DEPARTMENT OF ANATOMY & NEUROSCIENCE

2016 RESEARCH PROJECTS

(Honours, Masters and PhD)

Projects available in 2016

Department of Anatomy & Neuroscience

- Autonomic Neuron Development (Young Lab)
- Developmental models for Cancer (Quinn Lab)
- Digestive Physiology and Nutrition (Furness Lab)
- Kidney Development, Disease and Regeneration (Combes/Little Lab)
- Neural Development, Injury and Pain (Keast/Osborne Lab)
- Neural Regeneration (Turnley Lab)
- Neuron Development and Plasticity (Gunnersen Lab)
- Neurotrophin and Myelin Laboratory (Murray/Xiao Lab)
- Physical Anthropology (Pilbrow Lab)
- Sensory Neuroscience (Brock Lab)
- Sex Determination and Disorders of Sex Development (Wilhelm Lab)
- Stem Cells Genetics (Hime Lab)
- Visual Neuroscience Laboratory (Fletcher Lab)

Florey Institute of Neuroscience & Mental Health

- Arousal and Cognition Neuroscience (Gundlach Lab)
- Autonomic and Sensory Neuroscience (McDougall Lab)
- Behavioural Neuroscience (Nithianantharajah Lab)
- CNS Plasticity & Activity-dependent myelination (Staal Lab)
- Developmental Psychobiology @ the Florey (Kim Lab)
- Neural Plasticity @ the Florey (Hannan Lab)
- Neurobiology at the Florey (Howitt Lab)
- Neuroimmunology and Remyelination Lab (Kilpatrick Lab)
- Oligodendrocyte Function & Neuron-Glial Cell Interactions (Merson Lab)
- Presynaptic Dysfunction in Parkinson's Disease (Gordon Lab)
- Renal Denervation in Chronic Kidney Injury (Booth Lab)
- Systems Neurophysiology (Yao Lab)

School of Engineering

- Biomedical Engineering (Dr David Ackland)

Be sure to attend these sessions where you can meet with potential supervisors and discuss projects of interest

DEPT OF ANATOMY & NEUROSCIENCE

HONOURS INFORMATION SESSION

Be sure to attend this session where you can meet with potential supervisors and discuss projects of interest

FRIDAY, 18TH SEPTEMBER

12.00 – 1.30 pm HBA Museum, Level 3,

Medical Building

FLOREY INSTITUTE OF NEUROSCIENCE & MENTAL HEALTH

HONOURS/PhD INFORMATION SESSION

MONDAY, 7th SEPTEMBER

12.00-2.00 pm Kenneth Myer Building, Level 5

Research in the Department of Anatomy & Neuroscience

There are many opportunities for students to undertake research in the Department of Anatomy & Neuroscience that is based in the Faculty of Medicine, Dentistry & Health Sciences (MDHS). An Honours year is open to students who have completed an undergraduate degree, while students with a BSc (Hons) or BBiomed (Hons) can train for a career in science by undertaking a three to four year PhD or the shorter MPhil.

You can also contact the Departmental Honours and RHD Coordinator, Dr Peter Kitchener on 8344 6746 p.kitchener@unimelb.edu.au

What is an Honours Degree?

A BSc (Hons) or BBiomed (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. Your research project is included in the 75 point subject "Anatomy and Neuroscience Research Project". 80% of this subject is assessed by a 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 Neuroscience”.

Entry Requirements

You must have completed a suitable degree (BSc, BBiomed or equivalent) and achieved a Faculty Honours score of 65 or equivalent.

Thinking of applying?

- identify a number of potential projects and supervisors in this department or other MDHS departments which interest you (*there may be more applicants than places available for some projects or supervisors)
- lodge an application via your student portal with the Faculty of Science at http://www.sc.mdhs.unimelb.edu.au/how-apply
- lodge your project ***preferences*** with MDHS through the Honours Application and Tracking System (HATS).

What is a Master of Biomedical Science?

The Master of Biomedical Science program is an alternative to the Honours-to-PhD pathway. It is similar to Honours but is a 2 year course and therefore offers a greater number and range of coursework subjects and a more substantial research project. It comprises a research project of 125 points and 75 points of coursework. In

addition to discipline-specific coursework subjects, Master of Biomedical Science students take professional business and communication programs to complement and enhance their research capabilities and opportunities.

Entry requirements

You must have completed a suitable degree (BSc, BBiomed or equivalent) and achieved a Faculty Honours score of 65 or equivalent. You must also have the agreement of an academic staff member in the Department of Anatomy & Neuroscience to supervise your project.

Thinking of applying?

Detailed instructions on the MDHS website
http://www.medrmhwh.unimelb.edu.au/Courses/MSci.htmly

What is a PhD?

A PhD (Doctor of Philosophy) is 3 to 4 years full time supervised research. There are no exams or coursework. The research is written up as a thesis and assessed by two experts in the field.

Entry requirements

You must have a BSc(Hons), BBiomed(Hons) or equivalent. PhD scholarships are available to support you while you study. These are competitive and awarded based on your academic record.

What is an MPhil?

The MPhil (Master of Philosophy) is very similar to a PhD but carried out over a shorter period ~2 years. The research work is written up as a thesis which demonstrates your knowledge and contribution to the field of research. Entry requirements are the same as for a PhD.

Thinking of applying for either a PhD or MPhil?

- identify projects of interest and contact a potential supervisor who will assist you with the enrolment process

For further information re Research
Research Higher Degrees
http://gradresearch.unimelb.edu.au/handbooks/phd.intro.html
Department of Anatomy & Neuroscience
http://medicine.unimelb.edu.au/anatomy-neuroscience/research
Florey Department of Neuroscience & Mental Health
http://www.florey.edu.au/research/

Autonomic Neuron Development

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Dr Sonja McKeown (Rm E525, 8344 5783)
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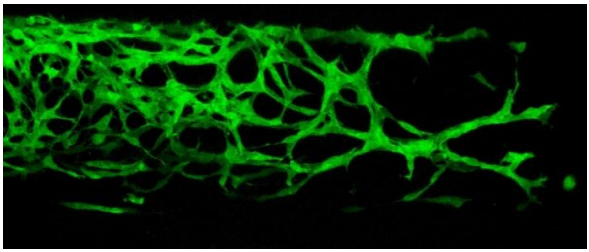
Dr Lincon Stamp (Rm E525, 8344 5783)
Email: lstamp@unimelb.edu.au

Web: http://medicine.unimelb.edu.au/anatomy-neuroscience/research/researchers/young_lab

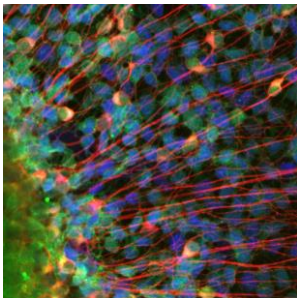
The peripheral autonomic nervous system arises from the neural crest. Our laboratory studies the mechanisms controlling the migration of neural crest cells to and within their target tissues, and the mechanisms controlling the differentiation of neural crest-derived cells. During the development of the 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 supervisors: Heather Young and Sonja McKeown)



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. We have recently shown that neural crest cells that "seed" a region (that is stop advancing) do not cease migration – they migrate circumferentially rather than directionally (Young et al., 2014). In this project you will use live cell imaging to determine why some neural crest cells keep migrating down the gut, whereas others settle down and remain to colonize each region.

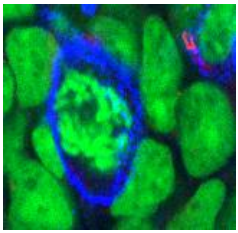
Project 2: Potential of stem cell therapy to treat Hirschsprung's disease
(Primary supervisors: Lincon Stamp, Sonja McKeown and Heather Young)



Infants born with Hirschsprung's disease lack enteric neurons in the distal bowel. Using a mouse model, we have recently shown that following transplantation, neural stem/progenitor cells migrate, proliferate and give rise to functional neurons (Hotta et al., 2013). In this project you will determine the optimal methods for culturing enteric neural progenitors and for transplanting neurospheres for the best proliferation, migration and differentiation.

Project 3: Proliferation of enteric neuron precursors
(Primary supervisors: Heather Young and Lincon Stamp)

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. You will examine cell cycle length and S phase length of enteric neuron precursors at (i) different ages, (ii) different distances from the migratory wavefront, and (iii) in mutant mice that have perturbed proliferation of enteric neuron progenitors.

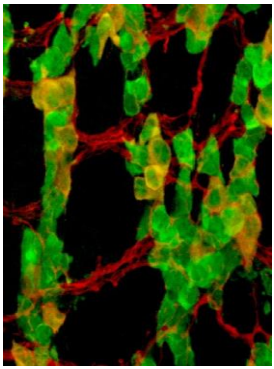


Further reading:

OBERMAYR, F., HOTTA, R., ENOMOTO, H. and **YOUNG, H.M**. (2013). Development and developmental disorders of the enteric nervous system. *Nature Rev Gastro Hepatol* 10:43-57

HOTTA, R., **STAMP, L.A**., FOONG, J.P.P., BERGNER, A.J., MCCONNELL, S.N., ANDERSON, R.B., ENOMOTO, H., NEWGREEN, D.F., OBERMAYR, F., FURNESS, J.B. and **YOUNG, H.M.** (2013**).** Transplanted progenitors generate functional enteric neurons in the postnatal colon. *J Clin Invest.* 123: 1182-91

McKEOWN, S.J., STAMP, L., HAO M.M. and **YOUNG, H.M.** (2013) Hirschsprung disease: a developmental disorder of the enteric nervous system. *WIREs Dev. Biol.* 2:113-29. doi: 10.1002/wdev.57



YOUNG, H.M., BERGNER, A.J., SIMPSON, M.J., **MCKEOWN, S.J**., HAO, M.M., ANDERSON, C.R. and ENOMOTO, H. (2014). Colonizing while migrating: How do individual enteric neural crest cell behave? *BMC Biol* 12:23.

Developmental Models for Cancer

Dr Leonie Quinn (Rm E632, 8344 5790)
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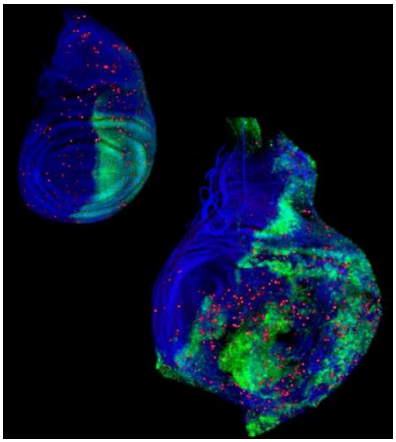
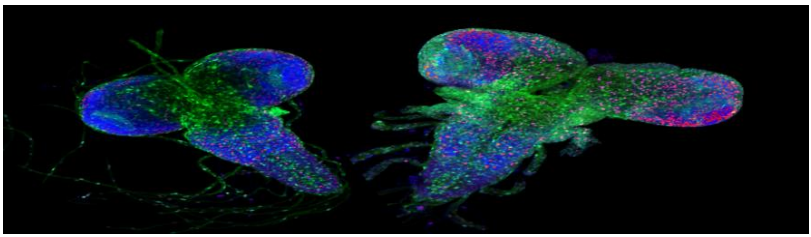
Project 1 - Transcriptional control of the MYC oncogene



The transcription factor and cell growth regulator MYC is upregulated in 70% of all human cancers. As therapeutically targeting MYC itself has proved unfeasible, we need to find new ways to indirectly target MYC in cancer. Most MYC-driven cancer is due to upregulation of expression, but the networks controlling *MYC* transcription in malignancy are largely unknown. The single stranded DNA binding protein FBP is essential for transcription of the *MYC* oncogene, and dysregulation of *FBP* is linked with a wide variety of cancers, eg. kidney, breast, liver, lung, bladder, prostate, gastrointestinal and brain. This research aims to use a combination of *in vivo* genetic models (*Drosophila* and mouse) and human cancer models to unravel the mechanisms for regulation of MYC expression by FBP.

Project 2 – Brain tumour models

With no effective drug treatments for malignant glioma these tumours are invariably lethal. One key discovery in glioma biology is that the EGFR/RAS/PI3K axis is activated in most gliomas. Indeed, preclinical trials are underway for therapeutics targeting PI3K/AKT and RAS/RAF in malignant glioma. Unfortunately, these studies have already revealed rapid acquisition of tumour resistance, which highlights the importance of understanding the activity of downstream targets. Elevated FBP and MYC correlate with poor patient survival, which suggests FBP and MYC



abundance/activity might also be drivers of glioma malignancy. This project builds on our exciting observation that FBP is a critical downstream target of EGFR/RAS/PI3K. We aim to use *Drosophila* (left), mouse and human glioma models to determine how the FBP-MYC axis drives MYC expression and brain tumour growth. Given the capacity of FBP knockdown to extinguish RAS-activated *MYC* expression, we propose that FBP is an attractive future target for developing novel cancer therapies.

Digestive Physiology and Nutrition

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Postdoctoral Fellows

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Projects
Concern the ways in which nutrients affect digestive function, the roles of gastrointestinal hormones and nerves, effects of poor diet and pathologies of the digestive system in animal models and in human. One project is on the anti-inflammatory effects of nerve stimulation in the intestine.

Technologies
Include: high resolution confocal microscopy; super resolution microscopy; molecular biology; *in vivo* physiology; physiology of isolated organs; receptor signalling and trafficking; immunohistochemistry; quantitative PCR and electrophysiology *in vivo*.

Nutrition: The control of gut hormone release by nutrients
Program Leaders: Dr Brid Callaghan, Mitchell Ringuet, Prof John Furness.
Contact: Dr Callaghan: b.callaghan@unimelb.edu.au

Control of gut hormones release is important to diabetes, weight control and digestive health. Additives from natural sources (micronutrients) are included in feed to accelerate animal rates of maturation and to increase muscle mass. We have made the discovery that hormones that are in the same cells are in separate storage vesicles. Our observation raises the strong possibility that the release of these, and other GI hormones that are colocalised in EEC cells, could be differentially controlled. In this project you will work with a team to investigate simultaneously the separate subcellular locations and the mobilisation of vesicles that contain 2 or more hormones in mouse enteroendocrine cells. Naturally-occurring and synthetic stimulants of nutrient receptors on enteroendocrine cells will be used to promote vesicle translocation and release.

References: Furness, JB, Rivera, LR, Cho, H-J, Bravo, DM, Callaghan, B: The gut as a sensory organ. *Nature Rev. Gastroenterol. Hepatol* **10**, 729-740 (2013).
Cho H-J, Robinson ES, Rivera LR, McMillan PJ, Testro A, Nikfarjam M, Bravo DM & Furness JB. Glucagon-like peptide 1 and peptide YY are in separate storage organelles in enteroendocrine cells. *Cell Tissue Res* **357,** 63-69 (2014).

Modulation of intestinal inflammation through nerve stimulation
Program Leaders**:** Prof John Furness and Dr Brid Callaghan, including collaboration with Prof Robin McAllen, Florey Institute.
Contact: Prof John Furness: j.furness@unimelb.edu.au

The innate immune system is the first response to invading pathogens. When challenged, the host needs an adequate inflammatory reaction but also needs to prevent collateral damage to tissues due to excessive systemic spread of inflammation and release of inflammatory mediators. Excessive inflammation underlies the pathogenesis of a range of disease syndromes, including sepsis, rheumatoid arthritis, inflammatory bowel disease and other inflammatory and autoimmune disorders. In this project you will investigate the endogenous mechanisms that prevent or neutralize excessive proinflammatory responses. This study aims to find novel therapeutic options for diseases associated with an excessive or chronic inflammatory states. The project will include establishing animal models of gut inflammation, *in vivo* nerve recordings and histology.

Reading: Sun P, Zhou K, Wang S, Li P, Chen S, Lin G, Zhao Y & Wang T. Involvement of MAPK/NF-κB signaling in the activation of the cholinergic anti-inflammatory pathway in experimental colitis by chronic vagus nerve stimulation. *PLoS One* **8,** e69424 (2013).

Novel drugs and receptors for targeting digestive function
Program Leaders: Dr Ruslan Pustovit, Dr Brid Callaghan, Prof John Furness.
Contact: Dr Ruslan Pustovit: Ruslan.pustovit@unimelb.edu.au &
Dr Callaghan: b.callaghan@unimelb.edu.au

One of the major problems of digestive function is failure of propulsive activity. This arises from a variety of neuro-muscular dysfunctions. The most common result is constipation that afflicts more than 10% of the population, many older Australians and most of those with spinal cord injury. We have discovered a new class of drug that can potentially be used to treat these conditions, and we have a clinical drug trial in progress. We are interested in further drug development. This project will provide you with the opportunity to conduct *in vivo* experiments and to learn much about whole animal physiology. You will work with a team of researchers to investigate the effectiveness and mechanisms of action of novel pharmacological tools.

Reference: Ferens DM, Habgood, MD, Saunders, NR, Tan, YH, Brown, DJ, Brock, JA, Furness, JB: Stimulation of defecation in spinal cord injured rats by a centrally acting ghrelin receptor agonist. *Spinal Cor*d, 49, 1036-1041(2011).

Pustovit RV, Furness JB & Rivera LR. A ghrelin receptor agonist is an effective colokinetic in rats with diet-induced constipation. *Neurogastroenterol Motil* 27, 610-617. (2015).

Gut Health: an intersection of food, environment, animal production and global health.

Program Leaders: Dr Brid Callaghan, Mitchell Ringuet, Prof John Furness, including collaboration with Dr Jeremy Cottrell, Agricultural Science.
Contact: Dr Callaghan: b.callaghan@unimelb.edu.au

Gut health can be defined as a condition in which mucosal function is normal and assimilation of nutrients is not compromised. When the gut is stressed, there are major effects on the mucosa. This is manifest as loss of structural integrity, increased mucosal permeability, malabsorption of nutrients, and mucosal inflammation. In addition to the mucosa, enteric neurons are susceptible to damage when there is stress, particularly oxidative stress, to the intestine (Pontell et al. 2009). We have developed a number of models for studying enteric neuropathies associated with inflammation (Nurgali et al. 2007; Pontell et al. 2009), ischemia/ reperfusion injury (Rivera et al. 2011b) and high fat diets (Rivera et al. 2014) and environmental heat (Maseko et al 2014). In study we will use ischemia/ reperfusion (I/R) injury as an acute stress to enteric neurons or a high fat diet regime, which has the hallmarks of accelerated ageing, as a more generalisable, chronic enteric neuronal stress as a screen, to test for drugs that might be used as neuroprotectors when oxidative stress is a factor, for example in intestinal transplant surgery

References: Nurgali K, Nguyen TV, Matsuyama H, Thacker M, Robbins HL & Furness JB. Phenotypic changes of morphologically identified guinea-pig myenteric neurons following intestinal inflammation. *J Physiol (Lond)* 583, 593-609. (2007)

Pontell L, Castelucci P, Bagyanszki M, Jovic T, Thacker M, Nurgali K, Bron R & Furness JB. Structural changes in the epithelium of the small intestine and immune cell infiltration of enteric ganglia following acute mucosal damage and local inflammation. *Virchows Arch* 455, 55-65. (2009).

Rivera LR, Leung C, Pustovit RV, Hunne B, Andrikopoulos S, Herath C, Testro A, Angus PW & Furness JB. Damage to enteric neurons occurs in mice that develop fatty liver disease but not diabetes in response to a high-fat diet. *Neurogastroenterol Motil* **26,** 1188-1199 (2014).

Rivera LR, Poole DP, Thacker M & Furness JB. The involvement of nitric oxide synthase neurons in enteric neuropathies. *Neurogastroenterol Motil* **23,** 980-988 (2011).

Maseko T, Dunshea FR, Howell K, Cho H-J, Rivera LR, Furness JB & Ng K. Selenium-enriched *Agaricus bisporus* mushroom protects against increases in gut permeability *ex vivo* and up-regulates glutathione peroxidase 1 and 2 in hyperthermal-induced oxidative stress in rats. *Nutrients* **6,** 2478-2492 (2014).

Kidney Development, Disease and Regeneration

Dr Alexander Combes
Dept of A&N and Murdoch Childrens Research Institute, (9936 6228)
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Professor Melissa Little
Dept of Pediatrics and Murdoch Childrens Research Institute, (9936 6206)
Email: Melissa.little@mcri.edu.au

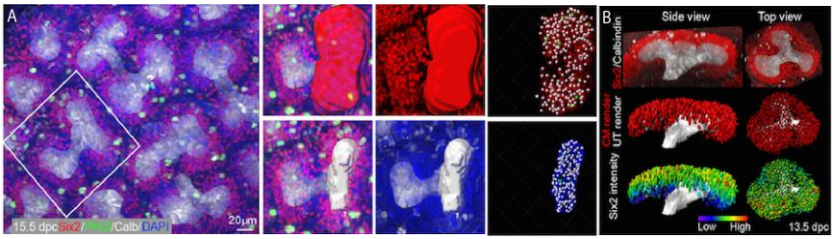
Web: https://www.mcri.edu.au/users/alexander-combes
https://www.mcri.edu.au/users/melissa-little

Regulation of organ size and stem cell hierarchy in the developing kidney

Transient stem/progenitor cell populations play essential roles in organ development and repair. The balance between self-renewal and differentiation in the nephron progenitor population plays a major, but poorly understood, role in regulating mammalian kidney development. Factors produced by undifferentiated progenitors promote organ expansion, whereas differentiation of these cells builds functional capacity. What is not clear is how the balance between self-renewal and differentiation is regulated in these cells, nor how the control of this fate decision impacts on optimal organ development. Our efforts focus on dissecting the molecular identity, regulation, dynamics, and influence of this stem cell population on kidney development. In addition, we seek to leverage our knowledge of kidney development to devise novel strategies for treating kidney disease through prevention, repair, and regeneration.

Project 1- Analyzing heterogeneity in the nephron progenitor population

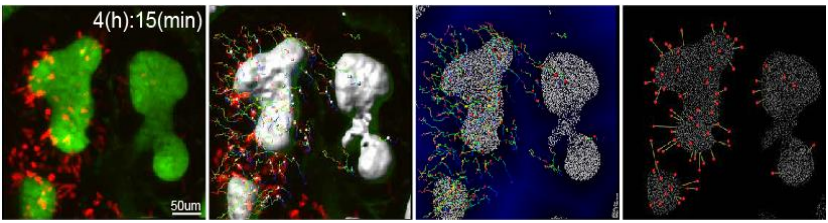
We have profiled kidney development across developmental time in unprecedented detail, using confocal microscopy and optical projection tomography to establish new standards for phenotypic analysis in the field (Short and Combes et al., Dev Cell 2014). As part of this study, we discovered a novel heterogeneity within the nephron progenitor population based on cell-cycle length. These fast and slow cycling nephron progenitor populations likely represent different states of differentiation. Understanding the molecular differences between these populations will build our knowledge of stem cell biology in this system and aid in our refinement of differentiation protocols (Takasato et al., Nat Cell Biol 2014).



Quantitative analysis of cell number (A) and protein distribution (B) in the nephrogenic niche

Projects will involve characterizing the spatial organization of the nephron progenitor niche using immunofluorescence and advanced 3D imaging. Known and novel markers that represent undifferentiated and differentiated states will be assessed by confocal and superresolution microscopy to construct a formal model of nephron progenitor differentiation and cell-cell signaling within the nephrogenic niche. Work performed in this study will contribute to a broader effort to quantify and mathematically model the molecular mechanisms underlying kidney development.

Project 2 – Assessing cell migration in the nephron progenitor population
The collecting duct system of the developing kidney grows through a dynamic program of branching morphogenesis driven by reciprocal interactions between the epithelial tips of the ureteric tree and surrounding 'cap' domains of mesenchymal nephron progenitor cells. The dynamics of ureteric tree branching are broadly appreciated. However, questions concerning how the cap mesenchyme is maintained at the tips of the tree during branching, and whether differentiation and interaction with stromal and tip-produced signals involves cell movement are unclear. Using live imaging of fluorescent reporters to label the cap mesenchyme and underlying tip compartment, we observed extensive cell motility in the cap mesenchyme. This is a novel finding which fundamentally changes the way we think of signaling and differentiation within the nephrogenic niche.



Quantifying cell migration in the nephrogenic niche: Left) Multiple transgenic mouse lines are used to label a subset of the nephron progenitor population (red)

and the underlying ureteric epithelium (green). **Remaining panels**) Cell and tissue movements are imaged by confocal microscopy, tracked in 3D, quantified and analysed through computational and statistical methods.

Projects will involve live imaging of genetically modified embryonic mouse kidney explants to further characterize cell migration in the nephrogenic niche and to analyse signaling pathways and cellular processes that have an impact on cell migration in this system. Organ culture methods will be optimized as part of this project to enable multiplex imaging and to determine the feasibility of 3D live imaging on light sheet microscopy systems. The results and methods will form the basis of longer term studies to determine the genetic drivers and functional significance of cell migration in the nephrogenic niche.

Project 3- Investigating the role of Snail proteins in the nephron progenitor niche
Snail proteins are critical regulators of epithelial to mesenchymal transitions during development and in cancer. Recent single cell sequencing data from the developing kidney has identified Snail 1 as being upregulated in the transition from an uninduced to an induced state in the nephron progenitor population (Brunskill et al., Development 2014) and Snail 2 was one of 8 factors used to reprogram HEK cells to a nephron progenitor-like state (Hendry et al., JASN 2013). Nephron progenitors undergo a mesenchyme to epithelial transition in their differentiation into an early nephron and given the involvement of Snail proteins in EMT/MET it seems likely that Snail 1 (and perhaps 2) are involved in this system. We propose that Snail 1 serves to restrict the differentiation of nephron progenitors by prepressing epithelialization in induced nephron progenitor cells.

Projects will include the conditional deletion and overexpression of snail proteins in the nephron progenitor population and assessment of the molecular and morphological consequences to kidney development using advanced imaging and genome-wide transcriptional profiling.

Neural Development, Injury and Pain Laboratory

Laboratory Heads:
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Dr Peregrine Osborne (Room E720, 9035 9716)
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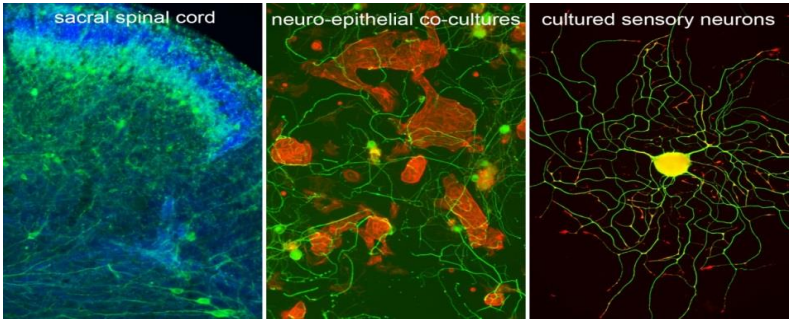
Postdoctoral fellows:
Dr Sophie Payne, Dr Casey Smith-Anttila, Dr Agnes Wong

Research Support Officers:
Ms Victoria Morrison, Ms Elsy Richardson

Web: http://medicine.unimelb.edu.au/anatomy-neuroscience/research/researchers/keast_lab

We utilise rodent models to investigate the development of spinal and peripheral nerve circuits, the neurobiological mechanisms underlying visceral pain and the impact of injury on visceral nerves (a common consequence of surgery to remove cancers of pelvic organs). We are particularly intrigued by the sacral component of the autonomic nervous system that not only has a strong level of sexual dimorphism but is also a site where visceral pain and autonomic pathways converge. This convergence is very relevant to a particularly challenging range of clinical problems (e.g., interstitial cystitis, endometriosis). We are especially interested in how the mechanisms that control the development of neuronal circuits could be re-activated in the adult to restore normal function. Techniques performed in our group include neuronal cultures, growth assays, immunofluorescence, 3D-imaging and reconstruction, tract-tracing *in vitro* and *in vivo,* micro-dissection of mouse embryos, use of reporter mice, FACs and RNA profiling. Our research based at the University of Melbourne is strongly collaborative with researchers in the US and Europe. Our work is supported by the NHMRC and the US National Institutes of Health.



Project 1: Development of autonomic and nociceptive nerve circuits

Urogenital function is regulated by autonomic neurons in the pelvic ganglia (known as the inferior hypogastric plexus in people) and sensory neurons in lumbosacral dorsal root ganglia. In comparison to other parts of the autonomic nervous system, the pelvic ganglia are very unusual. For example, they are very different in males and females, and they continue to be very sensitive to actions of steroids, even in adults. Most unusually, they are mixed sympathetic-parasympathetic ganglia, leading to questions of how these ganglia develop, and how their connections with two different regions of the spinal cord (lumbar and sacral) are determined correctly when they first form. Very little is known about how this part of the autonomic nervous system develops, but it is critical to understanding developmental abnormalities and may also point to mechanisms that can be activated in adults to repair axons after injury. Other projects are available to investigate the unique features of developing sacral nociceptive neurons that are later involved in pelvic pain conditions.

Project 2: Understanding neuro-epithelial communication using a novel co-culture system

The urothelium lining the bladder has two major functions: (i) a barrier, to protect the bladder tissues and their nerve supply from substances within urine, and (ii) as sensory transducer that responds to chemical and mechanical stimulation by releasing substances to activate sensory axons in the bladder wall. Whereas the barrier function is well accepted, the transducer properties have been inferred by strong, but indirect evidence. We have developed a neuro-urothelial co-culture system so can now directly study intercellular communication between urothelial cells and sensory neurons. This is relevant to understanding how the urothelium signals to nearby sensory nerve terminals and, conversely, how nerves may regulate epithelial growth and repair (relevant to conditions such as cystitis when the urothelium is damaged and, conversely, bladder cancer, when growth is dysregulated. One project will focus on the signalling from the urothelium to the sensory nerves, using compartmentalised cultures in microfluidic chambers, immunofluorescence and live-cell imaging. Another project will instead examine growth-promoting signals from the neurons that can impact on urothelial cell proliferation *in vit*ro and their ability to drive repair *in vivo*.

Project 3: Improving the recovery of injured visceral nerves

Visceral sensory and motor (autonomic) nerves that control urogenital function are often damaged by common surgeries such as prostatectomy, hysterectomy, bowel resection and other procedures performed on related organs. As this damage is a cause of long-term or permanent visceral and sexual dysfunction, we are studying how to promote functional recovery by stimulating and directing growth of the injured neurons. To do this, we have established robust microsurgical models that

can be used to study relevant types of neuronal injury. These are being utilized for *in vitro* studies of neurotrophic and steroid signalling pathways in isolated adult autonomic and sensory neurons maintained in culture; anatomical tracing and confocal microscopy studies to analyse the recovery of connections after injury; and *in vivo* studies to stimulate nerve growth using various physiological and pharmacological strategies. Another project in this area will investigate the source of trophic factors within the pelvic organs and their sensory nerve supply, and the impact of injury on trophic factor activity.

Project 4: Neuropharmacology of the GDNF family of neurotrophic factors in peripheral nerve regeneration and pain.

Nerve growth factor (NGF) is an endogenous neurotrophin that is released by tissues and targets peripheral sensory neurons to cause peripheral sensitization and pain. Strategies targeting NGF signalling are now being tested in clinical trials for the treatment of chronic pain. We, and others have found that a majority of sensory neurons also express receptors targeted by another class of neurotrophic factor, the GDNF-family ligands (GFLs). This has led to us to study the functions of GFL signalling in normal and damaged peripheral sensory neurons. In this project you will contribute to research performed in collaboration with Professor Mart Saarma (Institute of Biotechnology, University of Helsinki), using cultures of adult sensory neurons, growth assays and immunofluorescence.

Neural Regeneration

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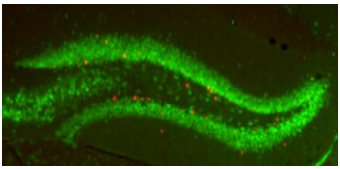
Traumatic Brain Injury and Regeneration
Supervisors: Ann Turnley and Nicole Bye plus additional co-supervisors as specified.

One of the reasons why injury or disease of the brain or spinal cord results in permanent disability is because nerve cells (neurons) die and those that remain can rarely reform the correct wiring of the nervous system to restore function. Effective neural repair is likely to require a multi-factorial approach, including blockage of neuronal death, replacement of lost neurons and oligodendrocytes by differentiation of neural stem/progenitor cells and regulation of appropriate subsequent neurite outgrowth, formation of correct synaptic connections and remyelination. These are also likely to be different depending upon the age when the damage occurs. Many neurodevelopmental processes are still occurring in the infant and child brain, such as myelination, astrocyte differentiation and maturation, interneuron migration and maturation, which may result in different outcomes to TBI than similar injuries in older brains. We are particularly interested in understanding what factors are involved in regulating neural differentiation, neurite outgrowth and axonal regeneration so that we can target these pathways to promote neural repair. We are currently using a traumatic brain injury (TBI) model in juvenile and adult mice to address these questions.

Each of the projects below may involve some or all of the following techniques: TBI surgery in mice, behavioural testing, brain dissection and sectioning, immunohistological staining and image analyses. The projects can be scaled or combined as appropriate to be suitable as BSc(Hons) or PhD projects.

Project 1 - What is the effect of infant, childhood or adolescent TBI on adult neurogenesis?
There is ongoing neurogenesis in the subventricular zone and hippocampus of the adult brain. Hippocampal neurogenesis, which is important for spatial memory, learning and anxiety, is susceptible to changing in response to a range of physiological and environmental factors. Given the ongoing cognitive and memory problems reported by TBI patients, this project will determine whether TBI at young



ages affects hippocampal neurogenesis in the adult. It will also assess behaviours associated with hippocampal function and these will be correlated with effects on hippocampal neurogenesis.

Project 2 - What is the effect of infant and childhood TBI on astrocyte development and astrocytic gliosis?

Astrocytes are still maturing in the juvenile brain. Little is known about the effects of TBI on these young cells – does it affect their maturation, do they remain reactive, is their response to injury different to adult astrocytes, does it alter their response to injury at later timepoints?

Project 3 - What is the effect of infant TBI on interneuron migration and maturation?

In the juvenile brain interneurons have not finished their development – many are still migrating to the correct location and do not express markers of their mature phenotype. What happens to these cells if the brain is injured while they are still developing? Do they migrate to their correct location, do they express their normal markers of interneuron phenotype?

Neuron Development and Plasticity

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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 activity, 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 cytokines and the Seizure-related gene 6 (Sez-6) protein family. The Sez-6 family consists of three similar proteins, implicated in synapse development and maintenance in the mature brain. Mutations in Sez-6 proteins are associated with autism spectrum disorders and febrile seizures and we are currently working to elucidate the relevant signalling mechanisms.

Sez-6 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. In addition to the possible links to epilepsy and autism spectrum disorders mentioned previously, the dendrite and synapse abnormalities seen when Sez-6 is lacking are common to a number of mental retardation and neurodegenerative conditions. The expression of Sez-6 proteins is also seen in the mature brain and recent data have implicated Sez-6 in the maintenance of excitatory synaptic connections. To study the complex molecular pathways regulating the development of neuronal branches and synapses, we use a range of experimental approaches. Synapses in developing neurons are fluorescently labelled using antibodies to pre- and post-synaptic markers. We are assessing protein functions in the mature brain and in specific types of neurons using an inducible tissue-specific gene knockout approach. We also investigate molecular

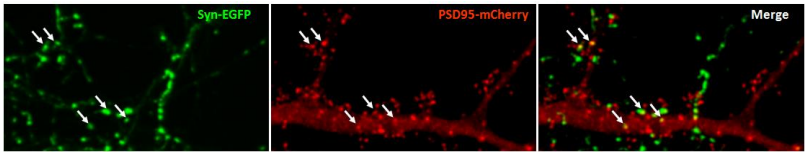
interactions and signalling pathways using molecular biological and protein biochemical techniques.

Project 1 - Role of Sez-6 in cocaine self-administration and extinction
Supervisors: Dr Jenny Gunnersen, Department of Anatomy and Neuroscience, Professor Andrew Lawrence, Florey Institute of Neuroscience and Mental Health

Pilot studies from our laboratory indicate that conditional deletion of the *sez-6* gene alters cocaine-induced reinstatement of conditioned place preference in mice, a model for relapse in human drug addiction. This project will test the *sez-6* conditional knockout mice in an operant conditioning paradigm involving intravenous self-administration of cocaine, extinction of cocaine-seeking and relapse to cocaine-seeking after extinction. Effects on neuron morphology in brain "reward centres" will be examined using immunohistochemistry and Golgi-Cox tracing.

Project 2 - How does GM-CSF prevent brain damage after traumatic injury?
Supervisor: Dr Jenny Gunnersen, Department of Anatomy and Neuroscience and Dr Sandy Shultz, Department of Medicine

Granulocyte-macrophage colony stimulating factor (GM-CSF) is best known as a haematopoietic cytokine however recent evidence suggests that it also acts to protect neurons after brain injury. GM-CSF can cross the blood-brain barrier and this cytokine has previously been found to be neuroprotective against stroke. Nevertheless, GM-CSF could be involved in acute inflammatory effects after brain injury and therefore could exert negative as well as positive effects at different stages. This project will measure effects of GM-CSF treatment on cultured neurons, astrocytes and microglia as well as investigating which cell types in the normal developing brain produce GM-CSF.



Overlap of fluorescently-tagged synapse marker proteins in live cultured neurons identifies developing excitatory synapses
Syn – synaptophysin, PSD95 – post-synaptic density protein 95 kDa

Neurotrophin and Myelin Laboratory

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Laboratory Overview:
Our research aims to understand the nature of signals that initiate, promote and maintain the interactions between neurons and glial cells that are vital for myelination during development and remyelination following a demyelinating insult. We use a variety of molecular, cellular, biochemical, genetic and confocal imaging techniques, as well as behavioural analyses, to investigate these events.

Project 1: Determining how BDNF signalling controls myelination
The neurotrophin family of growth factors exert diverse influences upon peripheral and central nervous system myelination. We have recently identified that brain derived neurotrophic factor (BDNF) plays an important role in promoting both central and peripheral nervous system myelination by activating distinct receptors: it activates the receptor tyrosine kinase TrkB to promote CNS myelination, but activates the p75NTR neurotrophin receptor to promote PNS myelination. This project aims to determine how these distinct BDNF receptors promote myelination through analyses of cells *in vitro* as well as conditional knockout mice *in vivo*.

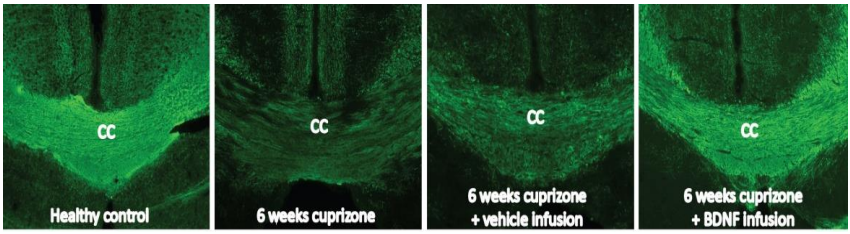

Figure 1: *BDNF infusion promotes CNS remyelination (green) in vivo.*

Project 2: Can mimetics of BDNF promote remyelination after injury?

We have developed novel low molecular weight peptides designed to selectively mimic the TrkB and p75NTR agonist properties of BDNF. This project aims to investigate whether BDNF mimetic peptides can promote myelin repair *in vivo* using animal models of central and peripheral nervous system demyelination.

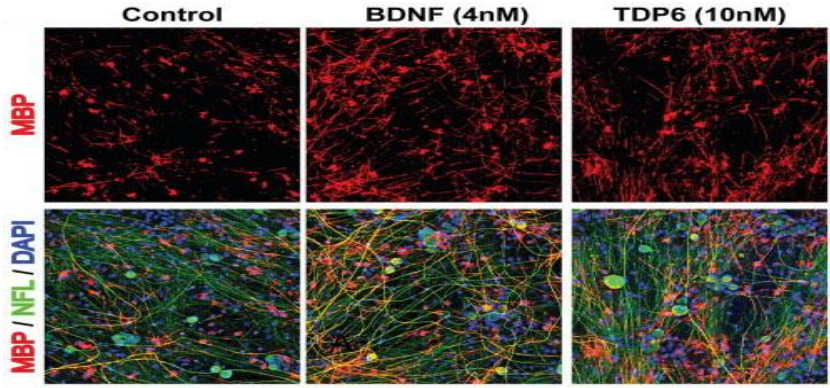

Figure 2: *The BDNF mimetic TDP6 promotes oligodendrocyte myelination (red) in vitro.*

Project 3: The role of Wnt/β-Catenin signaling on myelination and myelin repair

In Multiple Sclerosis (MS), one of the major factors contributing to disease progression is inhibition of oligodendrocyte differentiation in demyelinating lesions. Key components of the *Wnt* signalling pathway are upregulated in MS lesions, strongly suggesting that this pathway could control oligodendrocyte differentiation and remyelination in the context of MS. This project aims to identify how *Wnt* signalling regulates myelin development and remyelination via analyses of conditional knockout mice *in vivo*.

Physical Anthropology

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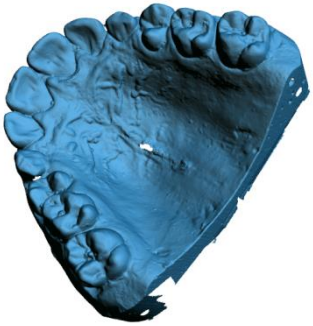
Introduction

In the Physical Anthropology Lab we use dental and skeletal morphology to address questions relating to human biology, behaviour, health, diet and evolution. We use morphological data to study gene flow and evolutionary diversification in fossil and ancient humans. We also reconstruct the physical features, diet, behaviour and health of past human populations. Our research materials are the osteological collections housed in the lab, ape dental material from museums around the world, and human skeletal material from archaeological sites.



3D scan of a human maxilla

Current projects available:

Master of Science

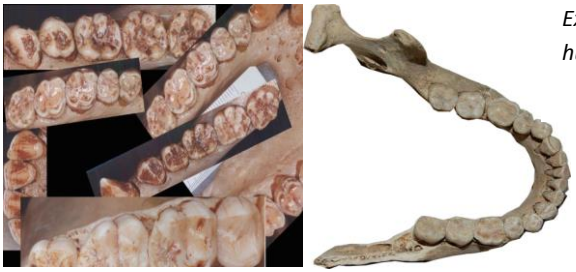
(1) Discrete dental trait analysis

Dental morphological features that are non-selective in nature get fixated randomly in populations. These discretely occurring dental traits provide an understanding of migration, admixture and genetic relationships. In this project we will study discrete dental traits in the ancient human population from Samtavro in the Republic of Georgia (3500 BC – 600 AD). The aim of the project is to determine the identity of the populations represented at the burial site of Samtavro, which lies at the cross-road of migration routes from Africa, Asia and Europe. The MSc candidate will have a background in anatomy, with sufficient knowledge of population genetics, dental morphology, evolution and biostatistics. The project will necessitate a visit to the field site in July.

Honours

(1) 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



Examples of ape and human teeth

evolutionary relatives. In this project comparative reference samples of present-day apes will be used as models to reassess the taxonomy, or species break-down patterns, of fossil hominids from the Plio-Pleistocene of Africa. This lab-based project will use scaled digital images of fossil and modern-day ape dentitions in the analysis.

Miocene ape teeth from Kenya National Museum

(2) Bone loading in metacarpals and metatarsals (in collaboration with Dr. David Ackland, Department of Mechanical Engineering)

Bone is genetically programmed to adapt to the load placed on it. Cortical and trabecular bone respond differently to mechanical loading. Their relative thickness and volume may be used to infer associated loading behaviours of extinct animals. This project will involve taking micro-computed tomography (micro-CT) scans of metacarpals and metatarsals of humans, chimpanzees, and old world monkeys to study how bone loading correlates with known hand and foot usage in these primates. A software analysis program will be used to measure parameters such as cortical bone thickness and volume fraction on each specimen. Imaging and analysis will be performed in collaboration with the Department of Mechanical Engineering. This study will form a baseline for future studies on the locomotor behaviour of extinct primates.

Human femur showing compressive and tensile stress

Sensory Neuroscience

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Project - How sensory signals are detected: Structure function relationships in peripheral sensory nerve terminals
Supervisors: Associate Professor James Brock, Dr Jason Ivanusic
Peripherally located small diameter sensory nerve terminals are critical for coding pain and changes in temperature, but our current lack of understanding of how they work limits our ability to target specific therapies associated with these sensory modalities. This study uses a combination of electrophysiological recording, neuroanatomical tracing, immunohistochemistry and high resolution confocal microscopy to provide new insights into how sensory signals are transformed into action potentials in sensory nerve terminals that code for pain and changes in temperature.

Sex determination and disorders of sex development

Head: Dr Dagmar Wilhelm
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The Wilhelm lab will be joining the Department in November 2015. Their current web site is here: http://www.med.monash.edu/anatomy/staff/wilhelm2.html

One of the most amazing biological processes is the development of a fertilized egg into a complex organism. It involves the orchestration of cellular processes, which is controlled by a delicate network of gene regulation. Disturbance of this network during development results in malformation and malfunction of organs, diseases such as cancer, and often lethality. Our laboratory studies mechanisms of gene regulation during embryonic development focussing on the development of embryonic gonads and their related disorders using mouse as a model system.

Disorders of sex development (DSDs) refer to congenital conditions in which the development of chromosomal, gonadal or anatomical sex is atypical. They are very common, with an estimate of approximately 1 in 4,500 births. They have profound psychological and reproductive consequences for the patient, which are also often prone to testicular or ovarian cancer later in life. Surprisingly, although many genes have been identified which play a role in these processes for almost 70% of cases we still do not know the underlying cause. Clearly, the identification of new genes and regulatory mechanisms involved in the formation of testis and ovary is critical for understanding the molecular pathology of DSDs.

Project 1: The role of non-coding RNAs in sex determination and gonad development
To really understand embryogenesis and its related diseases it is essential to integrate all levels of gene regulation, i.e. transcriptional control, post-transcriptional mechanisms such as mRNA stability, splicing, and editing, translational control and post-translational modifications. One novel regulatory mechanism is the regulation of gene expression by non-coding RNAs (ncRNAs). We are elucidating the function of non-coding RNAs using a series of *in vitro*, *ex vivo* and *in vivo* studies, integrating cell biology, developmental biology, biochemistry, and mouse genetics.

Project 2: The role of Hmgcs2 in mammalian gonad development
We have identified a new DSD candidate gene, *HMGCS2*, encoding mitochondrial 3-hydroxy-3-methylglutaryl coenzyme A synthase 2, in a patient with gonadal dysgenesis and male-to-female sex reversal. This project will characterize the contribution of *HMGCS2* to DSDs and will shed light on if and how diet can influence sex differentiation. We will test the **hypothesis** that *Hmgcs2* is critical for normal sex determination and foetal gonad differentiation, especially during fasting situations.

Project 3: Ovarian development and disease

The ovary plays a major role in women's health. Ovarian-related diseases are very common and include premature ovarian failure, which affects 1% of the female population, polycystic ovarian syndrome in 5 to 10% of all women in childbearing ages, female infertility (already 1% of women in their early 20's are infertile) and ovarian cancer, which causes 2.3% of all female death. Although the function and physiology of the adult ovary has been investigated in great detail, we know very little about the genes that control its development and early differentiation. This project will build on these discoveries to provide a new understanding of ovarian development and therefore the origin of ovarian disease.

Project 4: Post-translational control of cell fate decisions

Cell fate decisions are fundamental during the development of multicellular organisms. Arguably, one of the most unique cell fate decisions during mammalian development forms the basis of male *vs*. female identity. We have recently shown that the ubiquitin ligase NEDD4 is essential for sex determination. This breakthrough finding not only provides the first insight into a novel role for ubiquitination, but also more generally for post-translational modifications, in cell fate determination. This project will study molecular mechanisms of cell fate decision and the role post-translational modifications play in these processes using gonad development as an *in vivo* model system.

Stem Cell Genetics

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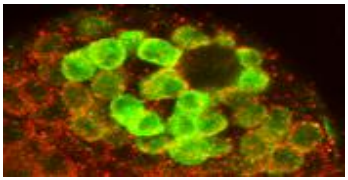
Identification of factors that regulate stem cell development in *Drosophila* and mice

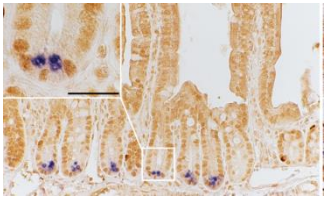
Stem cells are the key to organ regeneration and tumour growth
Many differentiated but renewable cell types in vertebrates are derived from relatively small populations of dedicated precursors, or stem cells. The ability to replenish differentiated cells depends on the continued survival and proliferation 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 tumo`urs 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* and mouse (male germ line and intestinal) as models for stem cell systems.



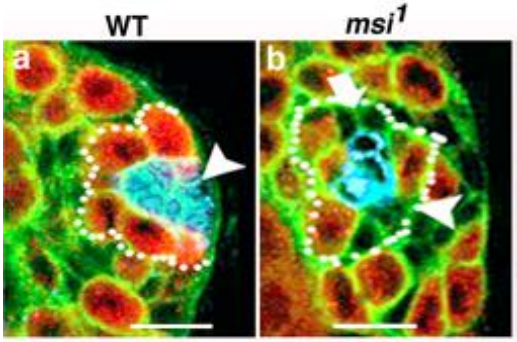
A rosette of germline stem cells (expressing a Snail family transcriptional repressor, green) can be observed surrounding the somatic stem cell niche in the *Drosophila*

Project 1 - Analysing the role of transcriptional repressors in *Drosophila* and mouse stem cells (in conjunction with Assoc. Prof. 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 to identify how these proteins regulate stem cell numbers and control the production of differentiated progeny 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.

Project 2 - Analysing genetic roles of RNA-binding proteins in *Drosophila* stem cells
We have identified that the RNA-binding proteins, Musashi, Enhancer of Rudimentary and Held-Out-Wings (HOW) 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)

Project 3 - Stem cell competition
If a mutation in a cell signaling pathway occurs in a stem cell can that cell outcompete other stem cells and result in the entire stem cell pool of an organ being derived from that single cell? What effects might this have on the ability of the stem cell to differentiate and produce functional cells? This project will use the genetic tools available in *Drosophila* to generate single mutant stem cells and follow their progeny. We will assay what proportion of the stem cell pool is generated from the

mutant stem cell over time and if the mutant cells can produce functional differentiated progeny.

Project 4 - How does alternative splicing regulate stem cell maintenance
Regulators of RNA splicing can lead to different isoforms of genes being expressed in stem cells. This project will use genetic methods, immunostaining and confocal microscopy to determine if different splice forms of signalling molecules affect stem cell maintenance and differentiation.

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Visual Neuroscience Laboratory

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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:
One of the major reasons for vision loss is the growth of new blood vessels in the retina (neovascularisation). This vascular pathology is evident in diseases such as diabetic retinopathy and Retinopathy of Prematurity (ROP). Approximately 10% of all diabetics experience vision threatening retinopathy which is characterised by pathological changes in neuronal, glial and vascular function. 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. We are investigating the retinal mechanisms that lead to vascular pathology and whether novel treatments prevent or slow vision loss thus improving clinical treatment.

Project 1: Pharmacological and laser therapies for age related macular degeneration
Dr Kirstan Vessey, Dr Andrew Jobling and Prof Erica Fletcher

Age related macular degeneration (AMD) is a major cause of vision loss in the older community. There are currently no specific treatments for preventing late stage AMD or slowing the progression of the disease to the later vision threatening forms. In this project we will characterise morphological and functional changes in the eye of a mouse model of AMD and test novel pharmacological and laser therapies to ameliorate these changes. This project will involve the use of wide ranging techniques such as assessment of visual function, immunohistochemistry and molecular biology. Ultimately, this study will help to answer whether novel pharmacological or laser therapies can be used as a preventative treatment for AMD.

Project 2: The regulation of glucose uptake in the diabetic retina
Dr Andrew Jobling and Prof Erica Fletcher

The retina is highly susceptible to damage arising from the high glucose concentrations present during diabetes. Glucose is known to be taken up into cells via membrane proteins known as glucose transporters (GLUTs). Currently there are 12 GLUTs that each have specific cellular expression profiles and/or glucose uptake characteristics. Within the retina, the cellular localization of the various GLUTs is unclear, as is the effect of diabetes on their expression. Additionally, how diabetes and altered glucose uptake effect neuronal and glial metabolism within the retina is not fully established. Understanding these changes is critical to explaining the retinal pathology that develops during diabetes. Using animal models of type I diabetes, this project will use in vivo imaging techniques, immunohistochemistry, protein chemistry and molecular biology to examine the changes in GLUT expression and cellular metabolism within the retina at various times during diabetes development.

Arousal and Cognition Neuroscience

Associate Professor Andrew L. Gundlach (Neuropeptides Division)
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http://www.florey.edu.au/about-florey/our-people/staff-directory/112/sherie-ma

Our laboratory is in the Neuropeptides Division of The Florey (FINMH) and our research is in the area of *systems neuroscience*. A primary research interest is in the role of neuropeptide/receptor signalling in the control of complex brain-behaviour interfaces such as arousal *and* motivation, stress *and* mood, and associated cognitive and memory processes, under normal *and* neuropathological conditions.

We are researching the neurobiology of the *relaxin-3/RXFP3* (peptide-receptor) system in brain, as well as the neural network driven by an area known as the *nucleus incertus*. Scientists and students in the laboratory undertake a range of distinct research projects that involve studies in animal models of health and psychiatric disease (including laboratory rats and normal and transgenic mice), using a range of biomolecular tools, including receptor-selective peptides and viral-vector delivered - neural tracing molecules, receptor-targeting peptides, 'DREADDs' (designer receptors exclusively activated by designer drugs) or optogenetic, light-activated channelrhodopsins.

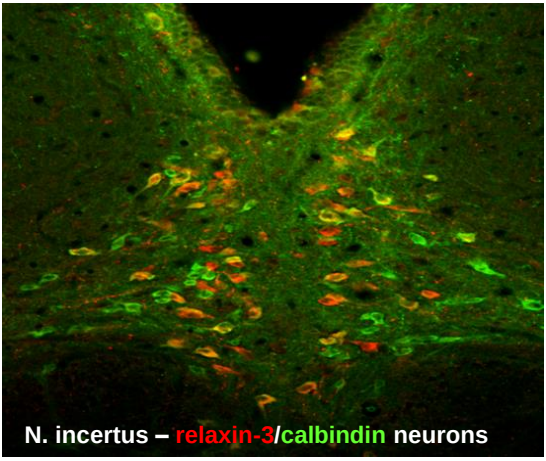
Different projects are suitable for Honours (and/or PhD) students and include:

Project 1: Nucleus incertus in control of arousal and motivated behaviours – pharmacogenetic and optogenetic studies
A/Pr Andrew L. Gundlach and Dr Sherie Ma
Our laboratory has identified and mapped the neurons that synthesise relaxin-3 and RXFP3 in the mammalian (rodent and non-human primate) brain. This peptide/receptor system has been conserved throughout evolution and is remarkably similar between rodents and primates, confirming the validity of using rodent experimental models to better understand the role of relaxin-3-releasing neural networks in human brain function. There is substantial experimental evidence that relaxin-3 systems regulates complex brain activity associated with arousal, emotion and cognition.

Relaxin-3 is expressed at high levels by large GABA projection neurons in the hindbrain 'nucleus incertus'. These neurons contribute to broad neural circuits that

regulate sensorimotor behaviours, arousal *and* motivation, feeding *and* metabolism, and emotional responses, along with associated cognitive processes.

Current projects employ viral-driven excitatory and inhibitory 'designer receptors exclusively activated by designer drugs' or *DREADDs* to reversibly increase and decrease the activity of nucleus incertus neurons and assess the effects on physiology (using telemetry recording of EEG and LFP) and behaviour in rodents. We are also establishing optogenetics, whereby light will be used to control nucleus incertus neuron activity in freely-behaving rodents. Anatomical studies aim to characterise the various peptide/transmitter populations of the nucleus incertus, and thus better understand the neurochemical heterogeneity of this brain region. Students will receive training in molecular and systems neuroscience, including neurochemical anatomy, physiology and behaviour, and neuropharmacology. Techniques include stereotaxic surgery and behavioural analysis in rodents, and associated immunohistochemical and biochemical analysis.



Project 2 - Relaxin-3/RXFP3 signalling in control of arousal and motivated behaviours – studies in normal and transgenic mice
A/Pr Andrew L. Gundlach and Dr Sherie Ma
'Arousal pathways' or neural circuits facilitate heightened awareness, attention and cognition, and are also implicated in 'reward signals' associated with food- and drug-seeking behaviour. Established arousal transmitter systems include serotonin neurons in the raphé nuclei, dopamine neurons in the ventral tegmental area, and orexin (peptide) neurons in lateral hypothalamus.

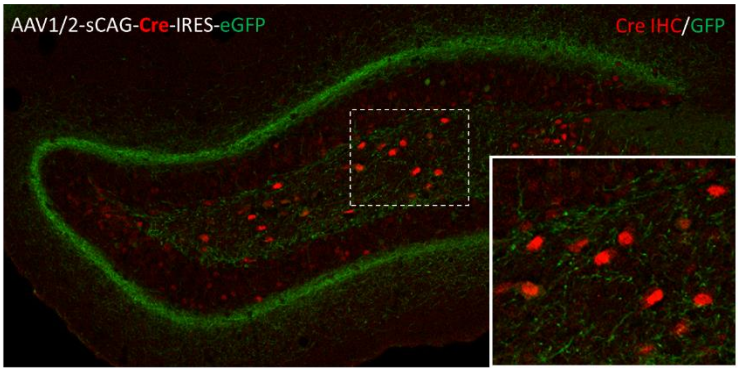
Anatomical and functional studies suggest relaxin-3 neurons in *nucleus incertus* (NI) and central grey (CG) represent an arousal pathway that modulates behaviours such as feeding, attention (vigilance), motivation and exploration. Mice genetically lacking relaxin-3 display an active-phase hypoactivity which is a reflection of aspects of clinical depression. Therefore, relaxin-3/RXFP3 systems represents a potential target

for drugs to treat conditions such as insomnia, anorexia, obesity, drug abuse and depression.

These studies will utilise unique *conditional* RXFP3 gene knockout (KO) and RXFP3-*Cre reporter* mouse strains, selective RXFP3 agonist and antagonist peptides and viral-driven peptide vectors, and validated behavioural paradigms to determine the effects of relaxin-3/RXFP3 deficiency.

As relaxin-3 and RXFP3 KO mice display hypoactivity on voluntary running wheels and other characteristics of mood disturbances, RXFP3 deletion will be virally-induced in conditional RXFP3 KO mice and the effects will be characterised by testing their performance versus 'wild-type' littermates on running wheels, and in motivated and mood-related behavioural tests. The ability of acute or chronic central RXFP3 activation to 'rescue' behavioural alterations in relaxin-3 KO mice can also be assessed using central injections of RXFP3 agonist peptide and/or a viral-driven RXFP3 agonist peptide.

RXFP3-*Cre reporter* mice are being used in studies to assess the identity of RXFP3-expressing neurons, as a prelude to more functional studies using the Cre/LoxP system to introduce light- or designer drug-sensitive channels/receptors into RXFP3 responsive neurons to further assess the neural circuits they regulate, including confirmation of a role in motivated behaviours.



Autonomic and Sensory Neuroscience

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Synaptic gating mechanisms involving inhibitory interneurons in the nucleus of the solitary tract.

The nucleus of the solitary tract (NTS) is the first central site to receive viscerosensory afferent input and is involved in mediating autonomic reflexes (e.g. baroreflex). Autonomic reflexes are highly flexible and dynamic. For example during exercise, where both blood pressure and heart rate both remain high over sustained periods, the baroreflex seemingly decoupled during this period. This project aims to determine the synaptic mechanisms underlying reflex strength and flexibility. Specifically, how inhibitory synaptic gating mechanisms affect synaptic transmission between viscerosensory afferents and NTS neurons utilizing a channelrhodopsin 2 expressing mouse line.

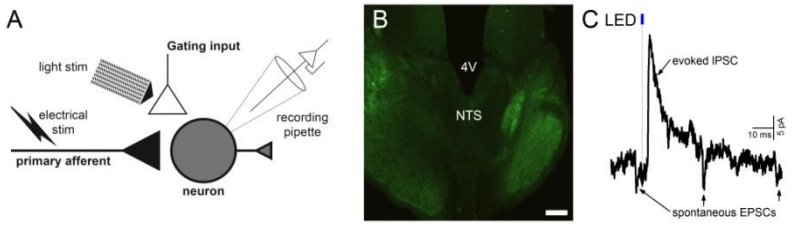

Figure. Using optogenetics to study the inhibitory network within NTS. **A.** Experimental concept. **B.** Horizontal medulla slice from a Sst-ChR2-yfp mouse. **C.** LED pulse evokes Inhibitory post synaptic currents in NTS neurons.

We are looking for a high achieving, intelligent and highly motived student to join our team. The successful student will learn advanced techniques including immunohistochemistry and slice electrophysiology. If you have a genuine interest in neuroscience research and want more information about the project (and honours in general) we would welcome an obligation-free, informal chat.

Behavioural Neuroscience

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Understanding the role of synaptic genes in cognition and disease

Sensory information from the environment is ultimately processed at the level of synapses, the connection between neurons that form the most fundamental information-processing units in the nervous system. Vertebrate synapses contain a large yet intricately organised signalling complex of proteins encompassing neurotransmitter receptors, scaffold proteins and cell adhesion proteins. In recent years, human genetic studies have increasingly highlighted that disruption of over 200 genes that encode postsynaptic proteins result in over 130 brain diseases. While it is accepted that postsynaptic proteins are fundamental for synaptic function, plasticity and thus behaviour, very little is actually known about the impact of postsynaptic gene mutations in regulating complex cognition and higher order processing.

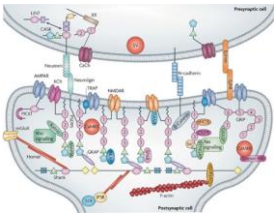

Figure 1

Modelling the complex cognitive processes that are routinely assessed in the clinical setting has been challenging in animal models, primarily due to limitations in the behavioural tests available. Bridging the gap between mouse and human cognitive testing, the recently developed touchscreen methodology provides an innovative tool for dissecting higher cognitive functions in rodents that is highly analogous to testing methods used in cognitive assessment of clinical populations.

Figure 2

Multiple projects are currently available. In our laboratory, we use mice as models carrying mutations in key postsynaptic genes - including the NMDA receptor, synaptic scaffolds and cell-adhesion proteins - to study how these genes regulate synapse formation, function and cognitive behaviours. In addition to in-depth behavioural analysis using novel methodology which our lab has unique expertise in, projects will involve training in key cellular and molecular techniques including immunohistochemical and biochemical analysis.

CNS Plasticity & Activity-dependent Myelination

Dr. Jerome Staal: (83448106)
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General Overview:
The brain is the source of behavior, but in turn it is modified by the behaviors it produces. This dynamic interplay between brain structure and brain function is the basis of cognition, learning and plasticity. Although the role of activity in regulating neuronal structure and subsequent function is well studied, relatively little is known regarding glial cells (the most abundant cell type in the brain). This is surprising as all types of glia can respond to and influence neural activity and structure. The question remains: how do glial cells influence the structure and function of the brain? And how does changes in functional activity, associated with disease (such as multiple sclerosis) or injury alter these neuron-glia interactions?

Projects available:
In the 1990s scientists made an intriguing discovery that astrocytes (a type of glial cell) respond to stimuli and the release of neurotransmitters via a special form non-electrical excitability. For almost a century neuroscientists believed that astrocytes had no function in information processing or transmission. We are only now beginning to understand the role of these glial cells in regulation neuronal structure and function.

Project 1: Synapses are the dynamic structures associated with learning and memory. We are interested to know how astrocytes, that are well known to be closely associated with synapses, regulate the remodeling of these structures during development, learning and following injury. This will be done using unique transgenic animal models that allow us to manipulate astrocyte function in living animals.

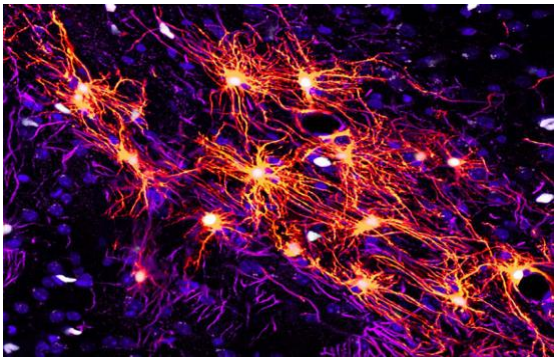

Figure 1. *Astrocytes surround neuronal synapses and form networks coupled by gap-junctions. (Image courtesy of Dr. Takahiro Takana, http://www.urmc.rochester.edu/labs/)*

Project 2: Oligodendrocytes are the glial cells responsible for producing myelin, which significantly alters the speed of signal transduction within neuronal networks. The amount and location of myelin production is dynamic but also very specifically regulated. It is known that neural impulse activity regulates the production of myelin by oligodendrocytes, yet little is known regarding the mechanisms associated with this. We are interested in studying how electrical activity in neurons regulates its interactions with oligodendrocytes. This will be done using unique co-culture electrostimulation models that allow us to determine how patterns of activity influence glial cell behavior (this project will be in collaboration with Dr. Merson).

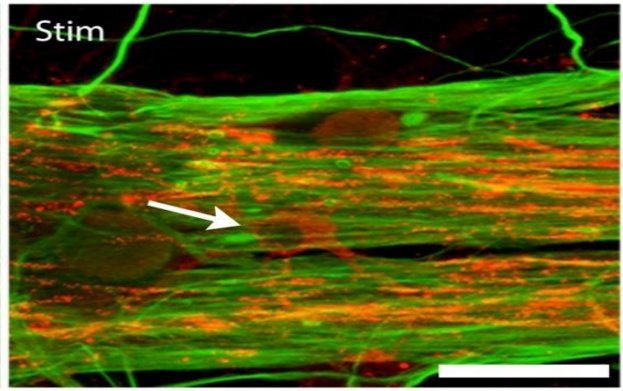

Figure 2. *A single oligodendrocyte (arrow) contacting and producing myelin (red) on multiple axons (green) after electrical stimulation*.

Developmental Psychobiology

Laboratory Head: Dr Jee Hyun Kim (9035 6623)

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Project 1: Early life stress and memory development
Primary supervisor: Dr Heather Madsen (heather.madsen@florey.edu.au)
Co-supervisor: Dr Jee Hyun Kim

Early life experiences play a pivotal role in shaping personality and psychosocial functioning into adulthood. For example, early life adversity in humans is associated with increased risk of developing mental illnesses such as depression and anxiety. Given the importance of these first few years of life, it is interesting that most adults fail to recall autobiographical events from their early childhood years. Infantile amnesia is the term used to describe this phenomenon of accelerated forgetting during infancy, and it is not unique to humans. In fact, infantile amnesia has been observed in every altricial species examined; that is, animals that undergo extensive post-gestational development.

Many investigations into infantile amnesia have used Pavlovian fear conditioning in rats as a model of learning and memory. While adult rats exhibit excellent memory retention following just a single conditioning episode, infant rats rapidly forget fear associations over short intervals. Recently it has been shown that exposure to early life stress improves retention of learned fear in infant rats. The aim of this project is to investigate the neurobiological changes that underlie this early transition to adult-like memory.

Project 2: Regulation of emotional memory across development
Primary supervisor: Dr Despina Ganella (despina.ganella@florey.edu.au);
Co-supervisor: Dr Jee Hyun Kim

Most anxiety disorders emerge during childhood, and individuals with childhood onset express more severe symptoms than do individuals who have adult onset. In fact, there is growing recognition that mental disorders may actually be developmental brain disorders and, as such, treatment strategies should focus on the young population. Currently, the effective treatments for anxiety disorders are cognitive-behavioural therapies that rely on inhibition of emotional memory. This project will examine inhibition of emotional memory throughout development using Pavlovian fear conditioning as a model of anxiety disorders in rats.

Neural Plasticity

Head: Professor Anthony Hannan (9035 6638)
Email: **anthony.hannan@florey.edu.au**

Web: http://www.florey.edu.au/about-florey/our-people/staff-directory/81/anthony-hannan

Neural Plasticity Lab Overview:
The human brain contains many billions of neurons that are interconnected by trillions of synapses, to form functional networks underlying our most complex cognitive, affective and motor functions. Our ultimate goal is to understand gene-environment interactions and experience-dependent plasticity in brain disorders involving cognitive and psychiatric symptoms, including Huntington's disease, schizophrenia, depression, anxiety and autism spectrum disorders. We use a range of techniques, including molecular biology, gene expression profiling, protein chemistry, immunohistochemistry, physiology, endocrinology, mouse behavioural analysis, as well as environmental and pharmacological manipulations, to understand how genes and environment combine to affect aspects of brain structure, function and behaviour. Our laboratory is located in the Florey Institute for Neuroscience and Mental Health, Melbourne Brain Centre, University of Melbourne.

Project 1: Gene-environment interactions in the regulation of cellular plasticity, cognitive function and behaviour
Supervisors: Dr Thibault Renoir (903 56614) and Prof. Anthony Hannan
Email: thibault.renoir@florey.edu.au ; anthony.hannan@florey.edu.au
The hippocampus is a dynamic brain structure believed to be critical to cognitive functions including memory consolidation and emotion regulation. One of its unusual features is that the dentate gyrus subfield is known to constitutively engage in the process of neurogenesis throughout adulthood. Interestingly, this process is not static, and behavioural manipulations such as housing animals in an enriched environment (EE), or allowing them to engage in voluntary exercise (VEx) on a running wheel, can increase the rate of hippocampal neurogenesis. The real impact of enhanced hippocampal neuroplasticity on cognitive functions remains unclear and very few studies have used genetically targeted animals to unravel neuroplasticity mechanisms associated with the effects of long-term environmental manipulations. Serotonin (5-HT) is known to influence adult neurogenesis, with recent studies suggesting that 5-HT in the hippocampus is more instrumental in cell proliferation than in cell survival; this might account for the differences in proliferation and survival observed between VEx and EE. Existing research suggests a sex difference in the regulation of hippocampal neurogenesis as well as sexually dimorphic neurochemical changes underlying the effects of environmental factors on hippocampal-related functions such as memory and emotion regulation.

Clearly, further studies are necessary to substantiate sex differences in gene-environment interactions on hippocampal-related functions. The role of 5-HT in the cellular changes induced by both EE and VEx needs to be explored more exhaustively to elucidate its involvement in the process of neurogenesis. Surprisingly, although they display valuable advantages, no study has yet used genetically targeted animal models with disrupted 5-HT signalling for such fundamental explorations. These questions will be the focus of this project.

A student taking on this project will have the opportunity to gain experience in at least one of the following experimental techniques:

- behavioural testing (modeling affective/cognitive symptoms) of wild-type and genetically targeted mice, and drug administration
- gene expression and protein analysis using quantitative real-time PCR, western blotting, ELISA , autoradiography, immunohistochemistry

Project 2: Investigating social communication in the Neuroligin 3 mouse model of Autism

Supervisors: Dr Emma Burrows (903 56629), Dr Scott Kolbe and Prof. Anthony Hannan

Email: emma.burrows@florey.edu.au ; anthony.hannan@florey.edu.au

Autism is a complex spectrum of disorders characterized by core behavioural deficits in social interaction, communication, and behavioural flexibility. The cause of ASD is unknown. Many gene mutations that contribute to ASD have recently been identified. These findings have lead to the development of genetic mouse models which display behavioural phenotypes mimicking ASD traits. This project takes advantage of a mouse model expressing a gene mutation coding for the Neuroligin-3 (NL3) synaptic protein identified in ASD patients. We have shown that NL3 mice show impairments in social interaction, a key criteria for validating mouse models of ASD, but other aspects of their phenotype, including communication, are not well characterised.

Rodents emit audible sounds (i.e. squeaks) but communicate among themselves predominantly in the ultrasonic range of sound frequencies. Male mouse ultrasonic vocalizations consist of The hippocampus is a dynamic brain structure believed to be critical to cognitive rapid series of ‘‘chirp-like’’ syllables, each call varies in duration, is uttered at rates of about ten per second and involves rapid sweeps in frequency. Once pitch-shifted these vocalizations are reminiscent of birdsong to the human listener. Of particular relevance to social behaviour are the ultrasonic vocalizations emitted by male mice in the presence of females or when they detect female urinary pheromones. It is believed that the vocalizations made by males are a critical step in initiating mating and represent a form of communication. It is for this reason that multiple groups assessing genetic mouse models of ASD have reported reduced

numbers of ultrasonic vocalizations as a proxy for the language impairment in ASD patients.

These studies have focused on number of calls and latency to call however there is evidence to suggest that shape or waveform pattern of the call influences behaviour and may more accurately represent differences in communication. A major challenge for quantitatively assessing differences between mutant and wild-type vocalizations lies in the quantity and complexity of data which must be analysed. Mice emit between 400-1200 calls during a typical 5 minute social interaction so manual detection and classification of calls is not feasible. Additionally, although specific call types have been identified previously in the literature, it remains unclear whether mutants and wild-types even produce the same types of calls. Therefore, a novel approach is required to first automatically identify individual calls, then to classify each call type.

This project will involve recording NL3 mice and WT mice communicating with female mice and then developing an automated detection and classification method using MATLAB to decode the calls. This will allow us to playback calls to female mice and search for relevant behavioural responses. We hope to identify specific genetic loci that play a role in species-specific vocalizations and are potentially implicated in disorders that involve social communication deficits.

Project 3: Utilising Touchscreen technology for preclinical modeling of attention in autism spectrum disorder

Supervisor: Dr Emma Burrows (903 56629) and Prof. Anthony Hannan
Email: emma.burrows@florey.edu.au ; anthony.hannan@florey.edu.au

Autism is a complex spectrum of disorders characterized by core behavioural deficits in social interaction, communication, and behavioural flexibility. Autism spectrum disorder (ASD) frequently presents with additional cognitive symptoms, including attentional deficits and perceptual processing deficits. Current estimates of comorbidity of ASD and attention deficit hyperactivity disorder (ADHD) range from 41–78%. Preclinical animal models are important tools for studying the behavioural domains and biological underpinnings of autism, and potential treatment targets. Many gene mutations that contribute to ASD have recently been identified. These findings have lead to the development of genetic mouse models which display behavioural phenotypes mimicking ASD traits. This project takes advantage of a mouse model expressing a gene mutation coding for the Neuroligin-3 (NL3) synaptic protein identified in ASD patients. We have shown that NL3 mice show impairments in social interaction, a key criteria for validating mouse models of ASD, but other aspects of their cognitive phenotype, including attentional performance and behavioural flexibility, are not well characterised.

This project will investigate attentional abilities and behavioural flexibility of NL3 mice and their WT littermates using a novel touchscreen testing apparatus. Mice will be trained in a step-wise process to touch a computer screen for a reward. Through increasing the complexity of stimuli on the screen mice will then be assessed for visual discrimination, reversal learning, and attention. Utilising touchscreen technology may not only uncover new phenotypes in NL3 mice, but will allow us to investigate brain changes that underlie attention and behavioural flexibility. This will inform the future development of treatments for attentional deficits and impairments in behavioural flexibility in brain disorders such as ASD.

Project 4: Investigation of paternal influence on offspring mental health
Supervisors: Dr Terence Pang (903 56316) and Prof. Anthony Hannan
Email: terence.pang@florey.edu.au ; anthony.hannan@florey.edu.au

As diploid animals, each individual human being is a genetic combination of their parents. Emerging evidence now indicates that the environment that our parents were exposed to can also influence us. The potential for maternal health and well-being to impact on the development of their offspring is well demonstrated and established. Stress during pregnancy has a negative impact on offspring by slowing developmental milestones, cognitive growth, and is also associated with adult-onset mental disorders. In contrast, the paternal influence on offspring development and mental health is largely unknown. Given the increasing exposure to stressors (physical , psychosocial and occupational) that are prevalent in our society, it is important to investigate the possible transgenerational effects of stress on the mental health of future generations.

Our lab has developed a model of elevated physiological stress in mice. Male mice are administered the stress hormone corticosterone via their drinking water, resulting in behavioural alterations associated with anxiety and depression. We have subsequently found that the offspring of these mice also exhibit alterations in anxiety and depressive tests. Interestingly, the effects on male offspring seem to be detrimental compared to female offspring that appear to be more resilient. Our lab is keen to expand on those findings by investigating potential environmental modifiers of these behaviours.

We have adopted several hypotheses which we are offering as potential projects. The first is a study of exercise and how the cognitive ability and response to stress of offspring born to exercising fathers might be changed. The second study will explore enhanced cognitive stimulation as a protectant for offspring born to stressed fathers. A third study would explore the benefits of regular exercise in preventing the transgenerational effects of stress in fathers.

Neurobiology @ the Florey

Supervisors: Dr Jason Howitt (9035 6322)
Email: jason.howitt@florey.edu.au

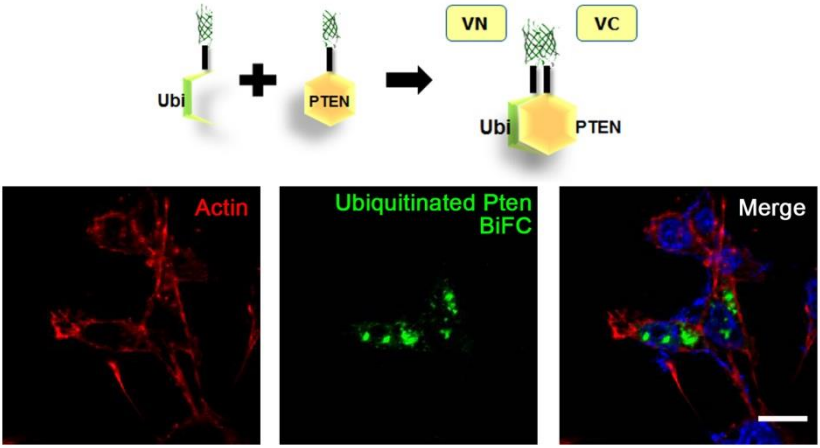
Professor Seong-Seng Tan
Email: sst@unimelb.edu.au

Web: http://www.florey.edu.au/research/protein-trafficking-and-signalling-laboratory

http://www.florey.edu.au/about-florey/our-people/staff-directory/209/seong-seng-tan

Project 1: Understanding molecular pathway changes in the autistic brain.
Throughout the world there is a rapidly increasing rate of autism diagnosis and currently we have little understanding of the molecular changes that occur in the brain that can result in the disorder. Genomic studies have shown a clear correlation between a handful of genes that may be causal to the disorder. One of these genes is PTEN, known predominantly as a major tumour suppressor protein that is lost or mutated in nearly all forms of cancer. This project aims to understand the functional changes that occur in PTEN mutations derived from autism patients.

This project will use a variety of techniques to analyse the location and function of PTEN mutations in the cell. These will include high end confocal microscopy,



Using BiFC techniques to invesiagte the location of modified PTEN in the cell

molecular biology and cell culture techniques.

Relevant publications*:*

Howitt J *et al*. Ndfip1 represses cell proliferation by controlling Pten localization and signaling specificity. *Journal of Molecular and Cell Biology*, 2015 Apr;7(2):119-31.

Li Y *et al*. Rab5 and Ndfip1 Are Involved in Pten Ubiquitination and Nuclear Trafficking. *Traffic.* 2014 Jul;15(7):749-61.

Howitt J *et al*. Ndfip1 regulates nuclear Pten import in vivo to promote neuronal survival following cerebral ischemia. *J Cell Biol.* 2012 Jan 9;196(1):29-36.

Project 2: The failure of ubiquitin pathways in neurodegenerative diseases.
Supervisors: Dr Jason Howitt and Professor Seong-Seng Tan

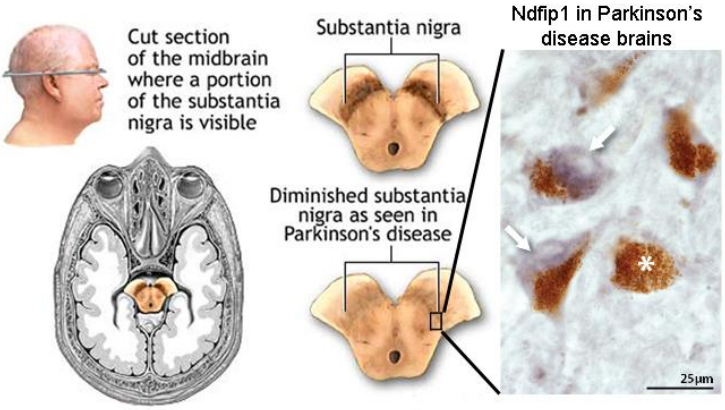
Project summary

In the brain, the control of metal homeostasis is critical as failure of metal regulation can have lethal effects on a cell by promoting side reactions that damage proteins and DNA. This fine balance is prone to disruption, leading to debilitating neurological diseases such as Parkinson's and Alzheimer's disease. This project is designed to understand an important pathogenic event in Parkinson's disease, the regulation of metal homeostasis by ubiquitin pathways.

Project details

Our laboratory has identified a key regulatory mechanism involved in metal entry into neurons. A protein called Ndfip1 regulates the metal transport protein DMT1 by tagging it with ubiquitin leading to the destruction of DMT1. Importantly we have identified that this process occurs in human neurons and in Parkinson's brains we have shown increased levels of Ndfip1 in the substantia nigra, the region of the brain that degenerates. This project aims to identify how this pathway functions in Parkinson's disease to determine if failure of Ndfip1 and ubiquitin function is causal to disease progression. Our laboratory has a wealth of tools that will be used for this study, including human tissue samples, knockout and transgenic mice, antibodies and many biochemical/molecular biology assays.

The ubiquitin system in Parkinsons' disease



In Parkinson's disease accumulation of metals in the substantia nigra causes cell death. We have found that the protein Ndfip1, that regulates the metal transport protein DMT1 using ubiquitin, is switched on in the substantia nigra of Parkinson's brains.

Relevant publications

Howitt J *et al*. Divalent metal transporter 1 (DMT1) regulation by Ndfip1 prevents metal toxicity in human neurons. *Proc Natl Acad Sci U S A.* 2009 Sep 8;106(36):15489-94.

Howitt *J et al*. Ndfip1 regulates nuclear Pten import in vivo to promote neuronal survival following cerebral ischemia*. J Cell Biol.* 2012 Jan 9;196(1):29-36.

Howitt J *et al*. Increased Ndfip1 in the substantia nigra of parkinsonian brains is associated with elevated iron levels. *PLoS One.* 2014 Jan 24;9(1):e87119.

Neuroimmunology and Remyelination Laboratory

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Michele Binder (9035 6636)
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We are multi-disciplinary group straddling the fields of immunology, genetics and myelin biology, with a particular interest in the intersection of these disciplines in MS susceptibility, severity and recovery. Our team works collaboratively to investigate questions ranging from basic biology, such as the effect of receptor tyrosine kinases on myelination by oligodendrocytes, through to translational research, developing novel therapeutic treatments for MS. We use a variety of techniques and mouse models of MS, as well as directly studying the human disease. We have a number of projects available that are suitable for both Honours and PhD students who would like the opportunity to explore the interface of genetics, immunology and neuroscience.

Projects Available*:*

TAMs receptors in demyelination and remyelination
Our group has a long-standing interest in a family of receptor tyrosine kinases known as the TAM receptors (Tyro3, Axl and Mertk), and we were the first group to link TAM receptor signalling to outcome in both demyelination and remyelination. The individual TAM receptors appear to affect different aspects of the demyelination response, with Tyro3 involved in direct regulation of myelination by oligodendrocytes, whilst Mertk is involved in regulating the response of the immune system. We have a number of projects available examining the effects of deleting either Tyro3 or Mertk upon demyelination and remyelination in mouse models of MS.

The ultimate aim of this work is to identify whether activation of the TAM receptors can either directly promote myelination by oligodendrocytes, or alter the immune response towards an anti-inflammatory phenotype, and thereby improve outcome following a demyelinating event. These studies are a crucial step in validating the TAM receptors as appropriate targets for future therapeutic development.

Functional Consequences of Multiple Sclerosis Risk Genes

Many of the genes that affect whether an individual is at higher risk of developing MS have been identified. We have ongoing projects exploring the effects of two of these genes, CD40 and MERTK, on the function of cells of the immune system. In order to better understand the consequences of the genetic changes in these genes associated with risk, we will collect the relevant cells from human participants and examine the function of these cells using a number of techniques, including an assessment of the inflammatory phenotype of these cells, as well as their phagocytic capacity. We also plan to use molecular techniques to directly examine the effect of gene changes on transcriptional regulation *in vitro.* This project aims to move beyond genetic associations, to an understanding of how gene changes alter cell behaviour, and how this altered behaviour can lead to disease in some people, and potentially how we may intervene to prevent or ameliorate disease.

Oligodendrocyte Function & Neuron-Glial Cell Interactions

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General Overview:
The Merson laboratory investigates the life cycle of myelinating glial cells in the nervous system, in particular how they are generated during development, how they are regenerated after injury and their role in supporting the function of axons. We are looking for students who are motivated to excel in research to join our close-knit team. We provide a stimulating and supportive environment to kick-start your research career.

Recent highlights from the laboratory:

- We have generated genetically modified mice to specifically deplete oligodendrocytes from the brain in a highly specific manner. Neuronal function is dramatically affected by oligodendrocyte depletion well before myelin is lost from axons. This has implications for understanding the function of oligodendrocytes and the role of functionally inert myelin in disease
- We have demonstrated that adult neural stem/progenitor cells play a major role in the regeneration of oligodendrocytes and myelin following the death of these cells. This research could translate to novel stem cell based therapies for Multiple Sclerosis and other myelin pathologies
- Our analysis of oligodendrocyte organisation in the brain is revealing fascinating new information about oligodendrocyte topography in white matter. The work has important implications for understanding how specific patterns of myelination restore neuronal function after injury and contribute to brain plasticity in adult life.

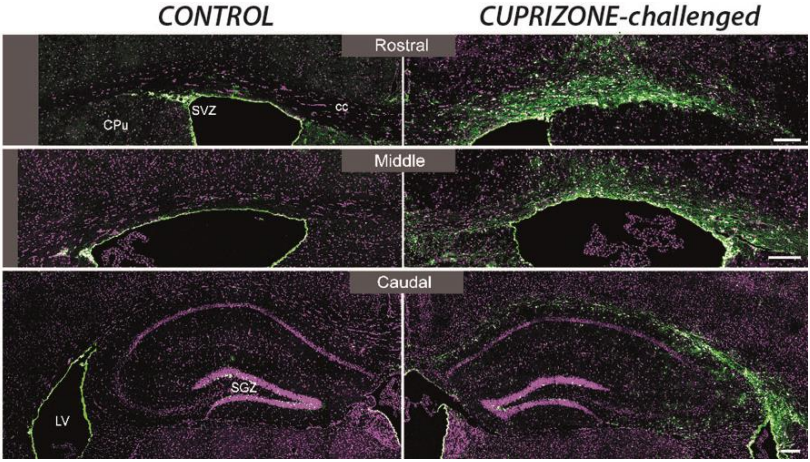
Projects available:
1. Role of alternate energy sources in overcoming oligodendrocyte dysfunction

2. Neural stem cell responses to close versus distant oligodendrocyte loss

3. Characterising oligodendrocyte and myelin organisation in the brain after social and/or sensory deprivation

4. Examining the role of neuronal activity in modulating CNS remyelination

Techniques:
Molecular biology, immunohistochemistry, confocal microscopy, animal behavioural analysis.



We use various transgenic tools in our laboratory. These images of adult mouse brain reveal that green labelled neural stem cells generate thousands of oligodendrocytes that migrate into the demyelinated white matter in response to cuprizone challenge. These new oligodendrocytes enable

Presynaptic Dysfunction in Parkinson's Disease

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Parkinson's Disease (PD) is the second most common neurodegenerative disorder, affecting 7 million people worldwide. The cause of PD is unknown. It is primarily a disease of ageing, affecting 1% of the population over the age of 60; this translates to 1 in 350 people in Australia living with PD. PD already costs the Australian health system almost $0.5 billion annually; with our ageing population, we can expect to incur an increasing health burden from PD. Considering the debilitating nature of this disorder, and the burden it poses to our healthcare system, it is imperative that the underlying cause of PD is identified so that therapeutics can be developed.

PD has a complex pathophysiology. Whilst there is no doubt that Lewy body formation and neuronal cell loss are end-points in PD, there is increasing evidence that disturbance in the nerve terminal precedes and possibly initiates the subsequent pathology in cell bodies. One of the earliest documented changes in both animal models of PD, as well as in patients, is synaptic dysfunction and/or loss.

Whilst the underlying cause of PD is unknown, key proteins have been identified that are likely to play a role in disease progression. The two most well-documented proteins to contribute to PD pathogenesis are alpha synuclein and leucine-rich repeat kinase 2 (LRRK2). Crucially, both proteins mediate presynaptic function and activity.

Several projects are on offer to examine the molecular mechanisms underlying the presynaptic functions of these key PD-related proteins. These projects will implement a variety of techniques, including molecular biology, biochemistry, primary neuronal cell culture from transgenic and wild-type rodent lines, fixed immunofluorescence imaging and live-cell fluorescent imaging, giving students the opportunity to master a range of key transferrable skills.

Project 1: Investigate how alpha synuclein regulates the synaptic vesicle cycle and neurotransmitter release.
Alpha synuclein has been proposed to modulate various aspects of the synaptic vesicle cycle. Importantly, it controls the presynaptic targeting of a key synaptic vesicle protein, synaptobrevin II, which is crucial for neurotransmitter release. This project will determine how alpha synuclein regulates the localisation and function of

synaptobrevin II and the implications this has for synaptic vesicle dynamics and neurotransmitter release.

Project 2: Investigate how LRRK2 and alpha synuclein functionally interplay at the presynapse.

Intriguingly, both alpha synuclein and LRRK2 regulate the recycling pool of SVs, by regulating the clustering or localisation of SVs at the presynapse, however the molecular mechanisms by which they perform this function is unknown. In addition to this correlation in presynaptic function, LRRK2 and alpha synuclein have also been shown to have a pathogenic interaction. This project will ascertain the function of alpha synuclein and LRRK2 at the presynapse, and determine if the proteins act within the same pathway.

Renal Denervation in Chronic Kidney Injury

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Destroying the nerves supplying the kidneys (renal denervation) using radiofrequency power transfer catheters is being trialled as a novel treatment in patients with hypertension and chronic kidney injury. The renal nerves surrounding the renal artery are destroyed via heat generated at the tip of the catheter, which is transferred across the artery wall. Our laboratory has recently developed a novel model of hypertensive chronic kidney injury in sheep. The current project will investigate the function of the renal nerves in our model of hypertensive chronic kidney injury and investigate whether kidney function and blood pressure are improved by renal denervation. The results of this study are likely to have a marked impact on the catheter-based denervation field and benefit for hypertensive patients with renal injury. Techniques that will be mastered during this honours project include – chronic recordings of cardiovascular variables and measurement of renal function in conscious large animals, quantitative immunohistochemistry, data analysis and statistical methods.

The specific aims of the project are:

- Investigate the changes in renal function, renin release and responses to cardiovascular challenges, such as changes in blood pressure, blood volume and ion balance in the hypertensive chronic kidney injury sheep
- Determine whether kidney function and blood pressure are improved by renal denervation
- Using immunohistochemical techniques, determine the levels of denervation acutely after catheter denervation and whether the nerves reinnervate 6-8 weeks after renal denervation

Systems Neurophysiology

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Project 1 – Unravelling the neural circuits that drive increases in sympathetic nerve activity in heart failure

Heart failure (HF) is a major global healthcare problem because of its high prevalence, morbidity, mortality and cost. Patients with heart failure are three times more likely to die within three years than patients diagnosed with cancer. While there has been some improvement in HF treatment over last 30 years, morbidity and mortality remain high. One of the major contributors to disease progression is the rise in sympathetic nerve activity. However, the brain areas responsible for this detrimental increase in sympathetic nerve activity has not been well characterised.

This project will investigate which cardiovascular brain centres are activated in the short and long term after myocardial infarction. This project will be relatively demanding and involve using a number of different techniques including surgery, echocardiography, neuroanatomy and electrophysiology if time permits. The successful completion of the project will expand our understanding of the neural circuitry driving sympathetic nerve increases in heart failure.

For this project we will be asking the following questions:

1. Which areas of the brain are activated in the short and long term after a myocardial infarction?
2. What are the chemical phenotypes of activated neurons?
3. Can this neuronal activation be prevented using the anti-inflammatory compound pentoxifylline?

Techniques involved:

- small animal surgery (induction of myocardial infarction)
- echocardiography
- tissue sectioning
- immunohistochemistry (DAB and fluorescence)
- microscopy (light, fluorescence, confocal)
- *in vivo* electrophysiology (if time permits)

Further Reading:

Ruchaya et al. (2014) Experimental Physiology, 99(1) 111-122.

Project 2 - Project title: Central cardiovascular control: uncovering the role of inflammatory cytokines in the area postrema

The area postrema is a circumventricular organ located in the brain stem. Because it lacks a blood-brain barrier the area postrema is exposed to a wide range of factors found in the circulation. There is much evidence to suggest that inflammatory cytokines are increased in a number of cardiovascular diseases such as hypertension and heart failure. However, the cardiovascular effect of these cytokines when exogenously applied to the area postrema is not currently known.

This project will investigate the effects of different cytokines such as CX3CL1 (fractalkine) and tumor necrosis factor-alpha (TNF-α) within the area postrema. The successful completion of the project will increase our understanding of the role of cytokines in driving changes in blood pressure at the level of the area postrema and how this signaling might be altered in disease states such as hypertension and heart failure.

For this project we will be asking the following questions:

1. What are the cardiovascular effects of CX3CL1 and TNF-α when applied directly to the area postrema?
2. Are the cardiovascular effects of these cytokines altered in hypertension and heart failure?

Techniques involved:

- small animal surgery (induction of myocardial infarction and hypertension)
- stereotaxic microinjections
- tissue sectioning
- microscopy (light, fluorescence, confocal)

Further Reading:

Ruchaya et al. (2014) Experimental Physiology, 99(1) 111-122.

Biomechanical Engineering

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Strength of a novel shoulder rotator cuff reconstruction technique
Rotator cuff tears are a common cause of shoulder pain and account for approximately 75,000 operations each year. The past decade has seen the evolution of rotator cuff tear management from open and minimally open repairs, to all-arthroscopic techniques. The now common use of suture anchors in shoulder surgery has revolutionised athroscopic rotator cuff repair. Despite technological advances, complications may occur with arthroscopic suture anchors, including failure of the tissue, suture, or anchor before healing has occurred. This project, in conjunction with the orthopedic company Smith & Nephew, will investigate the pull-out strength and stiffness of a novel cruciate suture repair technique. It is hypothesised that using a conventional suture anchor with a cruciate suture configuration will lead to improved tendon pull-out strength and stiffness compared to conventional single-row techniques. Experiments will be performed on shoulder cadavers using a material test system (MTS) and custom designed fixtures. The student will work with an orthopedic surgeon to perform the shoulder reconstructions and all experiments.

A biomechanical evaluation of clavicle fracture plate fixation techniques
Clavicle fractures are increasingly managed operatively, with plate fixation being the most common form of internal fixation. There have been recent concerns of vascular injury, including arterial and venous, occurring during screw drilling and insertion, particularly in the area just medial to the midpoint of the clavicle. It has been suggested that one way to avoid vessel injury is to ensure screws are uni-cortical (passing through one layer of clavicular cortical bone only). Clinical and biomechanical studies show that locked vs non-locked uni-cortical screws may not have the strength of the gold standard bi-cortical fixation. However, in practice, most surgeons use a hybrid construct comprising long non-locking ‘steerage’ screws and locked screws (Figure 1). At present, this hybrid fixation technique has not been biomechanically assessed nor compared to conventional uni-cortical fixation techniques. The aim of this study is to compare the bending and torsional strength of three fixations for clavicle fracture repair: a uni-cortical locking construct, a uni-cortical non-locking construct, and a novel hybrid uni-cortical construct utilising long non-locking steerage screws and locking screws. Thirty human clavicle specimens will be harvested, fractured, and repaired using the aforementioned fixation techniques

(3 groups of 10). Specimens will then be tested in bending and torsion using a Materials Testing System. The student will work with an orthopaedic surgeon to perform the surgical procedures.

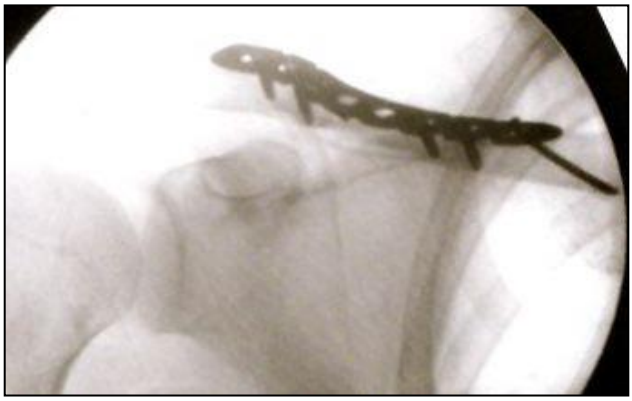

Figure 1: Hybrid construct comprising a long non-locking oblique 'steerage' screw and locking screws