Honours year in the Faculty of Science: Bachelor of Medical Science: BMedSc(Hons), Bachelor of Science: BSc(Hons)

After completing the requirements for award of a BSc or BMedSc, a student who has **majored** in Physiology or a related Discipline (**and have undertaken at least one Senior UoS in Physiology**) may be permitted to undertake a fourth 'Honours', year. To qualify for entry into these honours courses you need to meet the minimum requirements of the Faculty of Science and the Discipline. Honours in Physiology is primarily comprised of a research project carried out under the supervision of a member of the Academic Staff. Throughout the academic year honours students will attend timetabled honours sessions. These provide training in research and presentation skills as a cohort experience. Early in the course each student is required to write a review essay based on the literature of their research area and an introductory presentation. Examination is mainly by thesis, with the student's performance in the laboratory, honours sessions and in the end-of-year public seminar being considered.

Opportunities exist to gain teaching experience by casual employment as a demonstrator in under-graduate practical classes.

Application Process and Deadline

All students must apply to be admitted to an Honours year. Gaining entry into Honours is competitive - the standard you need to achieve must meet certain minimum requirements, but will also depend on the number and quality of other applicants in that year. **You are required to meet with the Hounours coordinator, A/Prof Assinder (stephen.assinder@sydney.edu.au), to discuss your intentions and lodge a completed expression of interest form with him prior to lodging an application form with the Faculty of Science.** An EoI form can be downloaded from the Discipline website at http://sydney.edu.au/medicine/physiology/students/honours/index.php). A copy of a completed and signed EoI is the only documentation you should submit as proof of contact with the Discipline, and that will be accepted. **Only those applicants that have attached a completed EoI and that have met with A/Prof Assinder prior to lodging their application will be approved.**

An application process checklist is provided below.

Domestic students - internal (currently enrolled) and external

Domestic students please note: As deferment of honours is not possible, you must apply in the semester prior to commencement.

Students applying for Honours will need to use the resources on this page to follow these steps:

1. Download and read the Honours Application Information
2. Meet with potential supervisors.
3. Those students intending to undertake honours in Physiology need to agree a project with a supervisor and obtain their agreement by signing the expression of Interest form (available on the Physiology website: http://sydney.edu.au/medicine/physiology/students/honours/index.php).
4. Fulfil any requirements of the school or department prior to lodging an application at the Faculty. This might include matching projects and available supervisors with students.
5. Those students intending to undertake honours in Physiology need to meet with the honours coordinator, A/Prof Assinder **prior** to lodging your application online.
6. If you are applying for Honours in more than one area, then list your areas in order of preference on the application form. You will need to repeat step 2 for each area you are

applying to. Please list the discipline area (eg. Biology, Cell Pathology) as appears on the coordinator contact list, or your application may not be considered.

7. Submit a completed online application form including relevant documentation (A completed and signed EoI is required by Physiology, by the due date (you will need to find your honours course among the list of courses, then click "Apply Now").

Honours Application Deadlines

Honours application deadline (for Honours commencement in Semester 1, 2018)	Thursday 30 November 2017

A step-by-step guide to the application process has been developed by the Faculty of Science and is available on the Faculty website under Honours.

Graduate Diploma in Science: GradDipSc

Students who do not qualify for the above Honours courses may be admitted to a program essentially identical to that of the BMedSc(Hons)/BSc(Hons) and obtain the diploma upon successful completion of this one year program. However, the GradDipSc is a full fee course while the Honours course incur a HECs payment.

Master of Philosophy(MPhil)

Another option for students who might not meet the requirements for honours. Medical and Science graduates may enrol for the degree of Master of Philosophy, by research and thesis. Candidature lasts from 1 to 2 years full-time; 2 to 4 years part-time. Enrolment for this degree is made through the Sydney Medical School. The candidate is expected to complete an appropriate research project and submit a thesis. You should discuss options and likelihood of acceptance with the Postgarduate Coordinator, School of Medical Sciences.

Scholarships

The Faculty of Science offers a full year and summer scholarships for Honours students. More information can be found: at http://sydney.edu.au/scholarships/current/honours_scholarships.shtml

The **Discipline of Physiology** offers a number of summer vacation scholarships for students who complete either an 8 week ($1000) or 4 week ($500) period of research during the summer vacation and who subsequently enrol in Honours and who hold no other scholarships. Award of the scholarships are made solely at the discretion of the Discipline. Your intention to claim a summer scholarship must be declared on your EoI form at time of lodging.

The **Faculty of Medicine** also offers Summer Research Scholarships to full-time students currently enrolled in Australian or New Zealand universities. For more information and to look at the wide range of projects available please go to the following website:
http://www.medfac.usyd.edu.au/research/srs/index.php

Honours Projects - 2018
A list of projects available to "Honours" candidates in 2018 is provided on our website (http://sydney.edu.au/medicine/physiology/students/honours/index.php). These one-year research programs are available in a number of themes that can accommodate a wide range of students. The Honours home page gives information on the current program and expectations.

A/Prof Stephen Assinder is the honours coordinator and should be contacted for general information at stephen.assinder@sydney.edu.au

Potential supervisors within the discipline should be contacted directly to discuss specific opportunities. The following research labs are associated with the discipline.

- Andrology Research Group – A/Prof Stephen Assinder
- Neurobiology Laboratory - Professor M.R. Bennett
- Epithelial Transport Laboratory – A/Prof Anuwat Dinudom & Professor D.I. Cook
- Developmental Physiology Laboratory – A/Prof M. Day
- Laboratory of Blood Cell Development – Dr Stuart Fraser
- Lipid Metabolism Laboratory – Dr Andrew Hoy
- Laboratory of Developmental Neurobiology – A/Prof C. Leamey
- Visual Neuroscience Research Group – Professor Paul Martin.
- VitaminD, Bone & Skin Cell Laboratory - Professor R.S. Mason
- Environmental Control of Physiology Laboratory - Dr Bronwyn McAllan
- Embryonic Stem Cell Laboratory - Dr Michael Morris
- Developmental & Cancer Biology Laboratory – A/Prof Matthew Naylor
- Molecular Neuroscience Laboratory – A/Prof W.D. Phillips
- High Blood Pressure research Group – Professor Paul Pilowsky.
- Vision Laboratory - Dr D. Protti
- Molecular Physiology of Membrane Transport – Professor Phil Poronnik
- Systems Neuroscience Laboratory - Dr Atomu Sawatari
- Laboratory of Motor & Sensory Systems – Dr Haydn Allbutt
- Retinal & Cerebral Neurobiology Laboratory – Dr Dan Johnstone and Professor Jonathon Stone
- Skeletal Endocrine Laboratory – Dr Tara Speranza
- Diabetes and Insulin Secretion Laboratory – Professor Peter Thorn

External Projects
The Discipline of Physiology may also accept into its Honours Course students who are performing their research in laboratories outside the Department. The project must be closely allied to physiology and ideally an internal supervisor who is familiar with the area must be prepared to act as the associate supervisor. The student must attend the teaching sessions for Honours students that occur weekly within the Discipline.

Previously students have successfully completed honours projects hosted by research groups of the:
Heart Research Institute
The Brain and Mind Centre
The Kolling Institute

Reasearch groups that have indicated their interest in taking such students are provided on our website. You should contact the supervisor of the project directly to express your interest.

OFFERED PROJECTS IN 2018
(listed by theme)

Nervous System, Senses and Movement:

MOLECULAR NEUROSCIENCE LAB

Supervisor: A/Prof Bill Phillips email: william.phillips@sydney.edu.au

We study the molecular mechanisms of synapse development and adaptation, focusing on the mammalian neuromuscular synapse. Muscle specific kinase (MuSK) is a receptor tyrosine kinase that coordinates the embryonic development of the neuromuscular synapse. We have a particular interest in learning how synaptic signalling systems such as the MuSK system maintain healthy synapses and how they might be targeted in new ways of treating neuromuscular diseases.

Project 1: Muscle Specific Kinase signalling and Duchenne muscular dystrophy
In Duchenne muscular dystrophy (DMD), deficiency of the muscle membrane protein, dystrophin, makes the muscle fibre vulnerable to damage with repeated rounds of fibre degeneration and regeneration leading to muscle atrophy. Recently we have found that increasing the expression or function of the muscle specific kinase (MuSK) using an adeno-associated viral vector protected dystrophic muscles from damage, in the mdx mouse model of DMD. This project will investigate, previously unknown functions of MuSK and the potential of the MuSK system to protect dystrophic muscles from degeneration. The project will involve analysing mRNA and immunolabelling to assess the impact of AAV-MuSK in muscle fibres.

Project 2: Role of MuSK in muscle-to-nerve homeostatic feedback signalling
Muscle specific kinase (MuSK) is a receptor tyrosine kinase that coordinates the embryonic development of the neuromuscular synapse. Many cases of myasthenia gravis are caused by autoimmune antibodies that interrupt the physiological function of MuSK resulting in loss of postsynaptic acetylcholine receptors. These same autoantibodies also block the physiological adaptation of the nerve terminal, which would otherwise increase release of acetylcholine to compensate for reduced postsynaptic acetylcholine responsiveness. This project will use intracellular electrophysiological recordings to investigate the effect of increasing the expression level of MuSK upon neuromuscular transmission. It's a project for someone good with hand-eye coordination, patience and an interest in developing electrophysiology skills. The project has the potential to identify an important new MuSK-mediated synaptic feedback mechanism relevant to progress in myasthenia gravis research.

Project 3: How do cannabinoids regulate neuromuscular synaptic transmission?
In the brain endogenous cannabinoids, such as anandamide, are produced by postsynaptic neurons when they are over-activated. They provide a feedback signal that then reduces the number of vesicles of transmitter released by presynaptic nerve terminals onto the postsynaptic neuron. Recent work in the lab by Marco Morsch found a very different effect of cannabinoids at the neuromuscular junction. Marco found that agonists of the cannabinoid receptor, CB1, had no effect upon the number of quanta released by nerve terminals but instead they increased the quantal size. His work suggests that cannabinoids increase in the amount of acetylcholine packed into each synaptic vesicle. This project will use intracellular electrophysiology to test the effects of other cannabinoid compounds and pharmacological antagonists of cannabinoid receptors to help clarify the signalling pathway. This project may help reveal a new physiological feedback system at the neuromuscular junction that helps maintain control of muscle during prolonged muscle contractions.

LABORATORY OF DEVELOPMENTAL NEUROBIOLOGY

Dr Catherine A. Leamey
Phone: 9351 4352
Email: cathy@physiol.usyd.edu.au
Anderson Stuart Bldg, Room N663

Neural connections underlie every aspect of our perception, behaviour and cognition and are a product of both genetic factors and environment/experience. The visual pathway is particularly useful for investigating the relative roles of specific proteins and experience in the assembly and function of neural circuits. We have recently discovered that a family of molecules, known as the Ten-ms or Teneurins, play important roles in wiring up binocular visual pathways. The miswiring present in the knockout strains leads to behaviorally measureable visual deficits.

Potential projects include:

1) ***The capacity for Environmental Enrichment to restore function in Ten-m3 KOs:*** The misalignment of projections present in Ten-m3 KOs leads to a form of functional blindness. Recent data suggests that enhancing the animals experience via environmental enrichment can lead to a recovery of vision. A number of projects exploring this important research avenue, including assessment of the degree of recovery following enrichment at different ages, investigation of underlying mechansims and mimicking enrichment by addition of pharmacological agents are available.

2) ***Impact of increased ipsilateral projections on binocular vision:*** Our preliminary data demonstrates that Ten-m4 KO mice have additional ipsilateral projections from dorsal retina. The chief aim of this study is to determine how these extra projections affect the visual ability of the mice using a new behavioural paradigm developed in the lab.

3) **C*ellular substrates of binocular vision:*** How does an environmental stimulus lead to a behavioural response? Recent work has demonstrated that ethologically-relevant visual stimuli trigger robust behavioural responses in mice. The circuits underlying these responses are not known, but provide enormous potential for understanding how visual input is processed and transformed to trigger an appropriate behaviour. The visual deficits present in Ten-m KO mice present additional windows into the function of specific cell types. Projects addressing these issues using multi-photon imaging, neural tracing and electrophysiological recording are available.

Some of these are offered as collaborative projects in association with Dr Atomu Sawatari and Dr Dario Protti's laboratories. Other projects may also be available on request.

VISION LABORATORY

Dario Protti, dario.protti@ sydney.edu.au, Anderson Stuart Bldg, Room N659, Telephone: +61 2 9351 3928

Our research work focuses on the function of the retina. Specific neuronal circuits provide ganglion cells, the output neurons of the retina, with excitatory and inhibitory inputs whose relative magnitude and timing determine the spatial and temporal properties of the electrical signals sent to higher visual centres in the brain. The relative impact of excitation and inhibition on ganglion cells output, however, is not well understood.
We are currently investigating the effect of cannabinoids, derivative compounds of marijuana, on the physiological properties of different types of ganglion cells in the eye.

Projects:
1) Cannabinoids effects on vision:
We have recently shown that exogenous cannabinoids modulate the transmission of visual information in the retina. In addition, we showed that the endocannabinoid system is active in normal conditions. This project will investigate how and where cannabinoids act on the retina by studying the effects of drugs that target different components of the cannabinoid system. For these studies we use genetically modified animals that express the light-sensitive membrane protein *channelrhodopsin* and other animals in which membrane channels have been knocked out.

2) The yin and yang of excitation and inhibition in the retina: Ganglion cells integrate excitatory and inhibitory signals from bipolar and amacrine cells respectively. The strength and relative timing of these inputs determine the output properties of ganglion cells. This project investigates how the balance of excitatory and inhibitory inputs impact on ganglion cell responses. To gain insight into this, we inject to various combinations of excitatory and inhibitory currents into ganglion cells and record their responses. This link to our video article can give you a good idea of the experimental approach:
http://www.jove.com/video/50400/implementing-dynamic-clamp-with-synaptic-artificialconductances
This is a joint project between Dr Dario Protti and Dr Jin Huang (N659. 9351 9065, jin.huang@sydney.edu.au)

The techniques used in these projects are patch-clamp recordings, optogenetics, dynamic-clamp recordings, confocal microscopy and computer modelling. For more information please contact Dr. Dario Protti: dario.protti@sydney.edu.au

Other projects are offered in collaboration with Dr. Cathy Leamey and Dr. Atomu Sawatari.

Cancer, Cell Biology, Reproduction and Development, Endocrinology

ANDROLOGY RESEARCH GROUP,

A/Prof Steve Assinder:
Medical Foundation Building Room G46 Telephone: +61 2 90363614
stephen.assinder@sydney.edu.au

Prostate disease is very common in the ageing male. Prostate cancer is the most commonly diagnosed cancer in men and second most frequent cause of cancer-related deaths. It is estimated that there is at least 1 death every 4 minutes worldwide attributed to prostate cancer. Benign prostatic hyperplasia (BPH) affects at least half of all men over the age of 60 years and presents a significant burden to health care costs. Evidence suggests that obesity is a risk factor for both BPH and prostate cancer.

Our research is focused on oxytocin, the hormone of love. Our recent work has indicated that oxytocin acts at the prostate to increase *de novo* steroidogenesis. It has also shown that this hormone might also affect tissues important in regulating energy balance.

Projects: How might the "hormone of love" mitigate obesity and type 2 diabetes?
Oxytocin is best known for its roles in maternal physiology. During childbirth this hormone is released from the mothers brain and acts on the uterus to induce and maintain contractions. Following birth oxytocin is important in stimulating the release of milk during breast-feeding. Since the description of these classical actions oxytocin has been shown to have key roles in modulating maternal behaviour as well as other social behaviours such as pair-bonding. Indeed, it is for these roles that oxytocin has been dubbed "the hormone of love" (reviewed in Tom and Assinder, 2010). More recent evidences suggest positive metabolic effects of oxytocin through improved glucose metabolism, circulating lipid profiles, and increased insulin sensitivity (reviewed in Elabd and Sabry, 2015; Altirriba *et al*., 2015). Hence, oxytocin is suggested to have pharmacological efficacy in treating obesity and type 2 diabetes.
Possible projects would determine: 1) how oxytocin modulates adipose and adrenal secretion of hormones important to maintaining energy balance; 2) how oxytocin affects lipid metabolism in adipose tissue and the liver and; 3) whether it modulates liver glucose metabolism (these projects are in collaboration with Dr Andrew Hoy); 4) whether oxytocin affects mitochondrial activity (in collaboration with Dr Ryan Davis, Koling Institute).

VITAMIN D, BONE AND SKIN LABORATORY

Anderson Stuart Bldg, Room N543, Telephone: +61 2 9351 2561 or +61 2 9351 4099

Professor Rebecca Mason.
The group has a particular interest in vitamin D and calcium physiology and the role of skeletal muscle in the maintenance of vitamin D status is being investigated in the offered project.

Project: The role of muscle in the maintenance of vitamin D status
Most vitamin D is made in skin as a result of a photochemical reaction between UVB light and 7-dehydrocholesterol. The vitamin D is then converted to 25-hydroxyvitamin D, the major circulating form of vitamin D, in the liver and then to the active hormone, 1,25dihydroxyvitamin D in the kidney and other tissues. The half-life of 25-hydroxyvitamin D in blood is inexplicably much longer than that of most steroids and much longer than its binding protein. Because of this and since there is relatively little vitamin D made in winter (not much UVB and not much skin exposed), there must be a mechanism that reduces the degradation of 25-hydroxyvitamin D, to allow for this long period in which 25-hydroxyvitamin D stays in blood without being degraded. How this happens

has not been investigated. Several lines of indirect evidence are consistent with a proposal that muscle somehow contributes to the longer than expected half-life of 25-hydroxyvitamin D. The project, which includes whole animal and cell culture studies, will test this hypothesis.

Abboud MA*, Puglisi DA*, Davies BN*, Rybchyn M, Whitehead NP, Brock KE, Cole L, Gordon-Thomson C, Fraser DR, Mason RS. Evidence for a specific uptake and retention mechanism for 25-hydroxyvitamin D in skeletal muscle cells. Endocrinology 154(9):3022-3030, 2013. *doi:10.1210/en.2012-2245*. * these authors contributed equally to the work.
Abboud M, Rybchyn MS, Liu J, Ning Y, Gordon-Thomson C, Brennan Speranza TC, Cole L, Greenfield H, Fraser DR, Mason RS. The effect of parathyroid hormone on the uptake and retention of 25-hydroxyvitamin D in skeletal muscle cells. J Steroid Biochem Mol Biol http://dx.doi.org/10.1016/j.jsbmb.2017.01.001 **(available online 16 Jan 2017)**

Direct inquiries can be made by email to: mark.rybchyn@sydney.edu.au or rebecca.mason@sydney.edu.au

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SKELETAL ENDOCRINE LABORATORY

Dr Tara Speranza: tara.speranza@sydney.edu.au or 9351 4099

Dr Speranza's laboratory investigates the role of the musculoskeletal system in macro nutrient metabolism, focusing on the proteins, receptors and pathways involved in this multi-system endocrine loop. Studies also include investigations into possible therapeutic agents on the skeleton, with a focus on the actions of bone forming cells (osteoblasts) and bone resorbing cells (osteoclasts).

Project 1: *The structural and cellular basis of skeletal fragility in type II diabetes mellitus*
Patients with T2DM have hyperglycemia and normal to high bone mineral density (BMD). This is usually associated with reduced fracture risk, yet patients with T2DM have a higher incidence of fragility fractures and an increased overall fracture risk. The increase in fractures in patients with T2DM is independent of factors such as age, sex, BMI, tendency to fall and visual impairment. This implies the increased fracture risk is driven by compromised bone quality. The aim of the current study is to test this hypothesis in mice and elucidate the specific mechanisms of action. Few rodent studies have assessed the total effects of hyperglycemia on the skeleton.
Mice will be allocated to a normal chow or a high fat (60%) diet. Insulin sensitivity and glucose tolerance will be monitored. The effects of hyperglycemia on the skeleton will be tested by analysing microarchitecture using uCT methods, histologically via fluorescent calcein staining for dynamic histomorphometry and for immunohistochemical analysis of the incorporation of AGE products: CML and pentosidine. Lastly, the molecular pathways for the basis of these findings will be analysed via qRT-PCR and western blotting to analyse the sclerostin content in bone and BMP7/Smad1/5/8 pathway.

Project 2: *The effects of hyperglycemia on human osteoclastic bone resorption in vitro*
Increased bone fragility and reduced skeletal muscle quality are under-recognised complications of long-term hyperglycemia in type 2 diabetes mellitus. As a result, patients have an increased risk of falls, fractures, and a reduced quality of life. Overall, human data thus far suggests a deterioration of tissue mineral quality and strength, likely brought about by adverse effects of long-term hyperglycemia on bone matrix and the bone cells. T2DM patients have reduced bone formation markers and some evidence that resorption makers are reduced: serum carboxy-terminal cross-linked telopeptide of type I collagen (CTX), indicating bone cells are adversely affected. This project is aimed at testing whether hyperglycemia directly reduces the activity of the bone resorbing cells, the osteoclasts. Human blood monocytes will be cultured and differentiated on coverslips and treated

with increasing concentrations of glucose over several weeks to form mature, bone resorbing osteoclasts. Cells will be stained for numbers and resorption markers and properties. Secondly, cells will be cultured and differentiated on slices of whale dentine in increasing concentrations of glucose. The dentine slices will then be analysed by electron scanning microscopy (SEM) to determine the amount of resorption carried out by these cells.

Project 3: *The role of osteocalcin in the modulation of whole body energy metabolism*
Osteocalcin is a bone-specific protein but recent evidence indicates that it plays a previously unsuspected role in the control of glucose and fat (energy) metabolism (Brennan-Speranza et al. JCI, 122:4172-4189, 2012). The mechanism by which the body senses osteocalcin is still unclear although evidence points to a Class C G-protein coupled receptor (the GPRC6A) as the osteocalcin receptor. This project aims to uncover the controversies surrounding osteocalcin-sensing by the body as well as further understanding the pathways by which this little protein from the skeleton controls whole body energy metabolism using molecular and cell biology techniques and mouse models.

LIPID METABOLISM LABORATORY

LIPID METABOLISM LABORATORY, Lab 3 West, The Hub, Charles Perkins Centre
Telephone: +61 2 9351 2514
Dr Andrew HOY
andrew.hoy@sydney.edu.au
@HoyLipidLab

The Lipid Metabolism Laboratory investigates the mechanisms linking perturbed lipid metabolism and a range of pathologies. Currently, our primary interests are in insulin resistance/type 2 diabetes/obesity and cancer including breast, pancreatic and prostate, particularly how these cancers behave differently in an obese patient vs a lean patient. The lab located in The Hub, Charles Perkins Centre where the following projects will be available.

Project 1: Novel proteins involved in fatty liver and insulin resistance
Insulin resistance is a unifying feature of the metabolic syndrome. The liver is an early site of perturbed insulin action and a critical regulator of whole body glucose and lipid homeostasis. The lipid droplet is the major site for storage of lipids and the movement of stored lipids out of this reservoir is highly regulated. We have recently identified proteins that locate to the lipid droplet whose abundance is altered in fatty liver and insulin resistance using innovative mass spectrometry and bioinformatic approaches. In this project these highly novel candidate targets will be characterised using techniques including cutting-edge microscopy, cell culture, biochemical and radiometric metabolic analysis, and genetic manipulation.

Project 2: Lipid metabolism and breast and prostate cancer
Lipid accumulation in both breast and prostate cancer is a common observation, especially in aggressive cancers. The vast majority of lipid is stored as triacylglycerols in lipid droplets within these cells. These lipid droplets are closely located to mitochondria, to serve a readily available supply of energy for tumour progression. This project will target the enzymes that regulate lipid flux at the lipid droplet, and elucidate their function and potential for therapeutic targeting in cancer. The project is part of a Movember-funded international program and will employ techniques including cell culture, genetic manipulation, radiolabel metabolic analysis, mass spectrometery, cutting-edge microscopy and cancer cell progression including proliferation, migration and invasion.

Project 3: Nutritional geometric framework and liver biology
Fatty liver is an initial starting point for a wide-range of pathologies. The seminal work of Prof Steve Simpson and Dr Samantha Solon-Biet has identified components of dietary intake, that for reasons yet to be determined, result in fatty liver. In collaboration with Prof Simpson and Dr Solon-Biet, this project will elucidate the influence of branch chain amino acids, non-branch chain amino acids and other factors on liver lipid homeostasis and its potential flow-on effect on hepatocellular carcinoma progression. The project will employ techniques including cell culture, biochemical analysis, gene expression, genetic manipulation, radiometric metabolic analysis and cancer cell proliferation.

Direct enquiries can be made by email to: Dr Andrew Hoy – andrew.hoy@sydney.edu.au

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DEVELOPMENTAL PHYSIOLOGY LABORATORY

Medical Foundation Building, Room 232, 92-94 Parramatta Rd Camperdown, Telephone: +61 2 9036 3312

Dr Margot Day
Roughly 3% of babies born in Australia result from assisted reproduction involving fertilization and then culture of the embryo in vitro. It is known that the embryo culture environment causes significant alterations in gene expression, epigenetics, metabolism and cell proliferation during preimplantation development and that these alterations may have effects on later life.

Our studies aim to help us to understand the impact of the culture environment on pre-implantation embryonic development in order to improve reproductive outcomes. We study the physiological processes involved in fertilization of the oocyte and proliferation of the cells in the preimplantation embryo.

To do this we use a range of techniques including in vitro fertilization, isolation and culture of preimplantation mouse embryos, gene expression, cell signalling, electrophysiology and live cell imaging.

Honours projects are available on the following topics:
- The mechanisms by which the amino acids improve blastocyst development in vitro.
- The role of cell cycle regulated K channels in proliferation of embryonic cells.
- The expression of scaffolding proteins during early embryo development (in collaboration with Prof. Phil Poronnik).

Direct enquiries can be made by email to: Dr Margot Day - margotd@physiol.usyd.edu.au

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EMBRYONIC STEM CELL LABORATORY

Medical Foundation Building

Dr Michael Morris
ph: 9036 3276; m.morris@sydney.edu.au
Room 139 Medical Foundation Building
How do mammalian, including human, embryos grow? And how can stem cells grown in the laboratory shed light on the highly complex mechanisms that control development? My lab focuses on understanding the complex molecular pathways and circuits that control early stages of development – from pluripotency to gastrulation to cells of the developing nervous system.

Project Title: *Modelling early embryo development and neurogenesis using embryonic stem cells*

Embryonic stem (ES) cells have the potential to differentiate into any cell type of the developing embryo and adult. So, they are invaluable in understanding the molecular mechanisms that drive normal development and can provide a window into what happens during abnormal development. ES cells also have great potential in treating a large number of currently incurable or poorly treatable human diseases and injuries, including neuropathies, brain and spinal injuries, muscular diseases, and diabetes. We use ES cells as an *in vitro* model to understand the key molecular mechanisms underpinning critical developmental milestones forming the nervous system.
We also develop protocols to direct the differentiation of ES cells to specific cell types that can be used in animal models of human disease and injury. In addition, we apply the knowledge we have gained from stem-cell behaviour *in vitro* to determine if the development of embryos themselves is controlled by the same or similar mechanisms. Thus, these projects examine the processes of development from pluripotency to germ layer formation to early neurogenesis and on to mature neural cells such as neurons, glia and neural crest cells that make up the central and peripheral nervous system. Our focus is on the many interacting signaling pathways and metabolic events that drive this directed differentiation.
Techniques to be used in these projects include tissue culture, cell signalling analysis, gene expression analysis, immunohistochemistry and fluorescence microscopy, flow cytometry, and measurements of aerobic and anaerobic metabolism.

ENVIRONMENTAL CONTROL OF PHYSIOLOGY LABORATORY

Medical Foundation Building (K25) Room G44; Telephone 9036 3615; Email: bmcallan@medsci.usyd.edu.au

Dr Bronwyn McAllan
Animal models are frequently used to understand physiological mechanisms. Comparative Physiologists use the diverse information discovered from a wide variety of non-laboratory animals to help formulate ideas about physiological processes. Our current research has focused on the environmental control of structure and function in mammals, especially marsupials. Research includes the seasonal physiological and endocrinological changes in mammals and their morphological implications. We use the small marsupials *Antechinus stuartii* (brown antechinus), *Sminthopsis macroura* (stripe-faced dunnart), and *S. crassicaudata* (fat-tailed dunnart) as animal models. Projects include collaborations with other research groups at USYD and the UNSW.

Project 1: *The regulation of reproduction and metabolism by photoperiod and temperature***.**
Seasonal changes in reproduction and torpor use (measured by open flow respirometry) are important for the survival of many small mammals. By exposing the marsupials *Sminthopsis macroura* and *Sminthopsis crassicadata* to different photoperiods and temperatures we can understand more about the survival responses of mammals to environmental change.

Project 2: *Understanding the common molecules needed for live birth in vertebrates.*
(With Prof Chris Murphy (Anatomy) & Prof Michael Thompson (Biol Sci))
The evolution of complex placentae is a fundamentally interesting but rare event, because it requires development of new structures and processes. With 100+ origins of viviparity, reptiles and mammals provide outstanding models for studying the evolution of a common vertebrate characteristic, namely viviparity and complex placentation. The honours project will focus on molecules which are important in the plasma membrane transformation of the uterus in preparation for implantation and later placental development in a marsupial mammal, *Sminthopsis crassicaudata*.

DEVELOPMENTAL & CANCER BIOLOGY LABORATORY

Anderson Stuart Bldg, Room N401 Email: matthew.naylor@sydney.edu.au; Tel: +61 2 9351 4267

A/Prof Matt Naylor

Research in the Developmental & Cancer Biology Lab focuses on understanding how normal development and cell function is controlled, and then, how this regulation is perturbed to result in human disease such as cancer. Specifically, research in the lab has focused on transcriptional and cell-matrix 'master' regulators of cell fate (eg. whether or not a cell undergoes proliferation or differentiation) in breast and prostate development and cancer. Using whole genome transcript profiling and subsequent mouse and human cell based models, we have identified several novel regulators of normal breast and prostate development and shown that altering the function of these genes can either speed up or slow down cancer progression.

Project Descriptions:

1) Investigate the role of Paxillin in breast cancer & metastasis. Breast cancer is the most common invasive cancer of women, with Australian women having a lifetime risk of 1 in 9 for developing the disease. Although prognosis for early or locally contained disease is good, patients diagnosed with metastasis have a long term survival rate of only 5-10%. We have previously shown that Integrins, which regulate the interaction between a cell an its local environment, control normal breast development and cancer progression. The role of paxillin, an integrin adaptor protein in this process remains unknown, but its expression is correlated with aggressive disease and cancer cell migration. This project will explore the role of paxillin in breast cancer cell function, tumourigenesis and metastasis. Techniques employed will include a combination of in vitro based techniques such as cell culture, morphology, migration and proliferation assays, shRNA, and in vivo based approaches such as genetic mouse models and xenografts.

2) Exploring the role of Paxillin in prostate cancer. Prostate cancer is the most common cancer of men and kills as many men as breast cancer does women each year. Similar to the Paxillin Breast Cancer Project, we have also recently demonstrated a role for a number of integrin and integrin related molecules in the progression of prostate cancer. This project will continue to explore the role of integrin signaling in prostate development and prostate cancer progression by using newly generated Paxillin floxed genetic mouse models, transgenic prostate cancer mouse models and by determining the effects of paxillin in prostate cancer cell function using cell culture, morphology, migration and proliferation assays, shRNA viral approaches, and further in vivo based approaches such as xenografts.

3) Metabolism and breast cancer. There is a clear link between metabolic disorders and obesity within a variety of different cancer types, including breast cancer. In addition, a key component in the progression of cancer is said to be the ability of a cancer cell to rewire its metabolic pathways to cope with increased energetic and biosynthetic demands required during tumour progression. We have demonstrated a novel role for ACC1 in this process. Using novel inhibitors in cell culture studies along with proliferation assays and mouse based carcinogenesis models, this project will investigate the effects of inhibiting lipogenesis and determine the subsequent effects on breast cancer cell growth and tumourigenesis.

4) Transcriptional regulators of mammary gland development and breast cancer. Control of cell fate and normal cell function is critical during development and is often perturbed during carcinogenesis and tumour progression. We have identified and developed a number of new mouse and cell based models to investigate or continue to define a completely novel function for a number of transcription factors not previously implicated in both the regulation of normal breast development or breast cancer. This project will utilise similar approaches and techniques to the projects previously described to determine the role of these transcription factors in the breast.

DIABETES AND INSULIN SECRETION LABORATORY

Charles Perkins Centre (contact, p.thorn@sydney.edu.au, Lab website, www.thornlab.com)
Professor Peter Thorn
Our group uses cutting-edge microscopy, transgenic and molecular approaches to understand how insulin secretion is regulated in health and disease. Our latest work suggests that insulin secretion from pancreatic beta cells is controlled through synaptic-like connections with the blood vessels of the islet. Our lab is based in the Charles Perkins Centre and consists of post-docs and students who will train and support the Honours students in these projects.

Project 1. Understanding how the pancreatic beta cell synapse controls insulin secretion.
Our discovery that beta cells secrete insulin via a synaptic-like connection with blood vessels in the islet challenges accepted models of insulin secretion. Ongoing work in the lab is showing the synapse changes in type 2 diabetes, suggesting it may be significant in disease. The next step in this work is to prove that functional interactions in the synapse have significance for the control of insulin secretion. To this end, in this project we will stain for the key proteins in the beta cell synapse and use super resolution microscopy to determine their relative position. This approach will be complemented by live-cell two-photon imaging of insulin secretion. The outcomes of the project will be significant for both understanding and treatment of diabetes.

Project 2. Refining cell-based therapies to cure type 1 diabetes.
We are working to engineer induced pluripotent stem cells to make them secrete insulin. Our experiments are testing some of the factors we are finding to be important in the control of beta cells in the islet with an aim to enhance the control of insulin secretion. For diabetic patients, cell replacement therapies have the promise, one day, to provide a cure for disease.

Energy Metabolism and Insulin Action Laboratory
Professor Gregory Cooney
Level 5 West, Charles Perkins Centre D17, (gregory.cooney@sydney.edu.au)
Dr Amanda Brandon
Level 3 West, Charles Perkins Centre D17, (amanda.brandon@sydney.edu.au)

The broad aim of our research is to understand how different aspects of genetic make-up, environment (temperature, nutrition) and behavior (exercise) contribute to altered energy balance and the development of obesity and metabolic disease.

Project: Role of environment and nutrition in the development of obesity-induced insulin resistance.
The exact mechanisms responsible for obesity-related metabolic disease such as diabetes and cardiovascular disease are not completely understood but are associated with the development of insulin resistance in liver, muscle and adipose tissue. Over-nutrition (including the macronutrient content of the diet) and environmental conditions like ambient temperature and exercise can impact on body fat accumulation and alter normal metabolism. We have developed dietary models of obesity in mice housed at different temperatures that differ in the degree of impairment of insulin action. This project will comprehensively examine the differences in insulin action in tissues from these mouse models using metabolic flux measurements, assessment of insulin signalling pathways and lipidomic and proteomic analysis to tease out what aspects of obesity predispose animals to insulin resistance and whether dietary or environmental interventions can reduce obesity-related metabolic disease.

Metabolic Cybernetics Laboratory

Professor David James
Charles Perkins Centre – Level 5 West
david.james@sydney.edu.au

We are a lively and interactive team of ~20+ Postdocs, PhD & Honours students and RAs with a broad range of expertise spanning biochemistry, cell biology, animal physiology, systems biology and bioinformatics. We study insulin action, exercise and metabolism in health and disease in a broad range of systems, including fat and muscle cells, mice, flies and humans. We use diverse methods ranging from single cell live microscopy, mass spectrometry, CrispR gene editing, screens in ~200 fly strains to bioinformatic analysis of complex Omics data.

Project 1: Mechanisms of insulin resistance
Insulin resistance is a risk factor for the development of a number of diseases including type 2 diabetes, cardiovascular disease and some cancers. Our group has discovered several links between how nutrients are processed and insulin resistance. This project aims to investigate the molecular basis for how mitochondrial metabolism affects insulin responses. Students will learn a wide range of techniques including molecular biology, cell culture, metabolic/biochemical assays, mitochondrial bioenergetics, microscopy and western blotting.

Project 2: The interaction between diet and the genome in flies and mice
How does our genetic background interact with the nutrients in our diet to promote a long and healthy life? The first studies on longevity demonstrated that calorie restriction increases lifespan in various model organisms including mice, worms and fruit flies. However, the mechanism by which caloric restriction increases lifespan remains unclear. Furthermore, increasing evidence suggests that it is the composition of macronutrients within the diet, not the reduction of total calories, that prolongs lifespan and that the effect of caloric restriction on lifespan is dependent on genetic background. This project aims to identify novel diet-dependent longevity pathways.

In this project, you will use the fruit fly (Drosophila melanogaster) as a model organism to identify genes that regulate longevity in a nutrient-dependent manner. The fruit fly is a great model organism for this type of diet-gene analysis because of the extensive genetic toolkit available and because the metabolic pathways are conserved between flies and human. You will be supervised by two post-docs who will teach you Drosophila genetics and assays for assessing longevity.

Project 3: Mapping new functions of insulin and exercise (Jacky Stoeckli, James Burchfield)
Insulin and exercise activate extensive signalling cascades to regulate an array of cellular processes. Identifying the composition of these signalling networks and the proteins responsible for eliciting specific functions of insulin and exercise is essential in understanding the defects that cause metabolic disease where insulin signalling is defective, and in harnessing the power of exercise to promote health. We have recently interrogated the insulin and exercise-regulated phosphoproteome, revealing the extent of these signalling networks and a number of new phosphorylation sites on proteins modified in response to these stimuli. This project aims to characterise the function of novel insulin or exercise-regulated phosphosites and to identify the upstream kinase. This project will involve cell culture, molecular biology, microscopy, immunoprecipitation, western blot and protein-protein interaction analysis.

Cardiovascular Physiology:

Comparative Cardiovascular Physiology

Dr Melissa Cameron: melissa.cameron@sydney.edu.au phone: 93515228

The regulation of blood pressure is tightly regulated in the resistance blood vessels within the body; therefore understanding how these vessels vasodilate and vasoconstrict is of considerable importance. My research studies the evolution of signalling pathways in the vasculature of vertebrates. In particular, I focus on nitric oxide and endothelium-derived hyperpolarisation in non-mammalian vertebrates and how these systems have evolved comparatively to mammals.

Project: Does endothelium-derived hyperpolarisation contribute to vascular tone in resistance blood vessels of non-mammalian species?
Endothelium-derived hyperpolarisation (EDH) has been shown in mammals to mediate vasodilation of resistance vessels. Currently, little is understood about the role of EDH in non-mammalian species. Recent findings from my laboratory have shown a link to EDH contributing to vasodilation in resistance blood vessels from amphibians; however, further exploration is necessary. This project will further the initial findings, with a possible expansion to other species such as fish, birds or reptiles. This project will use an integrative approach to the laboratory work, which include, but are not limited to, molecular biology techniques (gene/protein expression) and physiological techniques such as wire myography/organ bath experimentation.

External Honours Projects

The Discipline of Physiology on occasion accepts into its Honours Course students who are performing their research in laboratories outside the Department. The project must be closely allied to physiology and an internal supervisor who is familiar with the area must be prepared to act as the associate supervisor. Students undertaking projects off campus attend the all formal timetabled sessions for Honours students which occur weekly within the Discipline.

Supervisors and laboratories which have indicated their interest in taking such students are listed below. You should contact the supervisor of the project directly to discuss opportunities in the first instance.

Sensory systems and Integration | Dr. Aaron Camp
Aaron.Camp@sydney.edu.au

Project 1: A Noisy Nervous system: The impact of synaptic noise on sensory neuron sensitivity.
The output of individual sensory neurons is ultimately dependent on the combination of synaptic inputs and intrinsic neuronal properties We have shown that even small fluctuations (ones that do not cause either excitation or inhibition) in the membrane potential of sensory relay neurons produce dramatic changes in their subsequent output. This "synaptic noise" represents the impact of the network activation state within which sensory neurons are embedded, and presumably plays an important role in sensory neural signaling.

Using patch-clamp electrophysiological techniques you will investigate the sensitivity of neurons in the central vestibular (balance) pathway. This pathway provides an ideal model since neurons in the vestibular nuclei display a diverse suite of discharge properties, and are known for their capacity to undergo both synaptic and intrinsic "plasticity". Specifically, you will characterize the gain (sensitivity) of type A, B and C neurons in the Medial Vestibular Nucleus (MVN) to injections of current, and synaptic noise. This information is crucial to understand how individual sensory neurons code information about the outside world.

Project 2: The impact of visual stimulation on the vestibulospinal reflex.
Vestibular-mediated reflexes and the circuits that underlie them are embedded within a complex network of neural structures including the cerebellum, thalamus, and forebrain structures. As such it is likely that the output of the vestibular system is modulated by these structures in a context dependent manner. A question raised in this project is whether the cVEMP (a measure of balance function) can be altered in response to visual stimulation- a condition that occurs naturally, and ultimately whether there are differences in the way our nervous system modulates peripheral vestibular reflex function.

Vestibular evoked myogenic potentials will be recorded from target muscles in response to loud sound stimuli delivered via in-ear headphones. Myogenic responses will be measured using the Delsys dEMG system and data acquired using custom software prepared in-house using Matlab.

Neurogenetics Laboratory

Kolling Institute,
Northern Clinical School,
Royal North Shore Hospital,
St Leonards
Ph: 9926 4868

Dr Ryan Davis
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The Neurogenetics Laboratory bridges basic and clinical research into mitochondrial diseases, with direct access to patient-derived cell lines and samples. The main research focus is on improving mitochondrial disease diagnosis, understanding the pathophysiological basis of disease and identifying potential treatments in order to improve patient care and health outcomes.

Project: Light modulation of mitochondrial function in mitochondrial disease
Mitochondrial diseases are a large and diverse group of incurable, multisystemic, inherited disorders with the common pathological hallmark of mitochondrial respiratory chain dysfunction. There are currently no effective treatments for mitochondrial diseases. Evidence has emerged to suggest LED light exposure can improve mitochondrial function and thus improve mitochondrial disease manifestations.

In this project, mitochondrial disease patient-derived cell lines will be exposed *in vitro* to different light regimes (wavelength, duration, pulse) using a custom-built LED light source. Mitochondrial function will then be assessed to assess light-mediated improvements.

Potential methods to master during the project include cell culture, flow cytometry (general mitochondrial health assay), biochemical assays (e.g. cellular ATP synthesis rate), molecular biology assays (e.g. nuclear vs mitochondrial DNA content) and cellular bioenergetic analysis, in addition to general laboratory and research skills.

This project will be co-supervised by Assoc/Prof Stephen Assinder.

Dr Kristen Bubb
Senior Research Fellow, Oxidative Signalling group
Cardiothoracic and Vascular Medicine
University of Sydney, Kolling Institute of Medical Research
Level 12, Kolling Building, Royal North Shore Hospital, St Leonards
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We are interested in investigating oxidative modifications that lead to cardiovascular disease. We have recently discovered a role for the FXYD1 gene in regulation of cardiovascular function. This gene is highly expressed in the heart and is protective against cardiac fibrosis and vascular dysfunction.

There are several possibilities for projects within this laboratory.

1. Is FXYD1 protective against atherosclerosis development?
2. How does altering FXYD1 expression affect growth and fibrosis of vascularised cardiac spheroid "mini-hearts"?

Project 1 will involve the student investigating atherosclerosis development in mice to determine if it is worsened in the FXYD1 KO mice. We are currently crossing our colony of FXYD1 KO mice to APOE KO mice, that are more susceptible to developing atherosclerosis.
Methodology includes: Dissection of blood vessels, quantification of plaque area using histology, RNA and protein expression analysis of signalling molecules within plaques, serological quantification of cholesterol types, cell culture-based studies using macrophages and vascular cells, and advanced *in vivo* imaging to detect plaque stability. Dr Owen Tang will collaborate on this project.

Project 2 will involve developing vascularised cardiac spheroids from human or mouse heart samples, using cell culture techniques. In addition, these spheroids will be used as 'bio-ink' for bio-printing of heart tissue, using a 3D bio-printer. Analysis of spheroids and heart tissue will involve using confocal microscopy, RNA and protein expression. Silencing or overexpression of FXYD1 in specific cells within the spheroids will help to elucidate the role of FXYD1 within each cell type. Dr Carmine Gentile will collaborate on this project.

Cardiac Biology and Heart Failure Group

Scott Levick
Kolling Institute for Medical Research
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Our group uses an array of molecular, cellular, and whole animal techniques to identify mechanisms underlying adverse cardiac remodelling that leads to heart failure. Our recent work has identified a critical and complex role for neuropeptides in modulating cardiac structure and function is various disease states. Models of cardiac remodelling that we use include diabetes, hypertension, and myocardial infarction.

Project 1. Protection from Diabetic Cardiomyopathy by the Neuropeptide Substance P*:*
The diabetic heart displays a loss of the neuropeptide substance P. We have confirmed that this loss contributes to fibrosis in the diabetic heart with the finding that exogenous restoration of substance P reverses this fibrosis. We have data indicating that this is through direct effects on cardiac fibroblasts since substance P limits the excess extracellular matrix production by cardiac fibroblasts under high glucose (diabetic) conditions. The next steps are to determine the neurokinin receptor mediating the protective actions of substance P in the diabetic heart, and to identify the cellular and molecular mechanisms activated by substance P within the cardiac fibroblast that makes these cells resistant to the effects of high glucose. This project will utilise a mouse model of diabetes, measurement of cardiac function in vivo, as well as molecular approaches and mass spectrometry to identify key molecules being regulated in cardiac fibroblasts.

Project 2. The Peptide Catestatin as an Anti-Fibrotic in Cardiac Disease:
Catestatin is a peptide that is relatively understudied in the heart. Rodent studies have indicated that its administration can improve cardiac function following myocardial infarction. However, its ability to modulate cardiac fibrosis has not been examined. Cardiac fibrosis is critical because it underlies heart failure with preserved ejection fraction, which makes up approximately 50% of heart failure cases and for which there is currently no specific treatment. This project will use the angiotensin II mouse model of cardiac fibrosis to examine the ability of catestatin to protect against fibrosis. The project also includes measurement of cardiac function in vivo, as well as molecular approaches and isolated cardiac fibroblast experiments to identify key molecules being regulated in cardiac fibroblasts.

Pain Management Cellular Research Group

Kolling Institute, Northern Clinical School, Uni Sydney at Royal North Shore Hospital.
Supervisors: Dr Chris Vaughan & Dr Bryony Winters. Email: chris.vaughan@sydney.edu.au; bryony.winters@sydney.edu.au. Ph: 99264950.

Our group is examining the mechanisms underlying acute and chronic pain, and identifying novel therapeutic approaches. This work is carried out by using *in vitro* patch clamp electrophysiology and *in vivo* animal models. We are particularly interested in the endogenous cannabinoid system as a pain therapeutic target.

Project: Endocannabinoid modulation of descending inputs to the midbrain periaqueductal grey - using optogenetics and electrophysiology.

The midbrain periaqueductal grey (PAG) forms part of a descending analgesic pathway which receives inputs from higher brain centres, such as the amygdala, and projects via the medulla to the spinal cord. Endogenously generated cannabinoids produce pain relief from within the PAG by inhibiting synaptic transmission onto neurons within this brain region. The origin of these cannabinoid sensitive inputs is unknown.

This project will examine how the amygdala communicates with the PAG, and how this is modulated by endogenous cannabinoids. This project will use a combination of:
(1) patch clamp electrophysiology – to examine how endogenous cannabinoids regulate synaptic transmission in PAG slices.
(2) optogenetics – microinjection of channelrhodopsins (ChR2) into amygdala - to optically stimulate specific inputs from amygdala to PAG in the experiments described in (1).

Vascular Complications Group

Dr Mary Kavurma
The Heart Research Institute
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02 89088907

Our research is aimed at understanding fundamental mechanisms in molecular and cellular biology leading to complications associated with vascular diseases.

Projects currently on offer include:

1. **Investigating the role of Wnt signalling in vascular calcification**
   This project seeks to investigate the potential of targeting the Wnt signalling pathway in vascular calcification. We aim to test whether Wnt signalling contributes to calcification by regulating the expression of osteoprotegerin (OPG, an inhibitor of vascular calcification), receptor activator of nuclear factor-κB ligand (RANKL, an activator of osteoclastogenesis and vascular calcification) and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL, modulator of RANKL expression) in vascular smooth muscle cells (VSMCs) *in vitro* and *ex vivo*. A range of techniques will be employed including tissue culture, molecular biology/gene expression studies and biochemical assays.

2. **Identify the role of endothelial cell (EC) and vascular smooth muscle cell (VSMC) cross-talk in blood vessel develop**

This proposal extends on our published findings showing TNF-related apoptosis-inducing ligand (TRAIL) as a new molecule critical in generating stable blood vessels by simultaneously stimulating new blood vessel development, vessel stability and remodelling. How TRAIL does this is not fully established, and the cross-talk between EC and VSMCs in TRAIL-dependent blood vessel generation is unknown. Using world-first EC and VSMC-specific *Trail*$^{-/-}$ mice, we will identify the contribution of TRAIL coming from each cell type to blood vessel development *in vivo*. A range of techniques can be used in this study, including the *in vivo* Matrigel plug assay, histology, CT scanning, gene expression (PCR, Western blotting) and ELISA.

Platelet Group

Dr Freda Passam
Charles Perkins Centre, Level 3 East
Freda.Passam@hri.org.au

The Platelet Research group studies how platelets interact with clotting proteins and cells to form a clot. Our current focus is on the redox (reduction/oxidation) regulation of platelet receptors. We use a range of experimental approaches, including in vitro functional and biochemical assays, state-of-the-art imaging, and in vivo models.

Project 1: Regulation of platelet function by oxidoreductases

Platelets secrete a number of biologically active molecules during the clotting process, including a recently described group of enzymes, named oxidoreductases. Oxidoreductases catalyze oxidative and reducing reactions with proteins and cell receptors. We have identified a platelet oxidoreductase, named ERp5, which is released into the circulation from activated platelets. ERp5 interacts with the platelet receptor a2bb3 and promotes clot formation in vivo.

In this project we will study the role of ERp5 in platelet function and clot formation by using mice with genetic deletion of ERp5 in their platelets. We will investigate how this oxidoreductase regulates the interaction of platelets with clotting proteins (fibrinogen, von Willebrand factor) and vascular cells (endothelial cells and neutrophils).

This project will provide the opportunity to learn the method of intravital microscopy for the study of clot formation in mice. These studies will also employ platelet function tests, cell perfusion assays, flow cytometry and confocal microscopy.

Project 2: Control of integrins by functional disulfide bonds

Integrins are adhesive molecules that connect cells to their environment and to each other. For example, platelets use their major integrin, a2bb3, to attach to the surface of an injured vessel and to other platelets to make a clot which will stop bleeding. The purpose of this study is to understand how modifications of disulfide bonds within integrins, in particular the reduction and nitrosylation of disulfide bonds within integrin a2bb3, control platelet function.

A disulfide bond connects two cysteine residues in a protein. Post-translational modifications of cysteines in proteins are associated with altered protein function. Integrin a2bb3 contains 37 disulfide bonds, however, which disulfides are involved in the nitrosylation and reduction process is unknown. The cysteines in integrin a2bb3 will be mapped by mass spectrometry using cysteine alkylators developed specifically for this application. The functional consequences of nitrosylation and reduction of a2bb3 disulfides will be determined in vitro using platelets and cells expressing mutated a2bb3 for the cysteines identified.

This project will employ methods of protein chemistry, mass spectrometry, molecular and cell biology.

Thrombosis Research Group @ HRI:

Overall laboratory contact:
Associate Professor Simone Schoenwaelder
Thrombosis research Group
Heart Research Institute,
Level 3 - Charles Perkins Centre,
University of Sydney.
Email – simone.s@hri.org.au;

"*Atherothrombosis is a major healthcare problem in Australia, affecting more than 50 per cent of the adult population. We're hoping to develop innovative approaches to reduce the risk of blood clot in patients and ultimately save lives*"

Research undertaken in our laboratory is focused on determining the mechanisms underlying clot formation in healthy individuals; using this knowledge to better understand the mechanisms leading to platelet hyperactivity and pathological blood clot formation; and ultimately development of safer and more effective therapies to treat cardiovascular diseases including heart attack, stroke, diabetes and the metabolic syndrome.

Project 1: "Bad Blood": Unravelling the Link between Gut Ischemia and Remote Organ Injury - Ischaemic injury to vital organs is common in critically ill patients, producing deleterious effects on other organ systems. This is particularly common in the gut, with intestinal hypoperfusion inducing systemic inflammation and multiorgan organ dysfunction syndrome. In addition to promoting inflammation, prolonged ischemic injury to the intestines can also lead to the development of a systemic thrombotic response (pathological formation of blood clots), which is particularly common in the lung, and leads to a very poor prognosis (>90% mortality). We have identified a new mechanism of pathological blood clotting (thrombosis) and vascular occlusion that is triggered by dying platelets in the intestinal microvasculature. Our ultimate aim is to identify new therapeutic targets to improve microvascular perfusion and reduce inflammation and organ injury, which may represent an innovative approach to reduce remote organ injury in critically ill patients. This project will involve the use of animal models of ischaemia reperfusion, confocal microscopy, gut and lung histology, and other in vitro cell biology and biochemical approaches. (*Co-supervisors – Dr Mike Wu and Dr Yuping Yuan*)

Project 2: Solving a sticky clotting problem in Diabetes - The leading cause of death in diabetes is cardiovascular disease, with up to 70% of deaths relating to the development of blood clots supplying the heart (heart attack) or brain (ischemic stroke). Diabetic individuals are more prone to develop blood clots, and these clots are more resistant to standard anticlotting therapies. Our laboratory has discovered a new biomechanical clotting mechanism severely affected by diabetes that is resistant to the beneficial effects of commonly used antithrombotic agents. Studies ongoing in our laboratory aim to identify how high blood sugar levels (hyperglycaemia) can enhance this new clotting mechanism. To achieve this, we are using Biomembrane force probe ('BFP') technology, which allows us to study how a single platelet senses mechanical cues at the molecular scale. We will also examine the role chronic oxidative stress plays in amplifying blood clotting in diabetes, and the mechanisms by which oxidative stress may modify platelet receptors to enhance adhesion. These studies may identify novel targets with which to treat thrombosis associated with diabetes. This project will involve the use of

in vivo animal models, Biomembrane force probe ('BFP') technology, biochemistry and mass spectoscopy. (Co-supervisors – *Dr Sophie Maiocchi, Dr Arnold Ju*)

Project 3: New approaches to the treatment of ischaemic stroke - The development of a blood clot in the cerebral circulation (ischaemic stroke) is the third most common cause of death and the most common cause of adult disability globally. The central goal of stroke therapy is the prompt reperfusion of occluded blood vessels to minimise tissue death. The delivery of fibrinolytic agents modelled on tissue-type plasminogen activator (t-PA) is the only clinically approved means available to stroke patients. Despite this, the use of t-PA is associated with significant side-effects, limiting its widespread use. We are working on a novel approach to improve upon existing stroke therapies, making them safer and more effective. Ongoing studies using a novel mouse model of thrombolysis (iCAT) developed in our lab will determine whether cerebral damage and cognitive impairment associated with stroke are reduced using this approach. This project will involve the use of animal models of stroke, behavioural analysis, laser speckle contrast and Laser Doppler Flow imaging, histology, and cell biology approaches. (Co-supervisors – *A/Prof Simone Schoenwaelder and Dr Amelia Tomkins*)

Project 4: Investigating blood flow reductions in the brain after stroke - Acute ischemic stroke is a leading cause of death and disability worldwide. It is caused by the blockage of a major artery that supplies the brain. Injury occurs as a consequence of the reductions in blood flow and the longer the brain stays hypoperfused, the greater the damage inflicted. It has long been known that quickly restoring blood flow to the brain will limit the progression of cell death and improve patient outcome after stroke. However, there is evolving evidence that reopening the blocked artery does not always restore blood flow in the small vessels of the brain and correlates with worse prognosis for stroke patients. The causes of the continued hypoperfusion is poorly understood. Identifying the causes of these blood flow reductions despite large vessel reopening will provide targets for potential new stroke therapies. This project will involve the use of animal models of stroke, behavioural analysis, laser speckle contrast and Laser Doppler Flow imaging, histology, and cell biology approaches. (Co-supervisors – *A/Prof Simone Schoenwaelder and Dr Amelia Tomkins*)

Project 5: Platelet death as an important regulator of blood clot formation – The generation of a fibrin blood clot is driven by coagulation factors present in the plasma. These factors assemble on negatively charged endothelial surfaces, such as phosphatidylserine (PS), to facilitate thrombin generation and promote blood clot formation. Platelets are also capable of exposing PS on their outer membrane and promoting localized thrombin generation – a process referred to as platelet 'procoagulant' function. All currently employed anticoagulant agents indiscriminately inhibit blood clotting reactions at the injured vessel wall and throughout the body of a developing blood clot, resulting in increased bleeding risk for patients receiving these medications.

Our laboratory has demonstrated that procoagulant platelets are dying cells, undergoing a cell death process akin to necrosis, leading to PS exposure and thrombin generation. We have also found that the adaptor protein 14-3-3z plays an important role in regulating platelet death necessary for blood clot growth and stability. We aim to determine whether therapeutic targeting of this pathway, either alone, or in combination with necrotic cell death pathways, represents a safe and effective way of reducing thrombin generation in vivo without increasing bleeding risk.
This project will involve an array of in vitro biochemistry and platelet biology assays, along with in vivo models of thrombosis and confocal imaging techniques. (*Co-supervisors: A/Prof Simone Schoenwaelder and Dr Roxane Darbousset*).

HUMAN MOVEMENT AND NEUROSCIENCE

Discipline of Biomedical Science (Rm L217a) and NeuRA
Dr Joanna DIONG. joanna.diong@sydney.edu.au
Dr Martin HÉROUX. m.heroux@neura.edu.au

Our research aims to understand the mechanisms of normal human movement, impaired movement in people with stroke or other clinical conditions, and motor control during daily activities. We use transducers to measure force and angle (i.e. the study of kinematics and kinetics), and electromyography to measure muscle activity. We use scientific computing for biological and transducer signal analysis, and we are passionate about research reproducibility and good science.

Project 1: How does unwanted muscle activity change passive joint range of motion after stroke? Loss of passive joint range of motion (contracture) is common after stroke and leads to loss of function, disability and pain. Current best-practice protocols to measure joint range of motion are performed by asking people to relax before measuring joint angle when force applied to the joint is known. These protocols assume muscles are relaxed, but pilot data from people with stroke show that not all people are able to relax when passive joint range of motion is measured. This means measures of passive joint range of motion may not be valid, at least in some people, and findings from previous studies that measured passive joint range of motion without measuring muscle activity may be questionable. An important question is how much unwanted muscle activity is present in people who have stroke. This study will determine how much unwanted muscle activity is present in people who have stroke by using electromyography (EMG) to measure muscle activity when passively moving the ankle joint. Joint torque and angle will be measured using transducers. (Ethics approval has been obtained for this study.)

Project 2: How does force to control an object vary when performing functional tasks? The control of movement during activities of daily living depends on the ability to produce stable forces over a range of weak to strong muscle contractions, especially when moving and manipulating objects. However most laboratory studies only investigate steadiness in force production during static tasks, and it is not known whether these findings can be applied to realistic contractions at different physical loads. We will conduct a series of studies to investigate how the the central nervous system produces force during laboratory-based and functional tasks. Steadiness of force and muscle activity during static and dynamic contractions at different physical loads will be measured while subjects perform different tasks using custom-built equipment. Muscle activity will be measured using electromyography (EMG) and force and angle will be measured using transducers. (Ethics approval has been obtained for this study.)

James M. Shine
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I use multi-modal neuroimaging (such as fMRI and EEG) to understand the mechanistic fundamentals of cognition and attention, both in health and disease. I am particularly interested in understanding the pathophysiology of cognitive dysfunction in Parkinson's disease, and across a spectrum of dementia syndromes. Recent work in my lab has begun to explore how highly conserved neuromodulatory neurotransmitter systems impact the network structure of the brain to support cognitive processing, and how those systems might become impaired in Parkinson's disease.

Project: Noradrenaline and Cognitive Function – Investigating the forgotten symptoms of Parkinson's disease.

Although Parkinson's disease has been traditionally thought of as a disorder of movement, people with the disease also suffer from impairments in cognitive function. Unfortunately, the classical models that people have used to understand Parkinson's disease (which predominantly implicate the neurotransmitter, dopamine) are unable to adequately explain how or why these "non-motor" impairments occur, making them difficult to detect and to treat. Recent work has shown that the cognitive impairments in Parkinson's disease may relate to pathology within the autonomic 'arousal' systems of the brain. These systems rely on a different set of neurotransmitters, such as noradrenaline, which play a crucial role in coordinating normal cognitive function. Thus, impairments in these systems may account for the presence of cognitive impairment in Parkinson's disease.

To test this hypothesis, we plan to measure the network signature of the brain while simultaneously tracking ongoing changes in the autonomic arousal system, using non-invasive measures such as pupillometry and blood pressure monitoring paired with functional MRI. A successful student would help to collect peripheral autonomic measures while people with Parkinson's disease (along with others with different forms of dementia) perform challenging behavioral tasks during functional MRI scanning. We will then use this data to estimate the dynamic network signatures that underlie cognitive processing and relate to noradrenergic ascending arousal systems.

Overall, this project offers an excellent opportunity to learn functional neuroanatomy through novel investigations of the brain using advanced neuroimaging techniques. In addition, the project also represents a great chance to learn a range of clinical skills, to gain experience in the investigation of cognitive performance across multiple neurodegenerative disorders, to analyse physiological signals and to relate these changes to physiological autonomic signals in the brain.
