detection, ELISA, real-time PCR) and/or histological techniques. These studies will provide insight into ANX-A1-mediated rescue of myocardial viability and function after I-R injury in the intact heart, and the mechanisms involved. This project will be performed in A/Prof Ritchie's laboratory at the Baker IDI Heart and Diabetes Institute in Prahran. Development of therapeutic strategies for treating myocardial infarction after an unplanned ischaemic event (while reperfusion injury is still evolving), alone or concurrent with standard care, will ultimately reduce progression to heart failure and death in affected patients.

BARRIERS IN THE DEVELOPING BRAIN

Supervisor: **Prof NR Saunders**
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Facsimile: **8344 0241**
Location: **Department of Pharmacology**
Level 9 Medical Building
Co-Supervisors: **A/Prof KM Dziegielewska;**
Dr MD Habgood

The following project is offered as BSc Honours or MSc(RT)

MECHANISM OF MACROMOLECULAR TRANSFER ACROSS CEREBROSPINAL FLUID/ BRAIN BARRIER DURING DEVELOPMENT

Studies of transfer mechanisms into developing brain across protective barriers (the blood brain and blood-cerebrospinal fluid,CSF barriers) demonstrated that tight junctions at these interfaces close off the intercellular transfer even at the earliest stages of brain development. Instead, the transfer appears to be across a small proportion of choroid plexus epithelial cells with uptake into the brain from the CSF, rather than across cerebral blood vessels. Recent studies showed that protein is transferred by a different mechanism but also across a small proportion of choroid plexus cells. The next stage of the work involves molecular characterisation of these transfer mechanisms during different stages of brain development.

In this project you will examine expression of several protein transporters in the CSF/brain barrier in normal and pathological conditions. The techniques involved range from whole animal physiology and dissection to molecular biology and detailed morphology.

Reference:

1. Liddelow et al, 2011. Eur J Neurosci. 33(3):391-400

ROLES OF EXOSOMES IN AIRWAY BIOLOGY & DISEASE

Supervisor: **Dr Michael Schuliga**
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Telephone: **8344 8508**
Facsimile: **8344 0241**
Location: **Department of Pharmacology**
Level 8 Medical Building
Co-Supervisor: **Prof Alastair Stewart**

The following project is offered as BSc Honours only

There is a growing interest in the cell-cell communication roles mediated by secreted vesicles termed exosomes. The dual capability of exosomes to promote immunity or to induce tolerance has prompted their clinical use as vehicles for vaccination against different human diseases. In mouse models of asthma, exosomes attenuate allergen-induced inflammation[1]. It is believed exosome-based vaccines could represent an alternative to conventional therapy for allergic airway diseases such as asthma.

What are exosomes

Exosomes are nanovesicles originating from multivesicular bodies that are secreted by a variety of cell types. Exosomes are also found in vivo in body fluids such as blood, urine, amniotic fluid, malignant ascites, bronchoalveolar lavage fluid, synovial fluid, and breast milk. While the biological function of exosomes is still unclear, they can mediate communication between cells, facilitating processes such as antigen presentation and in trans signalling to neighbouring cells. In addition to a common set of membrane and cytosolic proteins, it is becoming increasingly apparent that exosomes harbour distinct subsets of proteins that may be linked to cell-type associated functions. Interestingly, the recent observation that exosomes contain both mRNA and microRNA, which can be transferred to another cell, and be functional in that new environment, is an exciting new development in the unraveling exosome saga.

Objectives

In this study we will examine exosomal release by airway epithelial[2] and smooth muscle cells[3], and their potential roles in asthma biology. Not only will we characterise their formation, but the inflammatory mediators that stimulate their release, and their roles in processes such as plasmin formation and matrix metalloprotease (MMP) activation. Furthermore, the effect of glucocorticoids, the mainstay treatment for chronic asthma, on exosomal release and function will also be examined.

Methods

Exosomal vesicles released by airway cells maintained in culture will be isolated by differential centrifugation and characterised further by mass spectrometry, flow cytometry, immunoblotting, electron microscopy, density-gradient centrifugation and light-scattering methods. Biochemical characterisation will be used to examine typical surface, cytoskeletal, and cytoplasmic proteins characteristic of exosomes, including the multivesicular and late endosomal membrane markers Tsg101 and CD63. Levels of RNA in the exosomes will also be evaluated. Exosomal functions to be examined will include their capacity to activate plasminogen and MMP-2.

References:

1. Imqvist N et al (2008) Serum-derived exosomes from antigen-fed mice prevent allergic sensitization in a model of allergic asthma. Immunology 125:21.
2. Kesimer M et al (2009) Characterization of exosome-like vesicles released from human tracheobronchial ciliated epithelium: a possible role in innate defense. FASEB J 23:1858.
3. Schuliga M, Harris T, Stewart AG (2011) Plasminogen activation by airway smooth muscle is regulated by type I collagen. Am J Respir Cell Mol Biol 44:831.
4. McCready J et al (2010) Secretion of extracellular hsp90alpha via exosomes increases cancer cell motility: a role for plasminogen activation. BMC Cancer 10:294.

AIRWAY REMODELLING IN ASTHMA

Supervisor: **Prof Alastair Stewart**
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Facsimile: **8344 0241**
Location: **Department of Pharmacology**
Level 8 North Room N802
Medical Building

The following projects are offered as BSc Honours only

MECHANISMS THAT SOFTEN AIRWAY SMOOTH MUSCLE DURING REMODELLING

Location: **Departments of Pharmacology & Chemical Engineering**
Co-Supervisors: **Dr Xuehua Zhang**
Department of Chemical Engineering

In asthmatics the muscle cells shorten too quickly and too much in response to chemicals that have little effect on muscle from non-asthmatic patients. This muscle shortening can be reduced by bronchodilator drugs. However, over-use of the bronchodilator drugs can make them ineffective and in patients with severe asthma,the bronchodilators may not work well enough to provide adequate airflows.

Our current work is probing airway smooth muscle cells from humans using a technique called atomic force microscopy. This technique has the unique capacity to probe structure and function of living cells on a nanometre scale.

We have discovered that a growth factor protein called bFGF that is able to soften the muscle cells, preventing them from becoming rigid and from shortening. We are now investigating how this happens, because amongst all the signals that enable this response, we believe there will be some excellent drug targets with which to develop completely new and safer bronchodilator drugs.

In this project you will learn culture of ASM, quantitative RT-PCR and Western blotting; use of live cell imaging and interventions using transient cell transfection and silencing RNA. You will apply atomic force microscopy to analyse cell hardness.

[this one of several Honours projects offered by the Immunopharmacology laboratory in 2012; depending on funding/supervision commitments up to 3 projects will be available]

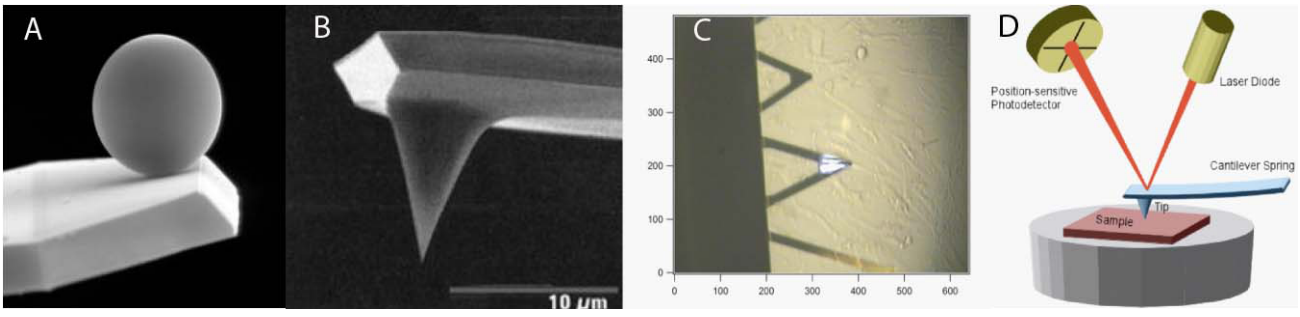
ANNEXIN-1, EXOSOMES, LIPOXINS AND BREAST TUMOUR EPITHELIAL CELL FUNCTION

The annexins are a large family of Calcium and phospholipid binding proteins with diverse biological activities (D'Acquisito et al., 2008). Annexin-1 has anti-inflammatory and inflammation-resolving activities, the latter being mediated through a G-protein coupled receptor, formyl peptide receptor 2 (FPR2) that is also activated by the lipid product, Lipoxin A.

Inflammation in tumours is regarded as a negative prognostic factor in many instances. On the other hand, activation of inflammation-resolving mechanisms in tumour environment may be expected to limit tumour growth and spread. Our initial studies have identified a role for endogenous annexin-1 in modulating the proliferative activity of both the estrogen receptor (ER) positive and ER negative cell lines: MCF7 and MDA MB-231, respectively (Khau et al., 2011). We now wish to ascertain the role of the FPR2 receptor in these actions of annexin-1, establish whether these activities are shared by the N-terminal annexin-1 peptide sequence (cleaved from annexin-1 in inflammation) and the lipid-derived, inflammation-resolving FPRL1 ligand, lipoxin A. We also wish to ascertain whether annexin-1 acts in an autocrine manner by release in exosomal particles. This project may extend to examination of the annexin-1 impact on tumour growth and metastasis in a nude mouse model using xenografts of MDA-MB-231 cells (Sutherland et al., 2005) or mouse mammary tumours (Konpoka et al., 2001) in transgenic animals.

The project will involve: culture of the MCF7 and MDA-MB-231 cell lines, quantitative RT-PCR and Western blotting; use of live cell imaging to track the translocation of GFP-tagged annexin-1; use of reporter constructs for activation of the estrogen response element (ERE) and interventions using transient cell transfection and silencing RNA.

Figure below: The AFM probes (A 5μm; B 20 nm) that will be used to map the tension in airway smooth muscle (C, ASM in culture with 20 nm probe [tip illuminated with LASER] engaged at ASM surface) and of the laser processes for tracking tip movement as the tip is advanced to probe ASM surface. AFM can provide high resolution, high accuracy measurements of sample stiffness in a physiological environment.



References

1. KHAU T, LANGENBACH SY, SCHULIGA M, HARRIS T, JOHNSTONE CN, ANDERSON RL, STEWART AG. (2011). Annexin-1 signals mitogen-stimulated breast tumor cell proliferation by activation of the formyl peptide receptors (FPRs) 1 and 2. FASEB J. 25:483-496.
2. KONOPKA TE, BARKER JE, BAMFORD TL, GUIDA E, ANDERSON RL & STEWART AG. (2001). Nitric oxide synthase II gene disruption: implications for tumor growth and vascular endothelial growth factor production. Cancer Res, 61, 3182-7.
3. SUTHERLAND TE, SCHULIGA M, HARRIS T, ECKHARDT BL, ANDERSON RL, QUAN L & STEWART AG. (2005). 2-methoxyestradiol is an estrogen receptor agonist that supports tumor growth in murine xenograft models of breast cancer. Clin Cancer Res, 11, 1722-32.
4. D'ACQUISTO F, PERRETTI M & FLOWER RJ. (2008). Annexin-A1: a pivotal regulator of the innate and adaptive immune systems. Br J Pharmacol, 155, 152-69.

[this one of several Honours projects offered by the Immunopharmacology laboratory in 2012; depending on funding/supervision commitments up to 3 projects will be available]

GLUCOCORTICOID RESPONSIVENESS IN THE LUNG: IMPACT OF INFLAMMATION AND INFECTION

Co-Supervisor: Dr Rosa Gualano

The high level of sensitivity of allergic inflammation to regulation by glucocorticoids (GCS) underlies the therapeutic success of this class of drugs in most cases of asthma, hayfever and urticaria. However, there is a partial resistance to control severe asthma by GCS and a more profound GCS resistance in COPD. A number of mechanisms for GCS-resistance have been advanced; including an up regulation of glucocorticoid receptor (GR) β, oxidative inactivation of histone deacetylase 2 (HDAC2) and our studies have suggested acquired resistance from extracellular matrix changes (collagen breakdown) signalling through integrins (Bonacci et al., 2006).

A In this project you will investigate the possible mechanisms of GCS-resistance that develop through chronic exposure to glucocorticoids. The project will involve: culture of the A549 and BEAS-2B cell lines and of normal and asthmatic-derived airway fibroblasts; measurement of gene expression changes by quantitative RT-PCR and Western blotting; use of live cell imaging to track the translocation of YFP-tagged wild-type and mutated GRs; use of reporter constructs and interventions using transient cell transfection and silencing RNA. Well-established pharmacological inhibitors will also be used in experiments designed to provide new insights into this important limitation on the effectiveness of GCS in chronic inflammatory diseases.

B A separate, but related project will examine the mechanism of glucocorticoid resistance induced by viral infection (respiratory syncytial virus, better known as RSV or Flu) using both human cell culture and mouse models. In this project you will learn to propagate viruses, infect airway epithelial cells and use techniques such as ELISA, western blotting and RT-qPCR to analyse glucocorticoid responses. Mice will be subjected to viral infection to examine the impact on viral load and gene expression responses to glucocorticoid treatment. The viruses are safe to handle and well-characterised (Wong et al., 2011).

The results you obtain will guide new approaches to reversing steroid resistance in chronic inflammatory diseases.

References

1. Fernandes DJ, Bonacci JB & Stewart AG. (2006). Extracellular matrix, integrins, and mesenchymal cell function in the airways. Current Drug Targets. 7:567-577.
2. Bonacci JV, Schuliga M, Harris T, Stewart AG. (2006). Collagen impairs glucocorticoid actions in airway smooth muscle through integrin signalling. Br J Pharmacol;149(4):365-73.
3. Wong ZX, Jones JE, Anderson GP, Gualano RC. (2011). Oseltamivir treatment of mice before or after mild influenza infection reduced cellular and cytokine inflammation in the lung. Influenza Other Respi Viruses: 5(5): 343-50.

[these two are some of several Honours projects offered by the Immunopharmacology laboratory in 2012; depending on funding/supervision commitments up to 3 projects will be available]

BRAIN NEUROTRANSMITTERS IN SCHIZOPHRENIA & DEPRESSION

Supervisor: **A/Prof Maarten van den Buuse**
Email: **m.vandenbuuse@mhri.edu.au**
Location: **Melbourne Brain Centre**
University of Melbourne
Pharmacology Supervisor: A/Prof Tony Hughes

The following projects are offered as BSc Honours or MSc(RT)

NEURODEVELOPMENTAL AND NEUROPHARMACOLOGICAL MECHANISMS IN MOUSE MODELS OF SCHIZOPHRENIA

Schizophrenia and other mental illnesses are likely to be caused by an interaction of genetic and early neurodevelopmental factors, leading to altered expression of trophic factors in the brain and changes in synaptic density and neuronal activity. We use mice with reduced brain expression of trophic factors such as BDNF (brain-derived neurotrophic factors) and Reelin. These mice are tested in behavioural animal models for aspects of schizophrenia, including locomotor hyperactivity, prepulse inhibition, social behaviour and cognitive deficits (short-term and long-term memory, avoidance memory). The focus is on the neuropharmacological regulation of behaviour, including the action of antipsychotic drugs. These studies may be complemented with receptor binding autoradiography, or with assessment of protein levels in the brain by Western Blot. This project is aimed at students who are interested in schizophrenia, bipolar disorder and depression, genetically-modified mice, pre-clinical psychopharmacology and behavioural neuroscience. The project will be done at the new Melbourne Brain Centre, University of Melbourne.

Selected references illustrating some of our work:

1. Van den Buuse M, Wischhof L, Lee RX, Martin S, Karl T (2009). Neuregulin 1 hypomorphic mutant mice: enhanced baseline locomotor activity but normal psychotropic drug-induced hyperlocomotion, prepulse inhibition regulation. International Journal of Neuropsychopharmacology 12(10), 1383.
2. Choy KH, de Visser Y, Nichols NR, van den Buuse M (2008). Combined neonatal stress and young-adult glucocorticoid stimulation in rats reduce BDNF expression in hippocampus: effects on learning and memory. Hippocampus 18(7), 655.
3. Gogos A, Bogeski M, van den Buuse M (2008). Role of serotonin-1A receptors in the action of antipsychotic drugs: comparison of prepulse inhibition studies in mice and rats and relevance for human pharmacology. Behavioral Pharmacology 19, 548.

ROLE OF OESTROGEN IN SCHIZOPHRENIA

Co-Supervisors: Dr Andrea Gogos & Dr Rachel Hill

There are gender differences in schizophrenia with respect to the age of first onset, symptom severity and treatment response. The reason for this difference is unclear but is often related to a 'protective' action of oestrogen and an opposite effect of high levels of testosterone. These effects may be mediated during puberty, when the brain undergoes extensive plastic changes and remodelling. This project will assess the modulatory effect of oestrogen and testosterone during puberty on behaviour in adulthood. The work will include the behavioural and molecular consequences of chronic administration of different clinically-used antipsychotic drugs in male and female rats. Behavioural tests include locomotor hyperactivity, prepulse inhibition, social behaviour and cognition. The studies will focus on indices of the activity of brain dopamine and serotonin, including receptor binding levels, receptor signalling activity, and neuroplasticity factors in relevant brain areas. The project will be done at the new Melbourne Brain Centre, University of Melbourne.

CANNABIS AND METHAMPHETAMINE AS RISK FACTORS IN SCHIZOPHRENIA

Cannabis and methamphetamine abuse are well-know risk factors in the development of psychosis. However, it is unclear exactly how these drugs cause such effects. The work in this project will focus on the effects of chronic treatment of adolescent or young-adult mice with cannabinoid receptor agonists, or methamphetamine. This could include mice with mutations in relevant brain signalling pathways, such as BDNF. Effects of the treatments will be analyzed at the level of locomotor hyperactivity, prepulse inhibition, social behaviour and cognition. The studies will also focus on indices of the activity of brain dopamine and serotonin, including receptor binding levels and neuroplasticity factors in relevant brain areas. The project will be done at the new Melbourne Brain Centre, University of Melbourne.

OXIDATIVE STRESS & LUNG DISEASE

Supervisor: **Dr Ross Vlahos**
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Facsimile: **8344 0241**
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Level 8 Room N814 Medical Building
Co-Supervisor: **Prof Gary Anderson**

The following projects are offered as BSc Honours only

ROLE OF ANTI-OXIDANTS IN ACUTE EXACERBATIONS OF COPD

Chronic Obstructive Pulmonary Disease (COPD) is a major incurable global health burden and will become the third largest cause of death in the world by 2020. It is currently believed that an exaggerated inflammatory response to inhaled irritants, in particular cigarette smoke, causes progressive airflow limitation. This inflammation involves the production of various cytokines and chemokines, induction of various proteases, oxidative stress, small airway fibrosis, mucus hypersecretion and emphysema. Patients with COPD are also prone to respiratory infections (commonly called acute exacerbations of COPD - AECOPD) that cause an accelerated decline in lung function, hospitalisation and even death. These respiratory infections consist of bacteria and viruses that get into the lungs of people with COPD. People with COPD find it extremely difficult to fight off these respiratory infections. We have developed mouse models of AECOPD that replicate the features of human disease. Oxidative stress plays a major role in COPD and AECOPD because cigarette smoke contains more than 1014 oxidants per puff, many of which are relatively long-lived. These oxidants give rise to Reactive Oxygen Species (ROS), which are a family of highly reactive molecules that are generated enzymatically by various cells in the lung in response to a variety of chemical and physical agents. However, the normal lung has developed defences to ROS-mediated damage, which include the anti-oxidant enzymes NADPH oxidase-2 (Nox-2) and glutathione peroxidise-1 (gpx-1). In this project you will investigate whether Nox-2 and gpx-1 ameliorates experimental AECOPD in a murine model of the disease. This will be achieved by using mice deficient in these anti-oxidant enzymes and pharmacological interventions. The significance of this will be that Nox-2 and gpx-1 may be novel targets that can be exploited therapeutically to treat exacerbations of COPD.

Skill acquisition: In vivo disease models, FACS analysis of cell populations, quantitative PCR, histology, virus and cell culture, ELISA, zymography and Western blotting.

ROLE OF INTERLEUKIN-17 IN INFLUENZA VIRUS-INDUCED LUNG DISEASE

Influenza A virus infection has claimed millions of lives worldwide and continues to impose a major economic burden on health care systems. Co-ordinated efforts to control this infection are problematic due to (i) resistance to anti-virals, (ii) the requirement for strain-specific vaccination and (iii) the ongoing threat of new pandemic strains of virus. Thus, new pharmacological strategies that ameliorate influenza viral lung pathology are urgently required. Animal and human studies provide compelling evidence that immune cells such as macrophages, neutrophils and T lymphocytes, which are critical for efficient viral clearance, initiate and exacerbate lung pathology following infection. An understanding of the mechanisms by which the various inflammatory cell types of the immune system cause such pathology may reveal novel targets for pharmacological modulation of host immune responses. Interleukin-17 (IL-17) is a newly discovered cytokine that has rapidly emerged as a major player in lung disease. In this project you will investigate whether IL-17 contributes to influenza virus-induced lung inflammation and injury. The significance of this will be that IL-17 may be a novel target that can be utilized for control of influenza virus-induced lung disease.

Skill acquisition: In vivo disease models, FACS analysis of cell populations, quantitative PCR, histology, virus and cell culture, ELISA, zymography and Western blotting.

CARDIOVASCULAR THERAPEUTICS UNIT PROJECT

Supervisor: **A/Prof Christine Wright**
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Telephone: **8344 8219**
Location: **Department of Pharmacology**
Cardiovascular Therapeutics Unit,
Level 9 Room N906 Medical Building
Co-Supervisors: **A/Prof James Ziogas**
Dr Ken Winkel
Dr Paul Soeding

The following project is offered as BSc Honours only

A project will be available for a suitable Honours student. The Cardiovascular Therapeutics Unit has interests in cardiovascular and autonomic pharmacology, as well as translation research in the following areas:

- novel angiotensin II receptor antagonists for the treatment of vascular disease (in vitro and in vivo approaches)
- snake and scorpion venom pharmacology (in collaboration with the Australian Venom Research Unit)
- pulmonary hypertension: in vitro pharmacology of human and animal tissues
- regional cardiovascular effects and cardiac toxicity of novel therapeutic agents (e.g. QT prolongation and Torsades de Pointes; in vitro and in vivo approaches)

Techniques available in the Unit include:

- analytical pharmacology in vitro (e.g. resistance blood vessels in myographs; cardiac tissues (atria and papillary muscle); and assays of sympathetic and vagal neurotransmission)
- haemodynamic measurements in vivo (e.g. blood pressure, heart rate, ECG and regional blood flows) in acute anaesthetised and/or chronic disease states such as hypertension
- cardiovascular reflexes in vivo (e.g. cardiac baroreceptor reflex)
- chronic surgical implantation of flow probes and catheters

Students with a strong interest in cardiovascular or venom pharmacology are encouraged to make contact to discuss potential projects for the Honours year. A project will be designed to suit the techniques in which the student is keen to gain skills, depending on current areas of pharmacological research in the Unit.

2012 COURSE OUTLINE

BSc HONOURS

PHRM40002 ADVANCED TOPICS IN PHARMACOLOGY (SEMESTER 1) 12.5 PTS

Manuscript Evaluation Examination	30%
Theory Project	70%

BIOM40001 INTRODUCTION TO BIOMEDICAL RESEARCH (SEMESTER 1) 12.5 PTS

2 Assignments	50% each

PHRM40001 + 40006 RESEARCH PROJECT 75 PTS

Oral Research Presentation I & II	15%
Literature Review	10%
Research Thesis	75%

Note: After each assessment, you will be given a grade on the LMS.

H1	80% +
H2A	75-79%
H2B	70-74%
H3	65-60%
F	Below 65

MSc (RT)

This is a 2 year degree comprising a 125pts research project and 75 pts of coursework subjects.
For details of the prerequisites and coursework subjects, see the handbook entry.

MSc RT Handbook is available on the following website:
https://handbook.unimelb.edu.au/view/2011/R05-RH

HOW TO APPLY

If you wish to be considered for Honours or MSc (RT) in 2012, and you would like to undertake your project and coursework in a MDHS Department or affiliated institute, you will need to carry out **THE FOLLOWING STEPS:**

HOW TO APPLY FOR HONOURS:

STEP 1:

Find the project or research area you are interested in apply for. This can be done by looking through this booklet.

Contact the potential supervisor to discuss potential projects.

Attend our Student Dinner on **8 September 2011** at University House.

STEP 2

Lodge an online application by Friday 18 November 2011

Applications for Honours are lodged online to MDHS via one of the following processes:

a) Local applicants to apply online via the following address:

https://prod.ss.unimelb.edu.au/student/S1/eApplications/eAppLogin.aspx?f=%24S1.EAP.LOGIN.WEB

b) International applicants to apply online via the following address:

http://www.futurestudents.unimelb.edu.au/admissions/applications/online-application-info

Please note the following:

All non-University of Melbourne applicants - Please provide an original or certified copy of your complete official academic transcript to the MDHS Student Centre as part of your application -

http://www.sc.mdhs.unimelb.edu.au/contact

Your applicant / student ID must be used for Step 3 below.

STEP 3

Lodge project preferences in Honours Applications and Tracking System (HATS) by Sunday 27 November 2011

Once you have decided on a project(s) and submitted your application via the method specified in Step 2, you will need to lodge your preferences for projects offered within MDHS departments through HATS.

Please note HATS will open mid-September 2011.

If you have lodged your online application for Honours, you will receive an email with your HATS password mid-September so you can lodge your project preferences.

THE KEY DATES ARE:

Sep-Nov 2011: Contact potential supervisors to discuss honours projects (Step 1).

18 Nov 2011: Closing date for Honours application (Step 2).

27 Nov 2011: Closing date for project preference submission through HATS (Step 3).

3rd week Dec 2011: First round of offer letters sent by mail to students.

4 Jan 2012: Closing date for acceptance/rejection by students of First Round offers.

9 Jan 2012: Second round of selection and mailing of offer letters begins.

13 Feb 2012: Honours 2012 begins (check with individual Departments/Institutes for specific starting date and other details.

HOW TO APPLY FOR MSc (RT):

STEP 1:

Find the project or research area you are interested in apply for. This can be done by looking through this booklet or you are welcome to go to our Research Lab pages.

Contact the potential supervisor to discuss potential projects.

Attend our Student Dinner on **8 September 2011** at University House.

STEP 2:

Once you have a potential supervisor and project, applications are made through the Melbourne Graduate School of Science on the following website:

http://graduate.science.unimelb.edu.au/apply.php

The MSc (RT) is a different degree to BSc Honours and applications are handled independently.

Note that MSc students do NOT lodge their project preferences through HATS.

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