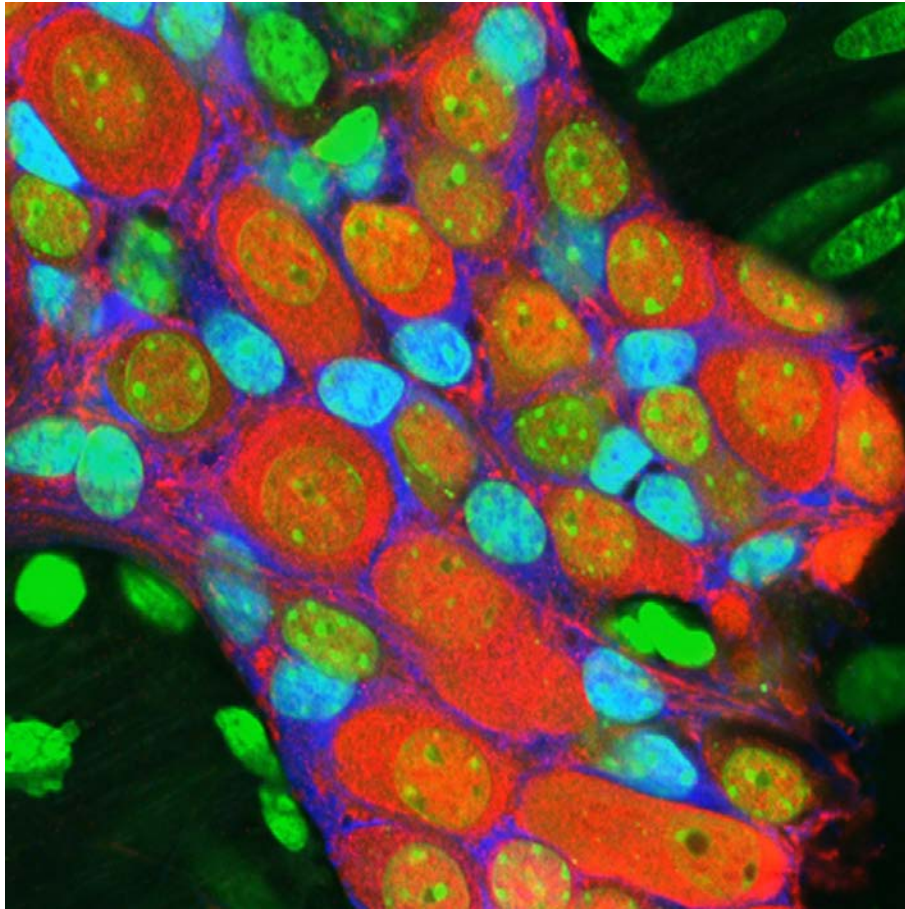


Research Projects for 2012 (Includes Honours and PhD)



Research in the Department of Anatomy and Cell Biology

There are many opportunities for students to undertake research in the Department of Anatomy and Cell Biology. An Honours year is open to students who have completed an undergraduate degree, while students with a BSc (Hons) can train for a career in science by undertaking a three to four year PhD. Medical students can enroll in an AMS year.

In this brochure, you will find a wide range of research projects that can meet the needs of potential Honours, PhD or AMS students. You should look through the projects and contact the Head of the laboratory of any projects that interest you. Alternatively, you can contact the departmental co-ordinators for Honours and PhD, Dr Peter Kitchener (8344 6746, pkitc@unimelb.edu.au).

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HONOURS

What is a BSc (Hons)?

A BSc (Hons) is a year of study following a Bachelor of Science or Bachelor of Biomedicine degree. It consists of a combination of a research project and course work. The research component is carried out under supervision in one of the research groups in the department. Your research project is included in the 75 point subject "Anatomy and Cell Biology Research Project". 80% of this subject is assessed via 7000 word research thesis submitted at the end of the year. Other assessment tasks include a literature review, a short oral presentation of your work as well as a grade from your supervisor. The remaining 25 points are from two 12.5 point coursework subjects, "Introduction to Biomedical Research" and "Seminars in Anatomy and Cell Biology".

The BSc (Hons) year starts in February and ends in November and is only available full time.

Entry requirements

You must have completed a suitable degree (B.Sc., B.Biomed. or equivalent) and achieved a faculty honours score of 65 or equivalent. You must also have the agreement of a supervisor in the Department of Anatomy and Cell Biology to supervise you in the project.

How to Apply

Detailed instructions on how to apply, and the circuital dates, are available at the MDHS student centre website:

<http://www.sc.mdhs.unimelb.edu.au/how-apply>

Essentially, there are three *sequential* steps in applying for honours.

Step 1: You will need to decide which projects / supervisor you would like to do your honours year with: because there may be more applicants than places available for particular projects or supervisors, it is important to identify a number of potential projects (within this department or other departments in the MDHS Faculty). Information about the range of honours research projects is provided on our departmental website, and also in hardcopy (this booklet). In addition, it is a good idea to come to the department's Honours Information Session, which is held mid September (details of the time and location will be advertised on our website from late August). This session is an opportunity to meet and talk with supervisors from all of the research laboratories in the department. Information about projects in this department, and other departments in the Faculty, will also be available at the MDHS faculty honours information session (also held in mid September).

Step 2: All applicants (local and International) must lodge an online application for Honours to the Faculty of Science. (See the website above for instructions and timelines.)

Step 3: After having decided on a project(s) and submitting your online application, you will need to lodge your project *preferences* with MDHS through the Honours Application and Tracking System (HATS). It is essential that you have already identified which projects you wish to apply for by speaking to potential supervisors (i.e. Step 1) and have applied for Honours through the Student Portal (i.e. Step 2) before you carry out Step 3.

PhD

What is a PhD?

A PhD is three to four years full time work doing supervised research in a laboratory. It is a necessary step in a career path to becoming a professional scientist. There are no exams or course work. The research work is written up as a thesis and assessed by two experts in the field. By the end of a PhD, you will be able to work as a professional scientist.

Why do a PhD in the Department of Anatomy and Cell Biology?

The Department of Anatomy and Cell Biology has long been a leading research department in the Faculty of Medicine, Dentistry and Health Sciences. We offer a range of research topics in anatomy, neuroscience, cell and developmental biology and molecular biology, and many of the laboratories collaborate with leading research groups around the world. The dynamic research in the department is due in part to the excellent facilities, as well as to the presence of a range of full time research-only staff.

Where does a PhD lead?

Following completion of a PhD in Anatomy and Cell Biology, you might move on to a job, often overseas, in a research institute or University, working full time as a scientist. You could remain working full time as a Research Scientist for the rest of your working life or eventually take a job as an academic in a University, doing both research and teaching. A PhD in Anatomy and Cell Biology may also lead to a range of other jobs, particularly if you have other postgraduate qualifications. These jobs could include patent attorney, clinical trials or research co-ordinator, or biotechnology manager

What to do if you are interested?

The first step is to identify projects of interest from this booklet and contact a potential supervisor, who will assist you with the enrolment process. For general information about postgraduate research in the department, contact the PhD Co-ordinator, Dr Peter Kitchener.

Entry requirements

Entry requirements to a PhD are that you have a BSc (Hons) or equivalent degree. PhD scholarships are also available to support you while you study. They are competitively awarded on the basis of your academic record.

Further information can be found at <http://www.anatomy.unimelb.edu.au/researchprojects/phd.html>.

Neuronal Differentiation Laboratory

Determining neuronal identity during development.

Assoc Prof Colin Anderson (Room E723, ph 8344 5807)

Email: c.anderson@unimelb.edu.au

Dr Kylie Cane (Room E503, ph 8344 3979)

Email: k.cane@unimelb.edu.au

Web Page: <http://www.anatomy.unimelb.edu.au/researchlabs/anderson/index.html>

The nervous system is the most complex of all biological structures. It contains many different types of neurons, which differ in the neurotransmitter they release, the receptors they express and the connections that they make. Yet many neurons that are distinctly different in their mature form originate from the same stem cells. Furthermore, this process yields the right number of cells in the right place and at the right time to create a functional network of neurons. Understanding the processes that control expression of neuronal diversity is one of the most challenging problems facing biologists today.

In our laboratory, we study autonomic neurons to determine how they adopt their distinctive, mature forms.

1. How do neurons and glial cells arise from a common pool of neural crest progenitors in a common environment?
2. What is the relationship between when a neuron is born and the phenotype it later adopts?
3. Does the developing sympathetic ganglion exhibit any topography in the distribution of stem cells and neuronal and glial precursors?
4. How is the axon of a developing autonomic neuron directed to its target?

Our approach is to use mice as models. This allows us to use a range of transgenic animals, with specific genes inactivated or with reporter genes indicating when specific genes are activated. In combination with modern culture and time lapse techniques, this gives us a powerful insight into development in a mammalian embryo.

Any one of these questions would make a suitable topic for a PhD, Honours, AMS or 516307 project. Projects can also be tailored to match the specific interests or goals of a particular student. All projects will provide training in key techniques widely used in modern biological sciences, including multiple-labelling immunofluorescence, organ culture and confocal microscopy and will provide the basis for a range of future career choices. Students should feel free to contact me to discuss possible projects in my laboratory, or to discuss Honours in general.

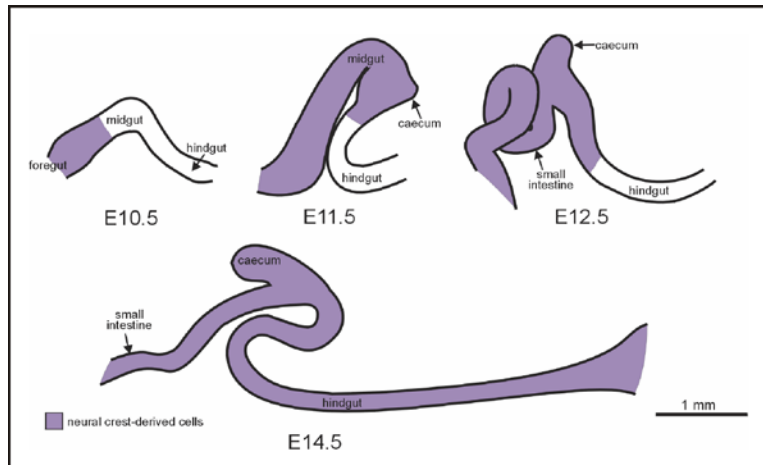
Cell Migration Laboratory

Dr Richard Anderson (Room E525, ph 8344 5783)

Email: r.anderson@unimelb.edu.au

Web Page: http://www.anatomy.unimelb.edu.au/researchlabs/anderson_r/index.html

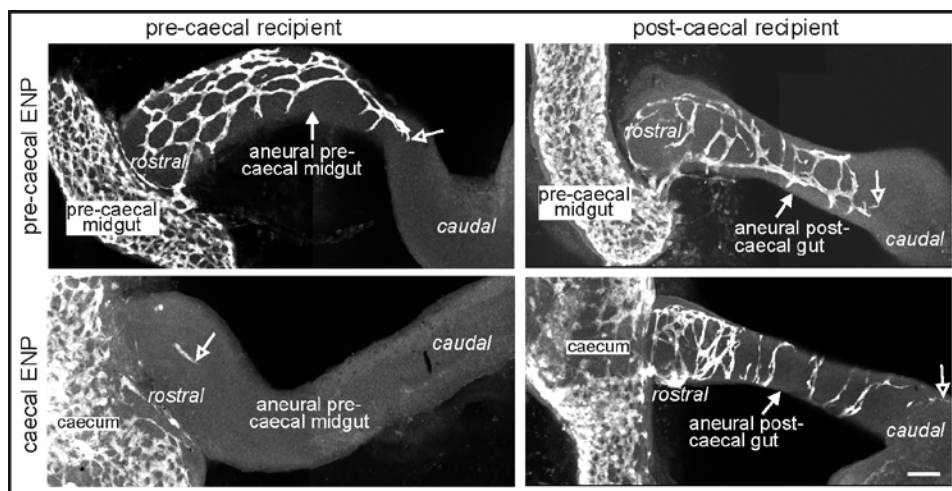
During the development of the enteric nervous system (ENS), neural precursors from the hindbrain must first migrate into and colonise the entire gastrointestinal tract (gut). This migration is very interesting because (a) it takes a long time (>4 weeks in humans and 4 days in mice), (b) the cells have to migrate very long distances, particularly those that colonise the colon and rectum, and (c) if cells fail to colonize the distal gut in humans, a disease called Hirschsprung's disease results which requires surgery.



Timetable of enteric neural crest cell migration

Project 1: Factors influencing the migration of neural crest cells within the caecum.

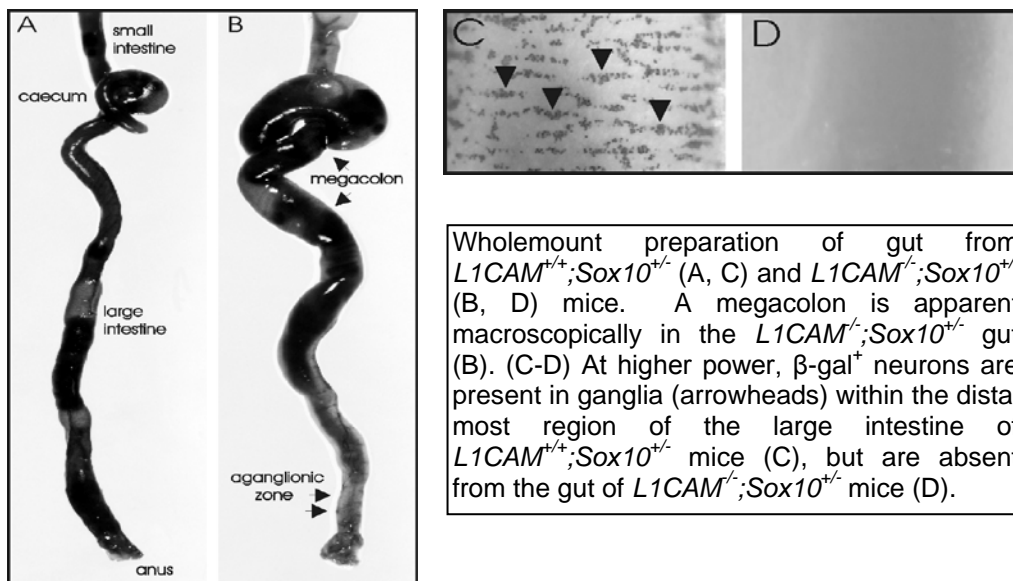
We have recently shown that when neural crest cells reach the caecum, their migratory properties are altered such that they are no longer able to migrate within the midgut. This suggests that the caecum possess molecules that can alter neural crest cell migration. In this project, you will identify molecules that are expressed within the caecum (using microarrays and in situ hybridisation) and then examine whether these molecules alter cell migration (using a variety of *in vitro* assays).



Comparison of distance that pre-caecal and caecal cells migrate in recipient pre-caecal and post-caecal gut. The most distal cell is indicated with an open arrow. Pre-caecal cells colonised both pre-caecal and post-caecal recipient gut equally well (top panel). Caecal cells colonised pre-caecal recipient explants poorly, but migrated a similar distance along post-caecal recipients to pre-caecal cells (bottom panel).

Project 2: Role of *L1CAM* as a modifier gene in Hirschsprung's disease.

L1CAM is a X-linked gene that encodes the cell adhesion molecule, L1. In humans, mutations in *L1CAM* have been implicated in a range of neurological disorders. Notably, some individuals with *L1CAM* mutations also have Hirschsprung's disease, suggesting a possible role for this gene in ENS development. We have recently shown that: (i) L1 is expressed by enteric neural crest cells as they migrate through the developing mouse gut; (ii) disrupting L1 activity retards enteric neural crest cell migration in explants of embryonic mouse gut in vitro; and (iii) *L1CAM* null mutant mice show a significant delay in enteric neural crest cell migration. However, the entire gastrointestinal tract is fully colonised prior to birth. This suggests that *L1CAM* may function as a X-linked modifier gene in Hirschsprung's disease. A modifier gene is defined as a gene that when mutated, is insufficient on its own to produce an effect. However, when coupled with another genetic mutation, it produces or enhances an effect. In this project, you will examine whether genetic interactions between *L1CAM* and other known Hirschsprung susceptibility genes results in a Hirschsprung-like phenotype. To do this you will use a variety of methods, including genetic and molecular analysis, microdissection, histochemistry and confocal microscopy.



Ocular Development Laboratory

Dr Robb de longh (Room E631, ph 8344 5788)

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Web Page: <http://www.anatomy.unimelb.edu.au/researchlabs/delongh/index.html>

Overview

Research in the Ocular Development Laboratory is directed at identifying the molecular mechanisms that regulate development, growth and pathology of the eye, particularly the lens. We use a variety of techniques to investigate the genes and signalling pathways involved in the cellular interactions and processes that control the development, structure and growth of the eye in the vertebrate embryo.

Project 1: Role of integrin-linked kinase in lens development and cataract.

During development of the vertebrate lens there are dynamic interactions between the extracellular matrix (ECM) of the lens capsule and lens cells. Disruption of these interactions can result in perturbation of lens development and cataract. Integrins are key receptors for ECM signals and various studies have documented distinct repertoires of integrin expression during lens development, and in anterior subcapsular cataract (ASC). One of the key mediators of integrin signalling in the cell cytoplasm is integrin-linked kinase. We have generated mice with conditional null mutation of *Ilk* and found that it affects cell proliferation and survival. However, our cell culture experiments indicate that over-activation of ILK can lead to an epithelial-mesenchymal transition (EMT), similar to that found in a form of human cataract. We have now generated transgenic mice that express a hyperactive kinase form of *Ilk* in the lens. This transgene can rescue the null phenotype and shows effects on lens epithelial cell morphology *in vitro*.

This project will further investigate the effects of the hyperactive *Ilk* transgene on lens cell phenotype. Techniques will include management of mouse colonies, PCR genotyping, and examining the responsiveness of epithelial cells to TGF β and FGF in tissue culture and examining molecular markers by RT-PCR, immuno-histochemistry/western blotting and in situ hybridisation. The project may also involve generating a new transgene for a kinase-dead form of *Ilk*, which will be used to generate a transgenic mouse line.

Project 2: Role of *Fz5* and *Fz7* genes in lens development.

We have already shown that the Wnt/ β -catenin pathway is central to regulating lens stem and progenitor cells during development.¹ Recent evidence indicates that the Wnt/PCP pathway also plays roles in lens development.² To investigate the contribution of Frizzled (Fz) receptors in mediating Wnt signals we are conducting Cre-LoxP experiments to conditionally delete *Fz5* and *Fz7* genes from the developing lens. Techniques include breeding and management of mouse colonies, histology, and investigating molecular markers of lens development by RT-PCR, immuno-histochemistry/western blotting and in situ hybridisation.

¹ Cain S et al., *Dev Biol* **321**:420 (2008); Martinez G et al., *Invest Ophthalmol Vis Sci* **50**:4794 (2009).

² Sugiyama Y et al., *Dev Biol* **338**:193 (2010).

Visual Neuroscience Laboratory

Dr Erica Fletcher (Room E721, ph 8344 3218)

Email: elf@unimelb.edu.au

Web Page: <http://www.anatomy.unimelb.edu.au/researchlabs/fletcher/index.html>

Dr Kirstan Vessey (Room E708A, ph 8344 5769)

Email: k.vessey@unimelb.edu.au

Dr Andrew Jobling (Room E708A, ph 8344 5769)

Email: ajj@unimelb.edu.au

Dr Una Greferath (Room E702E / E708A, ph 8344 4315 / 8344 5769)

Email: u.greferath@unimelb.edu.au

Web Page: <http://www.anatomy.unimelb.edu.au/researchlabs/greferath/index.html>

Retinal diseases are a major cause of blindness in the Western world. There are few treatments currently available, largely because the underlying mechanisms of disease are not well understood. Our laboratory investigates these underlying disease mechanisms. We are currently studying two broad classes of retinal diseases:

RETINAL DEGENERATIONS:

Death of the light-detecting retinal neurons, the photoreceptors, are associated with 50% of all cases of blindness in Australia, being a major contributor to visual impairment in Age-Related Macular Degeneration (AMD), and hereditary retinal degenerations including Retinitis Pigmentosa (RP). There are currently no treatments for RP or AMD. We are examining the mechanisms of photoreceptor death and whether specific treatments ameliorate or slow the loss of photoreceptors.

RETINAL VASCULAR DISEASE:

Retinopathy of Prematurity (ROP) is a major cause of visual impairment in children born prematurely. ROP is a vascular disease, caused by excessive growth of blood vessels on the surface of the retina in response to the combined effects of extreme immaturity of the retina and high levels of oxygen used for critical care of neonates. Currently, treatment targets the pathological angiogenesis. Despite treatment, many children suffer ongoing vision impairment. We are examining the major factors involved in the development of ROP in order to develop more successful clinical treatments.

Projects available:

Project 1: Investigation of potential therapeutic targets aimed at inhibiting photoreceptor death in a mouse model of retinal degeneration

Dr Kirstan Vessey and Dr Erica Fletcher

The excitatory neurotransmitter, extracellular ATP, acts via two classes of receptor, P2X and P2Y. The P2X7 receptor (P2X7-R) is a unique member of the P2X receptor family of ligand gated ion channels. It requires higher concentrations of ATP to become activated and when stimulated by ATP it not only conducts cations, but following prolonged stimulation can conduct larger molecules, ultimately causing cell death. As a result activation of the P2X7-R has been studied as a mediator of inflammation, cell death and neural degeneration. This project will examine whether pharmacological blockade or knock out of the P2X7-R slows photoreceptor loss and restores visual function in a mouse model of retinal degeneration.

Project 2: The role of microglia in Age-Related Macular Degeneration

Dr Andrew Jobling and Dr Erica Fletcher

AMD is a major cause of vision loss in the older community. Recent work has indicated that inappropriate activation of the immune response may play a role in the development of AMD. Retinal microglia, the resident immune cells within the retina, are thought to play two alternative roles, one being neuroprotective and the other resulting in neuronal cell death. Using a model system in which a major signalling mechanism is “knocked out” in retinal microglia, we will investigate whether these microglia are critical in both the protection of retinal neurons and what factors are altered that result in retinal neuronal death. This project will involve the use of wide ranging techniques such as immunohistochemistry, molecular biology and in vitro cell culture. Ultimately this study with detail why microglia are critical to normal retinal function and what factors are involved in the development of retinal degenerations such as AMD.

Project 3: The role of histamine in the development of the retina

Dr Una Greferath and Dr Erica Fletcher

Histamine is a biogenic amine involved in immune response and acts as a neurotransmitter in the brain. We have very new and exciting evidences that histamine plays a role in the development and correct lamination of the retina: mice, which cannot produce histamine (HDC-KO mice) have a disrupted photoreceptor layer and significant photoreceptor loss in adulthood. We aim to investigate the mechanism by which histamine controls photoreceptor development. We will need to localise histamine and its receptors during development, evaluate the effect of histamine on photoreceptors in vitro and try to rescue photoreceptor development by histamine supplementation. This project will involve the use of techniques such as immunohistochemistry, molecular biology and in vitro cell culture.

Autonomic Neuroscience, Pain and Sensory Mechanisms Laboratories

Principal Contacts

Assoc Prof James Brock (Room E238, ph 8344 5811)

Professor John Furness (Room E237, ph 8344 8859)

Dr Daniel Poole (Room E212, ph 8344 9994)

Dr Tony Frugier (Room E240, ph 8344 4782)

Post-doctoral Scientists: Dr Romke Bron, Dr Brid Callaghan, Dr Alan Lomax, Dr Trung Nguyen, Dr. Trent Reardon

See Our Web Page: <http://www.anatomy.unimelb.edu.au/researchlabs/furness/index.html>

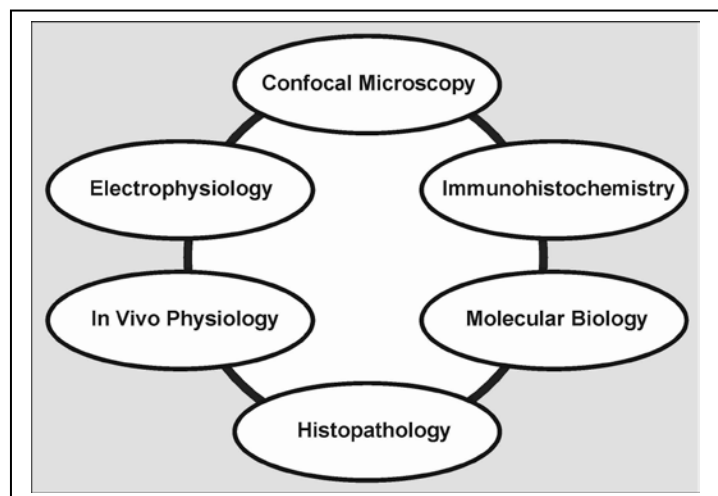
Major Project Areas

How neurons react to the challenges of Inflammation and Ischemia – The roles of ion channels in neurons – Visceral Pain – Spinal Cord Injury, seeking solutions to autonomic disturbances – The actions of ghrelin in spinal cord autonomic centres.

We also run a modern histopathology laboratory, primarily for mouse work: <http://www.apn-histopathology.unimelb.edu.au/>. There are opportunities for projects in this laboratory.

Experimental Techniques

Immunohistochemistry - confocal microscopy - molecular biology - patch clamp recording - intracellular microelectrode recording - in situ hybridisation - retrograde neuronal tracing - *in vitro* and *in vivo* reflex studies - biophysical analysis of neuron properties - whole animal physiology - behavioural testing - pharmacological analysis *in vivo* and *in vitro* - histopathology



Overview of Research Programs

Our research programs combine structural, physiological, pharmacological and neurochemical studies of the enteric nervous system, visceral afferent (sensory) neurons and digestive physiology, and central control of autonomic functions (cardiovascular, digestive, urinary). We focus on animal models of disorders of the nervous system, including irritable bowel syndrome, inflammatory bowel disease and the autonomic consequences of spinal cord injury.

Projects 1-3: Autonomic dysfunction in spinal cord injury

To most observers, the dominant impact of spinal cord injury (SCI) is impaired mobility. However, it is impairment of autonomic nervous system (ANS) function that socially isolates, increases dependence, precipitates hospital re-admission and causes premature death. We are applying a combination of unique clinical and preclinical approaches to develop novel approaches to treating ANS dysfunction in SCI. The autonomic centres in the spinal cord are disconnected from central control.

Project 1: Changes in vascular function after spinal cord injury.

Associate Professor James Brock, Mr Hussain Al Dera, Dr Trent Reardon

Cardiovascular disease is the greatest cause of premature death after the initial trauma of injury is overcome. Loss of blood pressure control means loss of a fundamental of human haemodynamics – maintaining brain blood flow when erect. Therefore, SCI patients are hypertensive when supine and hypotensive to the point of losing consciousness when erect. Their blood vessels are inadequately controlled and their physiological states are abnormal. In this project we are investigating the changes in neural control of blood vessels that occurs after spinal cord injury or local denervation. In this project you will record muscle tension in isolated vessels and you will conduct a pharmacological analysis of blood vessel responsiveness and nerve control.

Project 2: The effects of spinal cord injury on urinary bladder barrier integrity.

Dr Romke Bron, Dr Tony Frugier, Ms Rachael Abela, Associate Professor James Brock, Prof John Furness

A mysterious change occurs in the bladder lining following spinal cord injury. Although the injury is far from the bladder, the lining epithelium (the urothelium) loses its integrity and becomes highly permeable, allowing bacterial invasion of the bladder wall. Loss of neural influence on the bladder lining and breakdown of barrier function causes elevated rates of urinary tract infections in SCI; this is a major cause of hospital re-admission and loss of functional independence. In this project you will use techniques of molecular biology and immunohistochemistry to characterise the changes that occur in the bladder after spinal cord injury.

Project 3: Regulation of digestive function after spinal injury.

Ms Dorota Ferens, Dr Mark Habgood, Associate Professor James Brock, Prof John Furness

The daily need for assistance with defecation in spinal injured patients is unpleasant, time-consuming and expensive for patient and carer. Moreover, it does not avert the single most socially crippling consequence of SCI - uncontrolled defecation. In this project we are studying the mechanisms in the lower lumbar spinal regions communicating with the colon that could be manipulated to allow for controlled, timed and safely reproducible emptying of the bowel by patients. We have discovered a novel pharmacological method to trigger colorectal emptying. This project will test in animal models this and other therapies for treating injured patients. You will learn how to instrument animals and record from them, *in vivo*.

Projects 4-7: The roles of the hormone ghrelin in autonomic control and the biology of ghrelin receptors

We have discovered that the hormone ghrelin, best known for roles in growth hormone release and stimulation of appetite, has important functions in controlling autonomic organs from the spinal cord level and probably from the brain stem. In linked projects, we are investigating the roles of ghrelin using *in vivo* recording from rats, and the distributions of sites of action using *in situ* hybridisation, reporter mice and activity-dependent labeling of neurons.

Project 4: The cardiovascular effects of ghrelin.

Dr Daniela Sartor, Dr Trung Nguyen, Dr Brid Callaghan, Ms Dorota Ferens, Associate Professor James Brock, Prof John Furness

We have evidence that ghrelin affects the cardiovascular system at three levels: in the brain stem, where ghrelin, through unknown mechanisms, lowers blood pressure, in the spinal cord, where ghrelin acts on sympathetic preganglionic neurons to increase blood pressure, and at peripheral sites where it dilates blood vessels. At each site, the pharmacology of interactions with ghrelin receptors differs. In this project you will examine an aspect of the influence of ghrelin and ghrelin receptor agonists the cardiovascular system.

Project 5: The molecular basis for ghrelin receptor heterogeneity.

Dr Romke Bron, Ms Billie Hunne, Associate Professor James Brock, Prof John Furness

The receptor pharmacology of ghrelin is quite unusual. Despite there being only one molecularly defined receptor, the effects of agonists at different sites vary considerably. For example, at growth hormone secreting cells in the pituitary gland, ghrelin is a full agonist, whereas des-acyl ghrelin has no effect. At some blood vessels, both ghrelin and des-acyl ghrelin are agonists, and one site has been discovered in the spinal cord at which ghrelin is an agonist and des-acyl ghrelin is an antagonist. In this project you will use methods including laser microdissection to isolate regions of receptor variability and extract protein and mRNA. The protein will be separated by 2-D gel electrophoresis and immunoreactive proteins will be identified. If appropriate, these will be subjected to mass spectrophoretic analysis. mRNA will be characterised by size and sequence.

Project 6: Novel compounds that act at ghrelin receptors: pharmacological and functional analysis.

Dr Brid Callaghan, Prof John Furness, Dr Romke Bron, Dr Jonathan Baell, Ms Kung Ban, Ms Dorota Ferens

A range of ligands has been synthesized that act as ligands for the ghrelin receptor. Many of these have been reported in the patent literature without their biological activities being revealed. In this project you will collaborate with medicinal chemists to decide on strategies to prepare compounds. You will test these by pharmacological analysis using transfected cells and isolated tissues.

Project 7: Ghrelin receptor distribution revealed in reporter mice.

Ms Billie Hunne, Dr Romke Bron, Prof John Furness, Dr Alan Lomax

We have available mice that are engineered to express a fluorescent protein under the control of the promoter for the ghrelin receptor. Using these mice, you will determine the pathways in which the ghrelin receptor is involved. The reporter mice will be used to locate sites of electrophysiological recording to investigate the functions and roles of the ghrelin receptors.

Projects 8-12: Enteric neuropathy: ischemia-reperfusion injury

The changes caused by ischemia are recognised as important in causing gut dysfunction, but very little is known about effects at a cell level. We have recently found that effects on neurons are substantial and type specific, for example the nitric oxide neurons are swollen to almost double their normal volume. Moreover, there is a large, but generally unrecognised, inflammatory reaction associated with ischemia. A substantial part of the damage appears to be exerted by free-radical formation, including the production of nitric oxide. In these projects, we are investigating the mechanisms behind the damage, and are using transgenic animals and pharmacological interventions to investigate protection against ischemia-reperfusion injury.

Project 8: Consequences of ischemia-reperfusion injury in the human intestine.

Dr Tony Frugier, Mr Gene Venables, Dr Daniel Poole, Ms Michelle Thacker, Ms Louise Pontell, Prof John Furness, Dr Mehrdad Nikfarjam, Professor Robert Jones.

This project utilises human small intestine that has been subjected to ischemia and reperfusion. This project is part of an ongoing research program aimed at understanding the numerous biochemical and molecular changes following ischemia and reperfusion in humans. The availability of human small intestine tissue makes this project unique. Real-time quantitative PCR, ELISA, Western-blot and immunohistochemical methods are some of the techniques the prospective student will be trained for and use for this project.

Project 9: The reactions of enteric neurons and glia to ischemia-reperfusion injury.

Ms Michelle Thacker, Dr Tony Frugier, Mr Gene Venables, Ms Billie Hunne, Ms Louise Pontell, Prof John Furness.

This project uses immunohistochemical methods to investigate the changes that occur in the enteric nervous system when blood flow is interrupted and restored. This includes investigation of changes in neuronal and glial morphology and chemistry, including cytoskeletal disruption, the occurrence of cell death (using TUNEL staining) and the nitrosylation of proteins. You will learn how to conduct experimental surgery, methods of multiple labelling immunohistochemistry and confocal analysis.

Project 10: Calcium imaging of enteric glia.

Ms Michelle Thacker, Dr Trung Nguyen, Prof John Furness

Enteric glia have important roles in controlling the environments of neurons and restricting the changes in neuronal function that can be caused by accumulation of metabolites or by damage to tissues. Glial cells react to various factors, including cytokines and inflammatory mediators. Many of these signal through changes in cytoplasmic calcium. Enteric glia exhibit morphological changes in responses to stress, but how the stress signals are transduced is unknown. In this project you will learn how to record calcium signals in living cells and will use this technology to investigate the responses of glial cells to inflammatory mediators and the stress of ischemia/ reperfusion.

Project 11: Investigation of the role of nitric oxide in ischemia/ reperfusion injury using knock out mice.

Dr Tony Frugier, Mr Gene Venables, Ms Leni Rivera, Ms Michelle Thacker, Prof John Furness

We have good evidence that nitric oxide (NO), that is produced by the enzyme nitric oxide synthase (NOS), has a role in the enteric neuronal damage that is caused by ischemia-reperfusion. The main evidence is that NOS neurons are selectively affected and there is nitrosylation of proteins in the enteric nervous system. To test this theory, we will cause ischemia-reperfusion injury to mice in which neuronal NOS has been knocked out and to wild-type mice. Effects on the swelling of NOS neurons, on the nitrosylation of protein and on cell death will be measured.

Project 12: Intestinal ischemia/ reperfusion injury – protective strategies.

Dr Tony Frugier, Mr Gene Venables, Dr Daniel Poole, Prof John Furness

A major problem arising during intestinal transplantation surgery is ischemia/reperfusion (I/R) injury to enteric neurons that results in disordered gastrointestinal function. This problem could possibly be avoided or reduced by neuroprotective treatment before or at the time of surgery if the nature of the events was better understood and appropriate strategies are developed. Recent investigations have revealed that nitric oxide (NO) produced by damaged cells may have a key role in causing cell injury. In this project we will test the protection that results from inhibition of the NO-producing enzyme: nitric oxide synthase (NOS).

Projects 13-16: The enteric nervous system and the control of digestive function

Project 13: Changes in digestive function caused by pharmacological targeting of ion channels and receptors.

Ms Dorota Ferens, Prof John Furness

The enteric nervous system, which controls digestive tract movements, can generate a range of behaviours of the intestine, from strong propulsive movements to mixing activity. It would be useful, therapeutically, to be able to switch between movement patterns. This project investigates the ways in which drugs that target ion channels of enteric neurons can modify behaviour of the organ. In this project you will learn about recording physiological parameters, gastrointestinal motility, blood pressure and heart rate in an anesthetized rat model.

Project 14: Receptor Trafficking in Enteric Neurons of the Inflamed Intestine.

Dr Daniel Poole

Many G-protein coupled receptors (GPCRs) are internalized from the cell surface into endosomes in response to agonist binding. As this effectively controls the number of receptors available for subsequent agonist binding, endocytosis and recycling of GPCRs are key regulators of cellular responsiveness to agonists. During and following periods of intestinal inflammation there are significant changes in intestinal motility and secretion and this is partly attributable to the hyperexcitability of enteric neurons. Changes in trafficking of receptors expressed by these neurons have yet to be examined. In this project you will examine the distribution and trafficking of receptors in enteric neurons during and after periods of intestinal inflammation and stress, with an emphasis on the neurokinin 1, somatostatin receptor 2A and mu opioid receptors. Changes in the expression of key regulators of endocytosis will be examined by PCR and Western blot and changes in receptor signalling will be examined by Ca²⁺ imaging (NK₁R) and ERK assays.

Techniques: immunofluorescence, quantitative microscopy, Western blot, PCR, Ca²⁺ imaging.

Project 15: Effects of Milk-Derived Opioid Agonists on Enteric Neurons.

Dr Daniel Poole

The digestion of milk and milk products gives rise to casein-derived peptides (casomorphins), which can act as agonists at opioid receptors, leading to alteration in intestinal motility and secretion. These peptides may partly underlie milk intolerance in infants and 'cheese addiction'. In this project you will examine the effects of casomorphins on receptor endocytosis, using mu, delta and kappa opioid receptors heterologously expressed in cell lines. You will also examine the effects of casomorphins on endogenous opioid receptors expressed by enteric and central neurons and detect specific uptake using fluorescently-labeled peptides. Activation of cells by casomorphins will be examined using assays for cAMP production and ERK phosphorylation. The pharmacological effects of casomorphins on gut motility and secretion will be examined *in vitro* and *in vivo* and related to data from the microscopy studies.

Techniques: immunofluorescence, quantitative confocal microscopy, neuronal culture, cell culture, live imaging, peptide labelling, plate-based assays for cAMP and ERK activation, Western blotting, pharmacological assays, gastric emptying and bead propulsion.

Project 16: Developing Effective Transfection Methods for Enteric Neurons.

Dr Daniel Poole

The effective use of standard cell biology techniques in the field of enteric neurobiology has been greatly restricted by the relative inefficiency in which enteric neurons can be transfected. This has limited the use of e.g. siRNA knockdown of target proteins or the expression of tagged proteins. In this project you will optimize methods for transfection (electroporation, Ca²⁺ phosphate) of cultured myenteric neurons using eGFP as a marker of effective transfection and pan-neuronal markers to quantify relative efficiency. Neuronal viability following transfection will be assayed by Ca²⁺ imaging and effective knockdown of proteins will be examined by Western blotting. If time allows, you will then use these techniques to examine receptor trafficking in the soma and neurites of myenteric neurons using real-time imaging of fluorescently-tagged receptors and FRAP.

Techniques used: Neuronal culture, electroporation, Ca²⁺ imaging, Western blotting, live imaging, FRAP.

Project 17: Vascular neurobiology

Project 17: Changes in neurovascular transmission in diabetes.

Ms Niloufer Johansen, Dr Trent Reardon, Associate Professor James Brock

In both type 1 and type 2 diabetes there can be changes in the neural control of blood vessels leading to impaired regulation of blood pressure and tissue perfusion. In this project, we are concentrating on the early effects of diabetes on nerve-mediated contractions of small arteries supplying skin. Loss of neural control of these vessels has been suggested to be an early change that leads to microvascular disease in skin of diabetic patients, which impairs wound healing and increases the risk of gangrene particularly in the extremities (feet). In this project you will use blood vessel myography and smooth muscle electrophysiology to monitor changes in artery function and combine this with immunohistochemistry to assess changes in arterial innervation.

Project 18: Pain and sensory mechanisms

Project 18: The initiation of action potentials in corneal afferent endings.

Associate Professor James Brock, Dr Jason Ivanusic

The mechanisms whereby sensory stimuli are transduced into action potentials in the sensory nerve endings of nociceptors are poorly understood. This project uses an electrophysiological technique that allows electrical activity to be recorded directly from fine unmyelinated sensory nerve terminals. Using this technique we are investigating the location within the nerve terminal axons at which action potentials are initiated. This knowledge is fundamental to understanding the function of sensory nerve terminals. This project would be particularly suited those engineering skills.

Projects 19-20: Histopathological analysis

Project 19: Histopathology in mutant mice.

Ms Tina Cardamone, Ms Louise Pontell, Mr Steve Pouniotis, Prof John Furness

One of the most important skills needed for the analysis of mutant mice, where the mutation is not known, or the consequences of mutation are unexpected, is histopathological analysis. The skills to do this type of study are much in demand. In this project, you will be set the task of conducting histopathological analysis of three different mutants. You will learn how to prepare tissues, how to conduct a systematic analysis, how to use sophisticated imaging hardware and software and how to report histopathological results.

Project 20: Histological Investigation of Enteric Neuropathy and tissue damage.

Ms Louise Pontell, Prof John Furness, Ms Tina Cardamone

Damage to enteric neurons, and to other cell types in the intestinal wall, can cause death, and, if not death, results in considerable morbidity. We are using animal models of the intestinal damage that occurs in inflammatory bowel disease and in ischemia/ reperfusion injury to the intestine. We are also investigating changes in human intestine. This project aims at characterising the tissue damage that occurs and to relate the tissue damage to functional disorders. You will learn methods of histopathology and how tissues are analysed. You will also learn how to take electronic images and prepare histopathology reports.

Neuron Development and Plasticity Laboratory

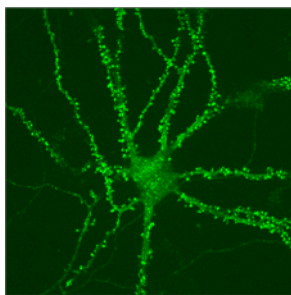
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How do neurons make connections in the developing brain?

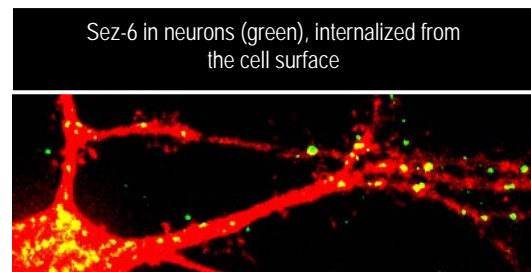


The aim of our research is to understand how neurons become connected to each other to form functional circuits. We investigate the formation of dendrites (branches) and inter-neuronal connections (synapses) in developing neurons in order to understand these processes in normal development and disease. Many neurological disorders are characterized by abnormal synaptic connectivity and changes in the number and strength of synaptic connections occur during learning and memory formation (termed plasticity). If we can understand how dendrites and synapses develop and change in the healthy brain, this knowledge will help us decipher the aberrant molecular pathways responsible for many cognitive disorders including mental retardation, epilepsy and schizophrenia.

Honours and PhD projects are available to investigate these questions, focussing on the roles of key molecules (e.g. Seizure-related gene 6 or Sez-6, Wnts, cytokines, Ndfip1) and the pathways in which they signal to regulate these important processes.

Sez-6 is a protein that is expressed in the developing brain and in adult neurons in regions important for learning and memory. To investigate the role of Sez-6, we produced a knockout mouse in which the Sez-6 gene was inactivated. Analyses of neurons in the cortex revealed that the dendrites of these neurons were abnormal, that the neurons were less easily excited by electrical stimulation and that there were fewer synapses providing excitatory input to these neurons. Abnormal Sez-6 function may be linked to the development of epileptic seizures and a Sez-6 family member is a candidate gene for autism. Furthermore, the dendrite and synapse abnormalities seen when Sez-6 is lacking are also seen in a number of mental retardation and neurodegenerative conditions.

To study the complex molecular pathways regulating the development of neuronal branches and synapses, we use diverse approaches, including real-time imaging using fluorescent markers of synapses, tissue-specific gene knockout or gene knockdown and a range of molecular biological and protein biochemical techniques.



Project 1: Molecular mechanisms of maladaptive plasticity in neuropathic pain

(Supervisors: Dr Jenny Gunnensen and A-Prof Christine Wright, Dept. of Pharmacology)

Neuropathic pain is a type of chronic pain caused by nerve damage. After traumatic peripheral nerve, spinal cord and brain injuries as well as diseases of the sensory nervous system, pain may persist for months or years after the original injury. In these cases, pain responses are amplified and even occur after stimuli that do not normally cause pain. In experimental pain models, it has been possible to measure changes in the structure and function (termed plasticity) of neurons that transmit sensory information to the brain. This "maladaptive plasticity" is now recognized as an important pathological mechanism contributing to neuropathic pain.

The drugs gabapentin and pregabalin are prescription medications that are effective against neuropathic pain. The receptor for these drugs is $\alpha 2-\delta$, a component of calcium channels or “pores” in neurons. It is currently believed that gabapentin and pregabalin act on $\alpha 2-\delta$ to reduce the level of active channels at the cell surface and, hence, neuronal excitability. Sez-6 can enhance excitatory synaptic connections through binding to $\alpha 2-\delta$. This project will examine the timing and levels of Sez6 expression in spinal cord neurons after sciatic nerve damage and relate these molecular changes to the development of neuropathic pain.

Project 2: Enhancing excitatory synapse formation through Sez-6 interaction with $\alpha 2-\delta$

(Supervisor: Dr Jenny Gunnensen)

Although $\alpha 2-\delta$ has recently been linked to the development of excitatory synapses (Eroglu C et al., 2009, Cell 139, 380-392), the molecular pathways mediating this effect are not known. Sez-6 binding to $\alpha 2-\delta$ can enhance excitatory synapse formation in a cultured neuron model and we have evidence that calcium signalling pathways are involved. This project will investigate protein-protein interactions and signalling events in order to characterize a new synaptogenic signalling pathway.

Project 3: Wnt signalling in the development of neuronal connectivity

(Supervisors: Dr Jenny Gunnensen and Dr Clare Parish, Florey Neuroscience Institutes)

Activity of the Wnt patterning and differentiation factors is vital for nervous system development. Their importance for promoting neuronal connectivity has recently been highlighted by the recent finding that neurons derived from induced pluripotent stem cells (iPS) from schizophrenia patients show aberrant Wnt signalling and reduced connectivity (Brennan KJ et al., 2011, Nature 473, 221-225). This project will compare the activities of different Wnt proteins and Wnt signalling pathways for promoting axon growth and synaptogenesis.

Project 4: The role of TCF7L2 (TCF4) in regeneration after spinal cord injury

(Supervisors: Dr Jenny Gunnensen and Dr Matthew Digby, Dept. of Zoology)

The transcription factor TCF7L2 (also called TCF4) is a downstream effector of the canonical Wnt pathway that is important for cell fate/differentiation in development. TCF7L2 is strongly upregulated in response to spinal cord injury and this upregulation is associated with the ability of the immature spinal cord to regenerate after injury (Mladinic M et al., 2010, Brain Res. 1363, 20-39). Interestingly, different forms of this transcription factor have different, even opposite effects. This project will investigate the different splice-isoforms of the messenger RNA for TCF7L2 in the mouse spinal cord in injury models.

Project 5: What does GM-CSF do in the developing brain?

(Supervisor: Dr Jenny Gunnensen)

While granulocyte-macrophage colony stimulating factor (GM-CSF) is best known as a haematopoietic cytokine stimulating production of blood progenitor cells, this cytokine and its receptor are found in the brain. Recent evidence suggests that cytokines, including GM-CSF, can exert neuroprotective effects after stroke or in other neurodegenerative conditions. Our data shows that GM-CSF can promote the growth of cultured embryonic cortical neurons however the role of GM-CSF in the developing brain is not known. This project will use cultured neuron and mouse models to study this question.

Identification of factors that regulate stem cell development in *Drosophila* and mice

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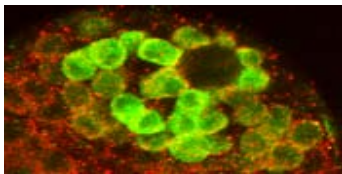
Web Page: <http://www.anatomy.unimelb.edu.au/researchlabs/hime/index.html>

Stem cells are the key to organ regeneration and tumour growth

Many differentiated but renewable cell types in vertebrates are derived from relatively small populations of dedicated precursors, or stem cells. The ability to replenish differentiated cells depends on the continued survival and proliferation of their respective stem cell populations. Stem cells are not only important for regeneration of healthy tissues but also play a key role in pathogenesis. Recent studies have demonstrated that all cells in solid tumours do not play equal roles but a small fraction of cells, the so-called cancer stem cells, contribute to the unlimited growth of the tumour and re-occurrence after tumour resection. If we are to realise the goals of re-programming tissue differentiation, growing organs for transplantation *in vitro*, regeneration of damaged organs *in vivo* and targeted effective treatments for cancer it is essential that we understand the molecules and mechanisms that stem cells utilise for renewal and differentiation.

Drosophila and mouse organs – complimentary models of stem cell function

The identification of mechanisms that regulate asymmetric division, daughter cell mitotic amplification and stem cell differentiation have been difficult to ascertain. These types of studies benefit greatly from the analysis of simple, genetically tractable systems. For these reasons we have chosen to focus on two stem cell niches in *Drosophila* (male germ line and intestinal) and one in the mouse (intestinal) as models for stem cell systems.



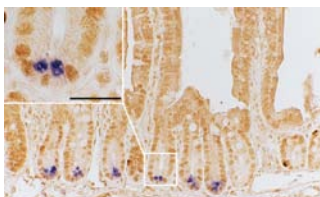
A rosette of germline stem cells (expressing the Snail family zinc finger protein Escargot, green) can be observed surrounding the somatic stem cell niche in the *Drosophila* testis

Projects are available within the following areas:

(1) Analysing the role of transcriptional repressors in *Drosophila* and mouse stem cells

(in conjunction with Dr. Helen Abud, Monash University)

We have shown that members of the Snail family of transcriptional repressors are required in diverse stem cell populations. This role has been conserved through evolution of animals as Snail family members can be found in stem cells from *Drosophila* to mouse. This project involves using genetically modified *Drosophila* or mouse strains combined with RT-PCR, microarrays and immunostudies to identify what factors are being repressed by Snail family proteins in stem cells.



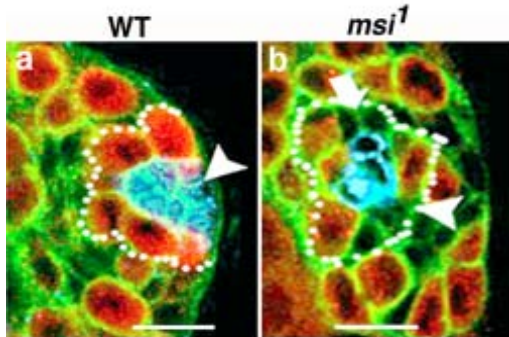
The mouse Snail family member, *Snai1*, is found in the nuclei of intestinal stem cells that are found adjacent to the Paneth cells (purple) as well as the nuclei of undifferentiated transit amplifying cells.

From Horvay et al., (2010)

(2) Analysing genetic roles of translational repressors in *Drosophila* stem cells

(in conjunction with Dr. Nicole Siddall)

We have identified three RNA-binding proteins, Musashi, Real Musashi and Held-Out-Wings (HOW) that are required to either prevent stem cells from differentiating in the stem cell niche or are required to regulate the cell cycle of stem cells. This project utilises a variety of genetic, molecular biology and immunostaining techniques to identify the roles of these proteins in different stem cell populations.



The ring of germline stem cells (red) can be observed surrounding somatic hub cells (blue).

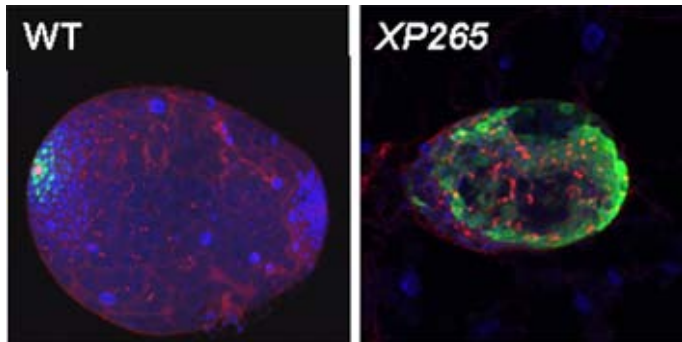
Loss of *musashi* (*msi*) function results in loss of stem cells as they prematurely differentiate and lose contact with the niche.

From Siddall et al. (2006)

(3) Analysing the role of a novel zinc finger protein in stem cell maintenance

(in conjunction with Dr. Greg Somers, LaTrobe University).

We have cloned the gene associated with a mutation that results in expansion of stem cells in the *Drosophila* testis. The gene encodes a novel protein that contains 21 zinc finger domains. We do not know what molecular processes are being regulated by this protein and the project will utilise molecular genetics and immunostudies to determine its role in stem cell maintenance.



Expression of the stem cell marker Escargot (green) is normally only observed in the stem cell niche. In the XP265 mutant we see stem cell markers throughout the testis and disorganised patterns of differentiation.

(4) How does regulation of nuclear importation affect stem cell maintenance

(in conjunction with Prof. K. Loveland, Monash University)

We have identified a nuclear importation factor that is required for maintenance of the germline stem cell population. We will use immunostaining and confocal microscopy to determine the effects of mutations of this factor on stem cell maintenance and conduct genetic interactions to identify proteins that are imported by this factor into the stem cell nucleus.

References:

K Horvay, F Casagrande, A Gany, GR Hime and HE Abud (2011) Wnt signalling regulates Snai1 expression and cellular localisation in the mouse intestinal epithelial stem cell niche. **Stem Cells and Development**, 20(4): 737-745

AC Monk, NA Siddall, T Volk, BA Fraser, LM Quinn, EA McLaughlin and GR Hime. (2010) The RNA-binding protein HOW is required for stem cell maintenance in the testis and for the onset of transit amplifying divisions. **Cell Stem Cell**, 6: 348-360

NA Siddall, EA McLaughlin, NL Marriner and GR Hime. (2006) The RNA-binding protein Musashi is required intrinsically to maintain stem cell identity. **Proc. Natl. Acad. Sci. U.S.A.** 103:8402-8407.

Neural Mechanisms of Bone Pain

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Pain associated with osteoarthritis, bone marrow oedema, bone cancer, fractures, osteoporosis and osteomyelitis causes a major burden on individuals and health care systems in Australia. This burden is expected to increase with advances in modern medicine that prolong life expectancy, because many of the conditions that cause the pain are intractable and develop late in life. The pain is poorly managed by current treatment strategies, and this likely results from our lack of understanding of the mechanisms that generate and maintain bone pain.

The broad objective of the work done in this laboratory is to determine the fundamental neural mechanisms that account for the perception of bone pain. This may lead to a better understanding of ways to treat bone pain.

A number of projects are being conducted by the [laboratory](#). The projects may be pursued as part of a PhD, Masters, BMedSc or Honours degree, and any interested parties should contact [Dr Jason Ivanusic](#) for further details.

Project 1: Changes in the neuro-chemical phenotype of primary afferent neurons that innervate bone in an animal model of inflammatory bone pain.

It is generally assumed that the primary afferent neurons that innervate bone have a similar neuro-chemical phenotype to primary afferent neurons that innervate cutaneous and muscular tissues. However, it is unclear whether the neurotransmitters and neuropeptides contained within bone-associated afferents are the same as in other fibre types, such as cutaneous, joint or muscle afferents. The aim of this project is to investigate the neuro-chemical phenotype of primary afferent neurons that innervate bone and to compare them to neurons that innervate skin, under normal conditions and in a model of experimentally induced inflammatory bone pain. Small volumes of neuro-anatomical tracers will be placed into a number of bony tissue locations (e.g. cortical, medullary and periosteal) or skin. After an appropriate survival time and subsequent processing, the distribution of labelled neurons in the peripheral sensory ganglia (DRG) will be determined using fluorescence microscopy, and the neuro-chemical phenotype of these neurons will be examined by using immuno-histochemical labelling of common neurotransmitters, receptors and neuropeptides. Changes in the neuro-chemical phenotype of these neurons will be examined in animals that have experimentally induced inflammatory bone pain, because such changes may reflect mechanisms that are (at least in part) responsible for chronic or persistent pain associated with inflammation. Students can expect to gain experience in animal handling, behavioural testing, anaesthesia and surgery, dissection, histological and immuno-histochemical processing, and fluorescence microscopy.

Project 2: Changes in neurotrophin signalling molecules in primary afferent neurons that innervate bone in an animal model of inflammatory bone pain.

Neurotrophins have a well documented role in the developing nervous system, have recently also been implicated in pain in the cutaneous and visceral systems, but are not well studied in models of bone pain. The aim of project 2 is to determine whether sensory neurons that innervate bone have the necessary receptors and proteins required for a role of neurotrophins in signalling bone pain, and to further determine if there is a change in neurotrophin receptor or protein levels subsequent to inflammation that could account for increased sensitivity to pain in an animal model of inflammatory bone disease. The same experimental approach described for project 1 will be used. Students can expect to gain experience in animal handling, behavioural testing, anaesthesia and surgery, dissection, histological and immuno-histochemical processing, and fluorescence microscopy.

Project 3: Investigation of the central nervous system areas associated with bone pain.

Whilst Projects 1 and 2 will provide valuable data regarding the first part of the pathways that relay sensory information from bone to the brain, it will not allow for investigation of the second and third order neurons of these pathways because the neuro-anatomical tracers used are incapable of moving across synapses. The aim of project 3 is to determine which parts of the CNS are involved in the relay of information about pain from bone to the brain. This will be achieved by examining the distribution of FOS expression in the CNS following stimulation of the rat tibia with noxious mechanical stimuli, and under the condition of experimentally induce inflammatory bone pain. FOS is a protein marker for neuronal activity and its distribution following activation of neuronal pathways can be examined following a standard immuno-histochemical staining procedure. We have results which show increased FOS expression in neurons of the dorsal horn of the spinal cord following noxious mechanical stimulation of bone, have combined this approach with retrograde tracing techniques to determine the target of these activated neurons, and plan to continue using these approaches to further define brain areas associated with noxious stimulation of bone. Co-localization of markers for common neurotransmitters and neuropeptides with the FOS protein may also be examined with the aim of identifying putative local neuronal circuits involved in the transmission of information about bone pain. Students can expect to gain experience in animal handling, behavioural testing, anaesthesia and surgery, dissection, histological and immunohistochemical processing and fluorescence microscopy.

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Project 1: Microglial invasion and migration into the embryonic brain

This project will attempt to elucidate the molecular mechanisms by which microglial precursors enter the embryonic central nervous system (CNS). This will involve 1) a spatiotemporal description i.e. when and where entry occurs; 2) a microscopic description e.g. what cell types are involved and their behaviour over the course of the process; 3) delineation of any temporal or other constraints on the events; and 4) investigating any candidate molecules that mediate the various aspects of the process. The primary methods used will be histology (including live cell tracking) and immunochemistry.

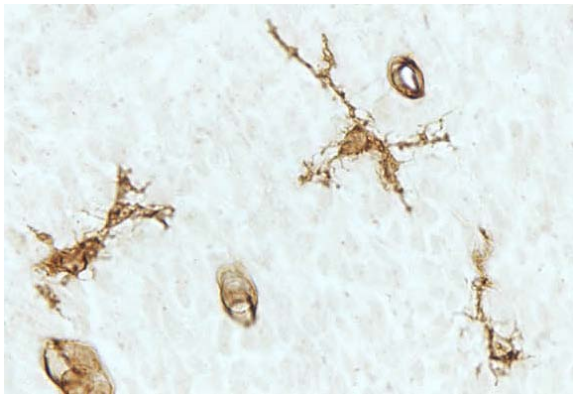


Figure 1. Three ramified microglial cells (and three capillaries) in the cerebral cortex of an Embryonic day 15 fetal mouse. These microglia are blood-derived cells that invade the central nervous system and take up permanent residence as it develops

Project 2: The formation of the cardiovascular control centres in brainstem

Dr Peter Kitchener and Assoc. Prof. Andrew Allen (Dept. Physiology)

The aim of this project is describe the development of the nuclei responsible for the regulation of vital functions, especially cardio-vascular regulation. We are interested in the emerging view that problems in cardiovascular regulation may have their origins in the early development of, and early environmental influences on, these brain regions. We wish to determine the periods of the key events: the birth, growth and differentiation of these neurons. With this knowledge, there can be a rational approach to manipulating the plasticity of these control systems, providing a better understanding cardiovascular regulation and also a promising avenue for understanding the pathogenesis of disorders such as essential hypertension.

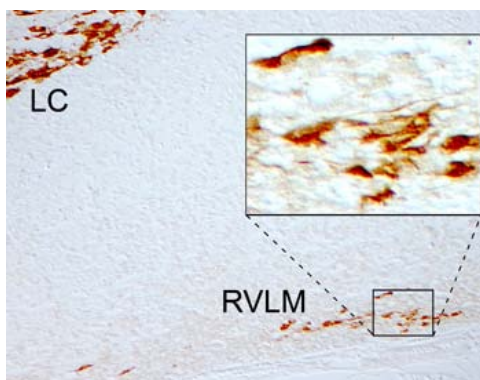


Figure 2. Tyrosine hydroxylase immuno-staining of neurons in the locus ceruleus (LC) and the cardiovascular-regulatory nucleus in the rostral ventral lateral medulla (RVLM) in an Embryonic day 18 mouse brainstem.

BEHAVIOURAL NEUROSCIENCE

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How do we think, feel, learn, remember and behave? We are beginning to investigate these questions on a number of different fronts. The research we are involved in is searching for fundamental mechanisms involved in learning and memory, emotions and emotional memory, and the basis of individual variation in peoples' behaviour.

Project 1: What circuits in the brain are involved in a particular behaviour?

The key way in which the brain works is as a complicated set of circuits, which link up various bits of information, combine them, process them and finally set the body to act. Some of the primary circuits in the brain, which are involved in a particular function, such as those involved in regulating movement, have been reasonably well characterized. However, for the brain functions, which underlie higher order behaviours, we have little idea of the brain circuits, which are involved. One of the reasons we know so little is because there have been no good ways of defining these circuits. We have generated transgenic animals, called fos-tau-lacZ mice, which will hopefully allow us to trace the circuits, which are used for a brain function. These mice have been designed to express a marker protein in the axons and dendrites of neurons, which have been activated by some functional stimulus. Thus, when the animal is doing something, the neurons, which are involved in that function will "light up", and so will their axonal projections throughout the brain.

Our current projects with these mice involve looking for circuits involved with learning and memory, processing of vision, and the processing of novelty.

Project 2: Plasticity in learning and memory.

What we hope to learn in our experiments above is which circuits are involved in the learning of a particular event. But to understand the learning process fully, we need to understand what changes occur in learning which result in the storage of that information in memory. Learning is thought to involve alterations in existing brain circuits. The key is the synapse. This is the connection between axon and dendrite, and it must be here that any changes in circuitry within the brain must be established. So far, there have been no direct demonstrations of synaptic change underlying a learning process. We are currently utilizing immunosorbent assays and confocal microscopy techniques to examine changes in the expression of synaptic proteins, in mice, following training on learning paradigms. We are using similar techniques to study the changes, which occur in the brain following exposures to novel environments. These studies are interesting because novel environments have powerful behavioural effects on animals, such as decreasing stress levels and improving their abilities in complex behavioural tasks.

Project 3: Genetics of Behaviour.

The final area of research involves inherent components of behaviour. There now seems no doubt that variation in types of behaviour, such as personality type, has a genetic contribution. Depending on the type of behaviour, the genetic component contributes from 40% to 70% of the variation. One such behavioural trait is associated with the way we cope with stressful events in our life. Our stress response is a natural and important part of our behaviour and contributes to our survival and well-being. However, a prolonged and heightened stress response can have very serious consequences such as long term anxiety states and panic attacks, depression, and susceptibility to cardiovascular disease and immune dysfunction. The way in which individuals respond to stress varies considerably and this variation has a genetic contribution of at about 50%. Clearly, it would be useful to understand which genes are involved in stress response, not only to gain a greater understanding of stress as behaviour, but also to understand how to modify our responses to stress. We are involved in a study, which will map the genes involved in stress response in an experimental animal model. The final aim of this study is to identify the genes which are involved in the stress response, and how presumed variations in these genes lead to different stress responses and different susceptibilities to the pathological conditions associated with stress.

Project 4: Methods to ameliorate cognitive decline and memory deficits in Alzheimer's disease.

This project is being conducted in collaboration with Assoc Prof Graham Barrett, Department of Pathology, University of Melbourne.

Alzheimer's disease (AD) is an extremely prevalent cause of dementia. One of the major consequences of AD is memory loss. It is characterized by death and degeneration of neurons, extracellular deposits of amyloid plaque, and intracellular fibrillary tangles. Cholinergic neurons in the basal forebrain comprise one of the earliest and most severely affected cell types in AD. Neurodegeneration of these neurons gives rise to a severe cholinergic deficit, which is believed to be instrumental in the impairment of memory and attention in AD. In the mature brain, the same cholinergic neurons are the only neurons that express a receptor for the neurotrophin growth factors, p75. In the cholinergic system, p75 signals the negative aspects of trophic regulation and thus acts as an inhibitor of cholinergic neuronal size, growth and activity. It can also cause apoptosis, particularly in response to injurious stimuli. We have previously shown that ablation of this p75 receptor has a remarkable effect on the cholinergic system in normal mice. Ablation of p75 results in cholinergic neuron growth, increases cholinergic innervation of the hippocampus, enhances hippocampal synaptic plasticity and improves spatial memory performance. Our hypothesis is that reduction and/or ablation of p75 in a mouse model of AD will alleviate the cognitive symptoms, primarily by its actions on the cholinergic system.

The project asks two closely-related questions; firstly, what is the role of p75 in the cholinergic neurodegeneration that occurs in AD? Secondly, does stimulation of the cholinergic system by removing or downregulating p75 produce an improvement in cognitive performance in AD? We have, in fact, previously shown that removing p75 in normal mice enhances the cholinergic system and memory performance in normal mice.

To help address these questions, we will conduct behavioural assays, including tests of spatial memory, in Alzheimer's mice with varying degrees of p75 downregulation. After these tests, the brains will be examined histologically and biochemically. We will assess the changes in the size, number and activity of cholinergic neurons. We will look for changes in cell death and amyloid deposition.

Neurotrophin Signalling Laboratory

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General Overview:

The focus of our laboratory centres on a family of growth factors known as the neurotrophins. We are interested in understanding the neurotrophins and how they signal, as well as the influence they exert on both peripheral and central nervous system myelination. We use a variety of molecular, cellular, biochemical and genetic techniques to investigate these events.

The laboratory is located on the second floor of the new Melbourne Brain Centre building.

Projects available:

Demyelinating diseases of both the peripheral and central nervous system have a devastating human impact. There is currently an incomplete understanding of the factors that initiate, promote and maintain the interactions between neurons and glial cells that are vital for myelination. Our laboratory is interested in increasing our understanding of some of the factors that regulate myelination, and dissect their primary mechanism of action.

Project 1: The influence of BDNF on CNS Myelination

We have recently identified that BDNF plays an important role in promoting central nervous system myelination. These data indicate that BDNF activates the receptor tyrosine kinase TrkB to promote oligodendrocyte myelination¹. We currently investigating the properties of a low molecular weight novel peptide designed to mimic the TrkB agonist properties of BDNF. In this project, we will undertake:

- (i) structural-based NMR studies to full characterise the structure of the TrkB agonist peptide, and investigate the key residues involved in binding to TrkB
- (ii) biochemical assays to investigate the capacity of the TrkB agonist peptide to activate TrkB and initiate key intracellular signalling cascades
- (iii) *in vitro* myelination assays to investigate whether the TrkB agonist can, just like BDNF, promote oligodendrocyte myelination.

This project will involve NMR spectroscopy, routine cell culture as well as the generation and co-culture of primary neurons and oligodendrocytes. Through analysis of these cultures by immunocytochemistry and Western blotting, we hope to identify whether our TrkB agonist can promote central nervous system myelination.

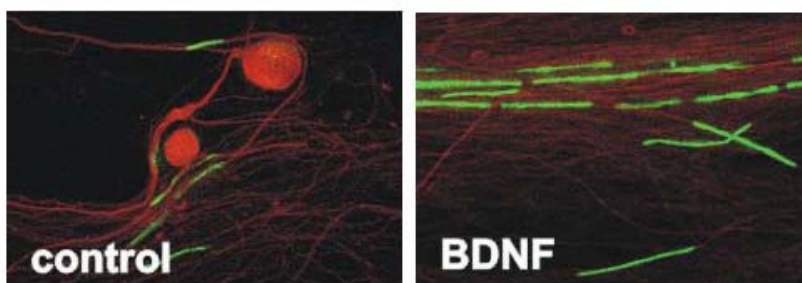


Figure 1. BDNF promotes Schwann cell myelination *in vitro*

DRG neurons and their axons are immunostained red, while myelinating Schwann cells are immunostained green. Untreated control cultures, left, myelinate at a low basal level. Following treatment with BDNF, right, an increase in the number of myelinated axonal segments is apparent in the cultures, indicating BDNF exerts a promyelinating effect *in vitro*.

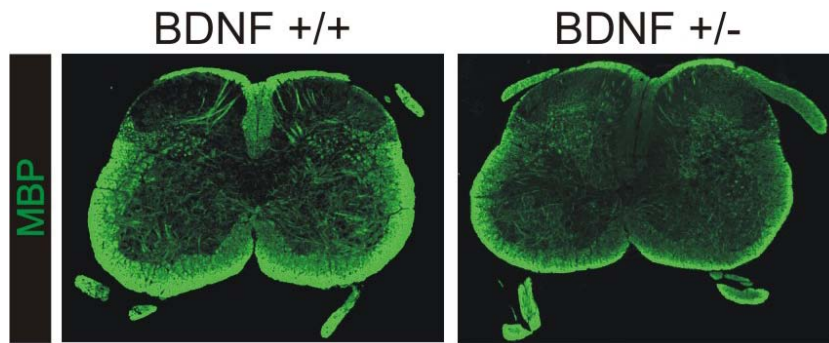


Figure 2. BDNF regulates myelination *in vivo*.

Transverse sections of spinal cord are immunostained with Myelin Basic Protein (MBP, green). Robust MBP staining is observed in white matter tracts in wild-type mice (BDNF+/+, left), whereas substantially less MBP is present in the spinal cord of BDNF heterozygous mice (BDNF+/-, right), indicating that BDNF has a dose dependent effect upon myelination *in vivo*.

Project 2: The role of *Fyn* signalling in CNS Myelination

Fyn is a non-receptor tyrosine kinase known to play a key role in central nervous system myelination, however the precise influence it exerts is unclear. We have clear evidence that BDNF can promote *Fyn* activation, and that blocking *Fyn* activation can also block the promyelinating effect of BDNF. These data suggest that *Fyn* activation is required in oligodendrocytes to promote myelination. In this project, we will undertake:

- (i) the molecular subcloning of mutant forms of *Fyn* in order to generate lentiviral particles, which will allow us to express these constructs in oligodendrocytes *in vitro*.
- (ii) assays to determine the influence that the expression of mutant forms of *Fyn* have on oligodendrocyte survival, proliferation and differentiation.
- (iii) *in vitro* myelination assays to investigate the influence that the mutant forms of *Fyn* have upon oligodendrocyte myelination, and also determine the impact they have on BDNF to regulate this process.

This project will involve molecular biology, routine cell culture as well as the generation and co-culture of primary neurons and oligodendrocytes. Through analysis of these cultures by immunocytochemistry and Western blotting, we hope to identify the precise role that *Fyn* exerts upon central nervous system myelination.

Project 3: The influence of BDNF on PNS Myelination

We have identified that BDNF can also promote peripheral nervous system myelination, but intriguingly our data identify that this is through mechanisms that are clearly distinct to its influences upon central nervous system myelination. We have undertaken computer aided structure-based design approaches to obtain a low molecular weight peptide based on the region of BDNF known to bind to the p75NTR receptor, the key receptor involved in peripheral nervous system myelination. In this project, we will undertake:

- (i) biochemical and pharmacological manipulation of this peptide, to generate modified analogues that are designed to increase bioavailability and retain efficacy in promoting myelination
- (ii) *in vitro* myelination assays to investigate the potency of the modified analogues in promoting Schwann cell myelination,

This project will involve pharmacological modifications to our BDNF peptide, routine cell culture as well as the generation and co-culture of primary neurons and Schwann cells. Through analysis of these cultures by immunocytochemistry and Western blotting, we hope to identify whether our BDNF can promote peripheral nervous system myelination.

Project 4: Mechanisms of growth factor signalling

Normal nervous system development requires highly specific and co-ordinated growth factor signalling. Precise control of signalling is dependent on a number of factors, such as presentation of the ligand, receptor structure and activation, the expression of co-receptors and adapter molecules, and the negative control or inhibition of signalling. One focus of our laboratory is to investigate growth factor signalling and how it is positively and negatively regulated. In particular, we focus on the neurotrophins Nerve Growth Factor (NGF) and Brain Derived Neurotrophic Factor (BDNF).

We are currently investigating aspects of neurotrophin signalling, including the form in which the neurotrophins are presented and a number of novel molecules that interact with neurotrophin receptors. We have several projects investigating how neurotrophin signalling is regulated by these molecules which include:

- (i) The precursor form of the neurotrophins, and their impact on cell signalling.**
- (ii) The Spred and Sprouty proteins, inhibitors of growth factor signalling.**
- (iii) The *Neurotrophin Receptor Homolog*, which modulates neurotrophin signalling.**
- (iv) The Sorting Nexin (SNX) proteins, which regulate the cellular location of receptors.**

Using *in vitro* approaches, the successful applicant will learn a variety of skills such as tissue culture techniques, cellular transfection, immunocytochemistry, SDS-PAGE and Western blotting, immunoprecipitation, PCR and cDNA mutagenesis.

These projects will identify some of the vitally important cellular events that regulate neurotrophin signalling and control the development and growth of the mammalian nervous system. Analyses such as these will provide new insights and develop into approaches that may be utilised to halt the progression of degenerative neurological diseases.

Physical Anthropology Laboratory

Dr. Varsha Pilbrow (Room E526, ph 8344 5775)

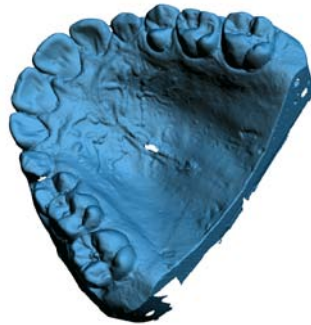
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Introduction

In the Physical Anthropology Lab we use dental and skeletal morphology to address questions relating to human biology, behaviour, health, diet and evolutionary relationships. Our particular focus is on understanding patterns of gene flow and evolutionary diversification in fossil humans and evolutionary relatives. We are also interested in reconstructing the biological profile, including physical characteristics, diet, behaviour and health of past human populations. Research is conducted on the osteological collections housed in the lab, ape dental material from museums around the world, and human skeletal material from archaeological sites.

We use a combination of traditional qualitative scores and caliper measurements, and more sophisticated 2-dimensional and 3-dimensional digital morphometric techniques. Our newly acquired 3D laser scanner allows us to scan entire jaws and apply state-of-the-art 3D geometric morphometric techniques to study dental data.



3D scan of a human maxilla

Current projects suitable for Honours, Masters and PhD students are:

Project 1: Population systematics of recent apes

Chimpanzees, gorillas, orang-utans and gibbons share a high proportion of genetic material with humans. Their evolutionary history and present day patterns of geographic distribution are of relevance for understanding the diversification of human ancestors. This project involves the use of dental specimens for studying the taxonomy of the apes. These provide the background for addressing questions regarding species designations and distribution patterns of fossil hominids.



Examples of ape and human teeth

Project 2: Discrete dental traits

Teeth are highly mineralized structures that preserve well in archaeological and paleontological contexts. Dental morphology thus plays an important role in understanding the systematics, evolutionary relationships, diet and behaviour of our ancestors. We have several projects that focus on non-metric, or discretely occurring, dental traits. Some involve the application of the Arizona State University Dental Anthropology System (ASUDAS), a dental trait scoring system for recent humans, while others require devising new dental trait scoring systems for recent apes.

Project 3: Reassessing fossil hominid taxonomy

Sample sizes for fossil hominids are typically small. To gain an understanding of patterns of variation and evolutionary diversification in our extinct ancestors we need to compare fossil hominid variation with variation in closely related evolutionary relatives. Using comparative reference samples as models we aim to reassess the taxonomy, or species break-down patterns, of fossil hominids. Two groups of hominids are of particular interest: Paranthropus from the Plio-Pleistocene of Africa, and Dryopithecus from the Miocene of Europe.



Miocene ape teeth from Kenya National Museum

Project 4: Paleopathology and paleoepidemiology

To document trends in prehistoric population health we are studying skeletal signatures for tuberculosis, osteoma, osteoarthritis, rickets and other such conditions in bones from the osteology lab and an archaeological site in the Republic of Georgia. This project run in collaboration with the Global History of Health Project (<http://global.sbs.ohio-state.edu/>) will document the history of human health from prehistoric to recent times.



Example of trepanation in Georgia

Project 5: Intentional cranial modification.

Artificial cranial deformation was practised in several parts of the world. We have several modified crania in our osteological collection and at the archaeological site in Georgia. We aim to use 3D geometric morphometric techniques to study the methods and comparative context of cranial shape modification in order to determine the genetic affiliation and behavioural practices of the people with modified crania.



Artificially modified cranium from Samtavro, Georgia

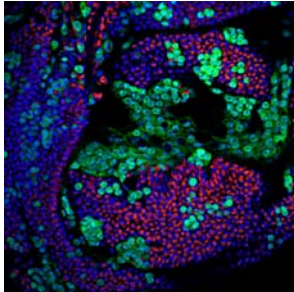
Animal Models for Cancer & Neurodegenerative Disease

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Our research involves developing models using *Drosophila* to understand the initiation and progression of human diseases, including cancer and neurodegeneration.

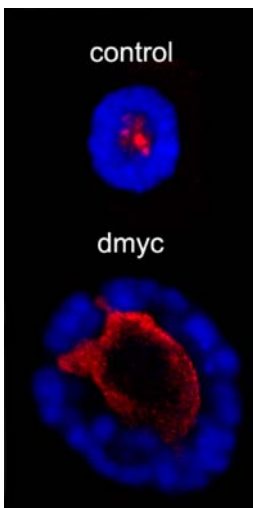
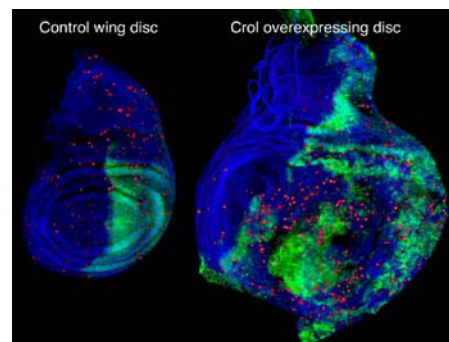


The cancer studies (Projects 1-5) focus on genes called oncogenes and tumour suppressor genes, which are required for tight regulation of the cell cycle.

For the neurodegenerative studies (Project 6) we have developed *Drosophila* models to investigate how proteins such as Huntington can aggregate in cells to disrupt neural functioning.

Project 1: Novel regulators of *Wg*, *dmyc* and cell cycle

Loss of cell cycle control is a fundamental first step in cancer initiation. Crooked legs (*Crol*) drives cell cycle progression and wing imaginal disc overgrowth (shown right), by leading to the upregulation of critical cell cycle genes. *Crol* is an important upstream regulator of the Wingless (*Wg*) signalling pathway. Future research is aimed towards determining how *Crol* regulates cell cycle progression via *wg* transcription.



*Project 2: To determine how *Hfp* regulates *dmyc*

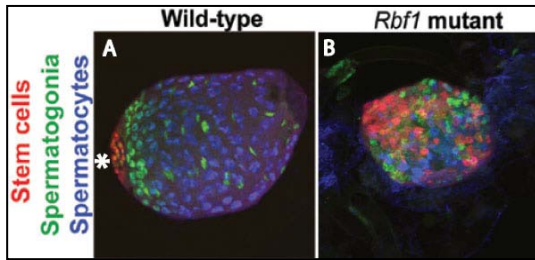
Myc drives cells to overgrow (see figure on the left) and as a consequence is a potent oncogene. Our work has shown that *Hfp* controls *dmyc* transcription and overgrowth via the DNA helicase, *Haywire*. Future research projects are aimed towards determining 1) how specific *Haywire* alleles abrogate *Hfp*-mediated *dmyc* repression and growth and 2) the signalling pathways upstream of *Hfp* and *Hay* that control *dmyc* transcription.

*Project 3: *Drosophila* models for ribosome biogenesis and growth

Up-regulation of ribosome biogenesis can lead to the uncontrolled proliferation and contribute directly to cancer. We aim to use *Drosophila* as a model to 1) determine how mitogenic signalling pathways including c-MYC modulate rRNA gene transcription and cell growth; 2) to determine the contribution of rRNA gene silencing to DNA recombination and genomic instability and 3) to investigate the mechanism by which ribosomal proteins can behave as tumour suppressor genes.

*These projects are a collaboration with Assoc. Prof. Ross Hannan at the Peter MacCallum Cancer Centre; <http://www.petermac.org/Research/GrowthControl>

**Project 4: The role of retinoblastoma in stem cell differentiation

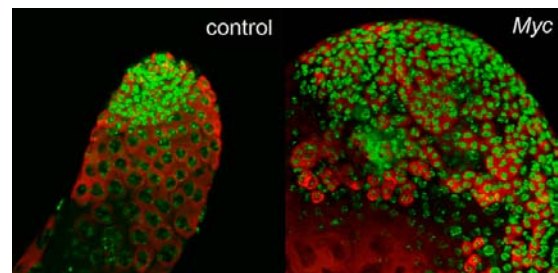


Stem cells are essential for tissue growth and maintenance throughout the lifetime of an organism. Conserved molecular signals from the local tissue environment are necessary for regulating stem cell divisions. Disruption to these signals can result in cancerous-like cell overgrowth. We have identified a novel pathway for the tumour suppressor protein *Retinoblastoma* in stem cell differentiation. This project will aim to examine the role of Retinoblastoma in

regulating stem cell development by identifying transcriptional targets as well as the molecular signals involved.

**Project 5: Myc regulation in stem cells

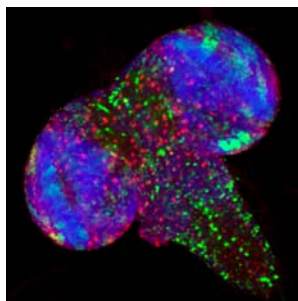
Recent studies on cancer stem cells (CSCs) have emphasized the importance of the interactions between stem cells and their microenvironment or "niche". We aim to use *Drosophila* stem cell models to identify the signals from the niche required to control levels of the Myc oncogene in the germline stem cells, which is important to prevent the formation of germline tumours (shown on the right).



**These projects are a collaboration with 1) Dr Greg Somers, La Trobe Institute for Molecular Science (LIMS); <http://www.latrobe.edu.au/genetics/staff/GSomers/index.html> and

2) Dr Gary Hime, Department Anatomy and Cell Biology, University of Melbourne <http://www.anatomy.unimelb.edu.au/researchlabs/hime/index.html>

***Project 6: *Drosophila* models for neurodegenerative disease



Proteins such as Huntington can misfold and aggregate in cells to disrupt normal cellular functioning, which is intimately associated with neurodegenerative disease. We have developed *Drosophila* models for Huntington's disease in order to determine how defects in protein conformation affect neural functioning. This project aims to determine the physiological mechanisms underpinning Huntington's disease *in vivo* using the *Drosophila* brain (shown on the left).

***This project is a collaboration with Dr Danny Hatters, Bio21 Institute, Melbourne University; <http://www.bio21.unimelb.edu.au/group-leaders/bio-chemistry/danny-hatters>

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The FZD7/Wnt Signalling Pathway in Hepatocellular Carcinoma

It is estimated that at least one-third (2 billion) of the world's population have been infected with hepatitis B virus (HBV), of which 400 million people have chronic disease (CHB), resulting in up to 1.2 million deaths annually due to liver cirrhosis and hepatocellular carcinoma (HCC). Recently it was shown that FZD7 plays important roles in HCC; however the effect of HBV infection in this context has not been examined. The Cancer Biology Lab has developed powerful tools for investigating FZD7 function in colorectal cancer. We now aim to utilise these tools to investigate the interplay between FZD7 signalling and HBV infection in HCC cells.

Specific questions to address:

1. Does modulating FZD7 signalling affect HBV infection in HCC cell lines?

We will determine the status of HBV infection in a human HCC cell line after FZD7/Wnt pathway stimulation or inhibition.

2. Does HBV infection alter FZD7/Wnt signalling in HCC cells?

We will determine the status of FZD7/Wnt signalling in a human HCC cell line after HBV infection.

3. What are the functional consequences of the interplay between FZD7/Wnt signalling and HBV infection in HCC cells?

To gain insight into functional effects, cell morphology and growth characteristics will be assessed.

This project will involve the analysis of fixed or inactivated material from the cell lines, for e.g. immunohistochemistry and confocal fluorescent microscopy on human cell lines, qRT-PCR, western blot, reporter assays. The project is suitable for an Honours project.

This project is in collaboration with Prof. Stephen Locarnini, VIDRL.

http://www.vidrl.org.au/locarnini/locarnini_professional.htm

Further reading:

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Frontiers in Bioscience 12, 4558-4567 (2007)

Vincan E, Darcy PK, Farrelly CA, Faux M, Brabletz T and RG Ramsay. Frizzled-7 dictates three-dimensional organization of colorectal cancer cell carcinoids.

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Clevers H. Wnt/beta-catenin signalling in development and disease.

Cell 127:469 (2006)

Kim M, Lee HC, Tsedensodnom O, Hartley R, Lim YS, Yu E, Merle P, Wands JR. Functional interaction between Wnt3 and Frizzled-7 leads to activation of the Wnt/beta-catenin signalling pathway in hepatocellular carcinoma cells.

J Hepatol. 48:780-91 (2008)

Autonomic Neuron Development Laboratory

Assoc Prof Heather Young (Room E524, ph 8344 0007)

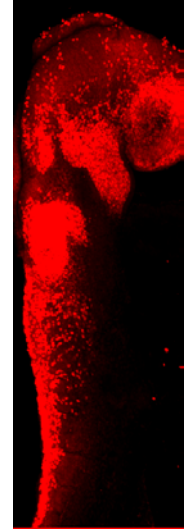
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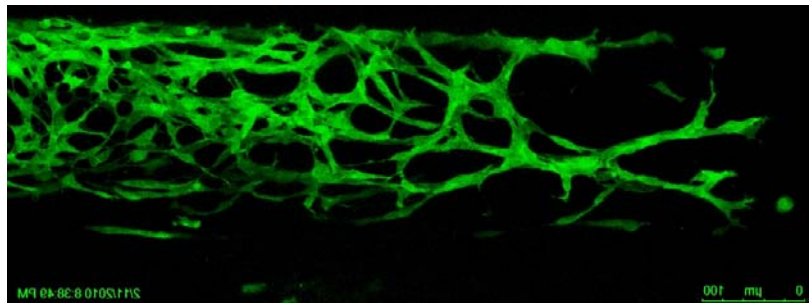
The peripheral autonomic nervous system arises from the neural crest. Our laboratory studies the mechanisms controlling the migration of neural crest cells to and within their target tissues, and the mechanisms controlling the differentiation of neural crest-derived cells. During the development of the enteric nervous system (ENS), neural precursors from the hindbrain must first migrate into and colonize the entire gastrointestinal tract (gut). This migration is very interesting because (a) it takes a long time (~3 weeks in humans and 4 days in mice), (b) the cells have to migrate very long distances, particularly those that colonize the colon and rectum, and (c) if cells fail to colonize the distal gut in humans, a disease called Hirschsprung's disease results which requires surgery.



Project 1: Live cell imaging of neural crest cell migration along the developing gut (Primary supervisor: Heather Young)

Our laboratory has devised methods for imaging the migration of neural crest cells along explants of embryonic mouse gut by using mice in which enteric neural crest cells express fluorescent proteins.

In this project you will use time-lapse imaging and a transgenic mouse in which neural crest cells within the embryonic gut express a novel fluorescent protein to identify (a) the rules that govern the migratory behaviour of neural crest cells within developing gut, and (b) the mechanisms by which endothelin-3 and Rho GTPases influence enteric neural crest cell migratory behaviour.

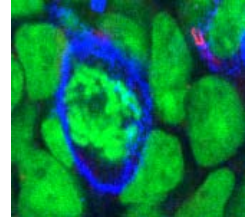


Project 2: Migration of melanoma cells in the neural crest environment (Primary supervisor: Sonja McKeown)

This research involves placing melanoma cancer cells into the neural crest environment of chick embryos. The neural crest are a population of cells that migrate extensively throughout the embryo and give rise to many different cell types including bones and cartilage of the face, neurons and glia of the peripheral nervous system and melanocytes, the pigment cells of the skin. When melanocytes become cancerous and form a melanoma they can become invasive and migrate extensively throughout the body. Why melanoma cells invade particular organs is currently unknown, but understanding of this process may ultimately lead to improvements in therapy of melanoma. Previous research has shown that melanoma cells follow the neural crest pathways when placed into chick embryos. This project would involve placing melanoma cells obtained from different metastatic sites into the chick neural crest pathway and determining differences in migration pattern from separate melanoma sub-populations. The project is a collaboration with Professor Jonathon Cebon at the Ludwig Institute for Cancer Research.

Project 3: Proliferation of enteric neuron precursors (Primary supervisor: Heather Young)

Enteric neuron precursors undergo massive proliferation in order to generate the mature enteric nervous system. In this project you will investigate the role of various cell cycle parameters in wild-type mice and a variety of mutant mice with defects in the enteric nervous system.



Project 4: Potential of cell therapy to treat Hirschsprung's disease (Primary supervisors: Heather Young and Lincon Stamp)

Infants born with Hirschsprung's disease lack enteric neurons in the distal bowel. In this project you will examine potential sources of cells to generate enteric neurons for use in cell therapy. Potential sources of enteric neurons will be tested in vivo using a mouse model of Hirschsprung's disease.

Further reading:

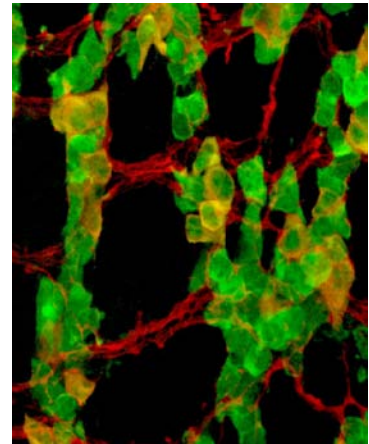
YOUNG, H.M., BERGNER, A.J., ANDERSON, R.B., ENOMOTO, H., MILBRANDT, J., NEWGREEN, D.F. and WHITINGTON P.M. (2004) Dynamics of neural crest-derived cell migration in the embryonic mouse gut. *Dev. Biol.* **270**: 455-473.

HAO, M., ANDERSON, R.B., KOBAYASHI, K., WHITINGTON, P.M. and **YOUNG, H.M.** (2009). The migratory behaviour of immature enteric neurons. *Dev. Neurobiol.* **69**:22-35.

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HOTTA, R., PEPDJONOVIC, L., ANDERSON, R.B., ZHANG, D., BERGNER, A.J., LEUNG, J., PEBAY, A., **YOUNG, H.M.**, NEWGREEN, D.F. and DOTTORI, M. (2009) Small molecule induction of neural crest-like stem cells from human embryonic stem cell-derived progenitor cells. (*Stem Cells*, in press, accepted 16.08.09)

MCKEOWN SJ, LEE VM, BRONNER-FRASER M, NEWGREEN DF, FARLIE PG (2005) Sox10 overexpression induces neural crest-like cells from all dorsoventral levels of the neural tube but inhibits differentiation. *Dev Dyn* 233:62-76.



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