Orals
Subsection 1: Plenaries

Plenary 1 – ANS Plenary Lecture – Prof Jurgen Gotz (Queensland Brain Institute, The University of Queensland)
Abstract still to come

Plenary 2 - Eccles Plenary Lecture – Prof Kathryn North (Murdoch Children’s Research Institute)
Abstract still to come

Plenary 3 – International Plenary Lecture – Prof Junichi Nabekura (National Institute of Physiological Sciences (NIPS) Japan)
Abstract still to come

Plenary 4 – The Lawrie Austin Plenary Lecture – Prof Andrew Lawrence (Florey Institute of Neuroscience & Mental Health)
Abstract still to come
THE NEW NEUROTECHNOLOGIES: IMPLICATIONS FOR SCIENCE, MEDICINE AND SOCIETY

Rafael Yuste, Columbia University

In physical systems built with many components, emergent properties, such as ferromagnetism, are often generated from the interactions among these particles. These emergent properties are by definition invisible when observing individual particles, since they depend on large-scale interactions between them. Likewise, the function of the brain has been mostly studied by examining the responses of individual neuron, yet probably is based on emergent functional properties which arise from the coordinated activity of large numbers of neurons in its neural circuits.

To capture this emergent level of brain function, we proposed a large-scale, international public project, the Brain Activity Map Project, aimed at developing new methods to measure and control neural activity across complete neural circuits in experimental animals and human patients. This technological effort, sponsored by the White House as its flagship BRAIN Initiative, an interdisciplinary project that incorporates into neuroscience many methods and approaches from the physical sciences and nanotechnologies. The data obtained with these new methods could prove to be an invaluable step towards understanding fundamental and pathological brain processes. Finally, the novel technologies developed by this project, like it happened with the Human Genome Project, could give rise to new areas of economic and industrial development and likely alter our society, requiring novel ethical guidelines for the application of these technologies.

Shigeo Okabe (University of Tokyo)
Abstract still to come

Sung-Jin Jeong (Korea Brain Institute)

The development of the field of neuroscience has been propelled by the advent of novel technologies and neuro-tools, and the pace of technological evolution has accelerated dramatically in the past decade. Along with this paradigm shift in neuroscience, Korea announced the national flagship brain project that aims to decipher the brain functions and mechanisms that mediate the integration and control of higher brain functions that underlie decision-making.

At the core of the project is a dual-track strategy of innovative “R&D Project” that places emphasis on the development of novel neurotechnologies for functional brain mapping associated prefrontal cortex at the level of multi-modal and multi-scale. It will be applied to advance AI and BMI/BCI technologies and understand the neurological diseases as well as the emotional disorders. Korea Brain Initiative also includes “Reinforcement of the Neuroscience Ecosystem” that focuses on fostering brain research environments and educating multidisciplinary next generation.

We expect to contribute to impactful cooperation with the other global networks that are involved in related with International Brain Station and neuroethical alliance.

Christoph Ebell (Human Brain Project)
Abstract still to come

Caroline Montojo (Kavli Foundation)

INTERNATIONAL BRAIN INITIATIVE: IT TAKES THE WORLD TO UNDERSTAND THE BRAIN

Over the past few years, countries around the world have launched large-scale brain projects, including the U.S. BRAIN initiative, the Human Brain Project, and the Japan Brain/MINDS project. These projects share a common goal of advancing our understanding of the healthy and the diseased brain. Several neuroscience initiatives are on the horizon in other regions of the world,
such as in Australia, China, and South Korea planning to launch their own. With the emergence of these large-scale brain research projects, academic, government, and funding communities recognized that increased cooperation and collaboration is critical for the complex task of understanding the brain. The Kavli Foundation has played a unique role in the development of the U.S. BRAIN Initiative, as well as in more recent efforts to facilitate the International Brain Initiative, an effort to promote cooperation and collaboration across global brain projects.
Subsection 3: Campbell Award Keynote
Dr Stephen Abbott
Abstract still to come
Subsection 4: Kondelos Award Keynote
Professor Naomi Wray

NEW INSIGHTS FROM HUMAN GENETIC STUDIES OF BRAIN-RELATED TRAITS

Naomi R Wray
Institute for Molecular Bioscience & Queensland Brain Institute
The University of Queensland, Brisbane Australia

New technologies of the last decade have generated novel data from human studies that can contribute back into basic research. Direct evidence for the genetic complexity underpinning adult-onset neurological, psychiatric and cognitive ageing disorders imply polygenic genetic architectures. At an individual level, this means that each of us harbour disease risk loci and that each affected person likely carries both a higher burden, and a unique portfolio, of risk alleles. The classical paradigm of functional follow-up of genes associated with disease that underpins research in Mendelian single-gene disorders is not viable or relevant for polygenic diseases with thousands of risk variants. Novel statistical and bioinformatics analyses are filling this void, merging independently collected data sets of SNP-disease, SNP-gene expression (eQTL), SNP-DNA methylation (mQTL) and genomic annotations to build a picture of the biological causality pathways, including specific cell types of relevance for disease. New technologies provide opportunities for neuroscience to embrace the complexity of human genetic variation which is fundamental to understanding the human brain.
Subsection 5: Symposia (Symposium 1-22)

Symposium 1
Getting nervous about immunity

William A. Banks (University of Washington, USA)

THE BLOOD-BRAIN BARRIER AS AN INTERFACE BETWEEN THE BRAIN AND THE PERIPHERAL IMMUNE SYSTEM

Banks, WA1,2
1. Geriatric Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, Washington, USA. 2. Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington School of Medicine, Seattle, WA, USA.

The blood-brain barrier (BBB) prevents the unrestricted exchange between the blood and the CNS of immune-related and immune-active substances and cells, thus establishing the immune privilege of the brain. However, the BBB then acts as a blood-brain interface, re-establishing communication between the CNS and the immune system by participating in a number of neuroimmune axes. Three of the five known neuroimmune axes that involve the BBB will be discussed in this lecture: i) the ability of the barrier cells to transport cytokines; ii) the ability of immune secretions to modify functions of the barrier cells; iii) the ability of the barrier cells to secrete substances either into the brain or into the circulation. The first axis is responsible for relaying information regarding peripheral immune events and is, for example, a mechanism by which circulating interleukin-1 induces deficits in learning and memory. The other two axes work independently and together to facilitate cell-cell crosstalk among the components of the neuroimmune axis. Examples of the latter include the ability of immune stimuli to regulate P-glycoprotein activity (a BBB efflux transporter important in CNS drug delivery) and LRP-1 (an efflux transporter regulating amyloid beta peptide levels in brain), to enhance the passage of HIV as free virus from blood-to-brain, and to enhance insulin transport across the BBB by nitric oxide dependent pathways. These axes are increasingly useful for drug delivery as exemplified by the use of interleukin-1 receptor antagonist in the childhood diseases of FIRES and NOMID.

Erica K. Sloan (University of California Los Angeles, USA)

NEURAL REGULATION OF LYMPHATIC VASCULATURE: NEW MECHANISMS OF IMMUNE REGULATION AND TUMOR PROGRESSION

Erica K. Sloan

Drug Discovery Biology Theme, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria 3052, Australia

Chronic stress induces signalling from the sympathetic nervous system (SNS) and drives cancer progression, although the pathways of tumour cell dissemination are unclear. To address this we used state-of-the-art intravital microscopy and preclinical models of cancer to show that chronic stress restructures lymphatic networks within and around tumours to provide pathways for tumour cell escape. We show that VEGFC derived from tumour cells is required for stress to induce lymphatic remodelling and that this depends on COX2 inflammatory signalling from macrophages. Pharmacological inhibition of SNS signalling blocks the effect of chronic stress on lymphatic remodelling in vivo and reduces lymphatic metastasis in preclinical cancer models and in patients with breast cancer. These findings reveal unanticipated communication between stress-induced neural signalling and inflammation, which regulates tumour lymphatic architecture and lymphogenous tumour cell dissemination. These findings suggest that limiting the effects of SNS signalling to prevent tumour cell dissemination through lymphatic routes may provide a strategy to improve cancer outcomes.
Peter M. Grace (University of Texas, USA)

CENTRAL IMMUNE SIGNALING AS A REGULATOR OF PAIN CHRONICITY
Grace PM

Division of Anesthesiology, Critical Care, and Pain Medicine, University of Texas MD Anderson Cancer Center, Houston TX, USA.

Crosstalk between neurons and immune cells has emerged as a key mechanism of chronic pain in rodent models. Immune mediators released by leukocytes and microglia can promote neuronal hyperexcitability in pain pathways. Evidence will be presented that tuning the immune system with exogenous challenges can dramatically alter the course of chronic neuropathic pain in rat models. On the one hand, administration of opioids—which can act as a pro-inflammatory challenge in the central nervous system—exaggerates microglial activation after peripheral nerve injury, leading to a doubling in the duration of neuropathic pain. On the other, exercise before peripheral nerve injury stimulates anti-inflammatory signaling throughout the pain neuraxis. This prior anti-inflammatory challenge strikingly suppresses three months of subsequent neuropathic pain. The cellular mechanisms of these phenomena will be discussed. Importantly, these insights may lead to novel immune-targeted therapeutics for treatment of neuropathic pain, and strategies to prevent the transition to chronic pain.

Asya Rolls (Israel Institute of Technology)

ACTIVATION OF THE BRAIN’S IMMUNE SYSTEM BOOSTS IMMUNITY

Rolls Asya1,2
1Department of Immunology, 2Department of Neuroscience, Rappaport Medical school, Technion, Israel Institute of Technology, Haifa, Israel

Thoughts and emotions, can impact physiology. This connection is evident by the emergence of disease following stress, or recovery in response to placebo treatment. Nevertheless, this fundamental aspect of physiology remains largely unexplored. We have recently shown that activity of the brain’s reward system, which is active during positive emotional states and positive expectations, boosts anti-bacterial immunity. We used DREADDs to activate the dopaminergic neurons in the ventral tegmental area (VTA) and analyzed the effects on the immune system under different challenges. In this talk, I will discuss how reward system activity can regulate antibacterial immunity and anti-tumor immune response and the potential implications to cancer therapy. Given the crucial role of the reward system in emotional processes, our findings offer a new mechanistic insight to the association between the patient’s psychological state and disease progression.

Symposium 2
Breaking the boundaries of myelin pathology: what can we learn from different disease states

Anna Williams (Centre for Regenerative Medicine, Edinburgh, UK)

“REMYELINATION STRATEGIES IN MULTIPLE SCLEROSIS”

Dementia is a major social and economic problem to our ageing population. One of the commonest forms of dementia in the elderly is vascular dementia, commonly caused by cerebral small vessel disease (SVD), which also trebles the risk of stroke. In life, white matter abnormalities are typically seen on MRI, but the mechanistic link between blood vessels and the white matter changes is not understood. Sporadic SVD was thought to be secondary to hypertension but more recently, an alternative hypothesis suggests dysfunction of the blood brain barrier (BBB) as the primary cause. Here, using a rat model of SVD, we show that the earliest change in development of the disease is endothelial cell (EC) dysfunction, causing BBB weakening. These dysfunctional ECs secrete heat shock protein 90-alpha, which blocks oligodendroglial differentiation, contributing to impaired myelination. We also see evidence of EC and oligodendroglial dysfunction in human post mortem brains with early asymptomatic SVD pathology. Furthermore, treatment of our SVD model rat with drugs reversing dysfunction in ECs also reverses these EC and oligodendroglial pathologies. We
show that the cause of the EC dysfunction in our SVD model rat is a loss-of-function mutation in ATPase11B, and link this to human SVD, by identifying a SNP in ATPase11B associated with MRI white matter abnormalities. We suggest EC dysfunction as the cause of SVD white matter vulnerability and provide a therapeutic strategy to treat SVD, aiming to improve outcome in vascular dementia.

Liz Milward (The University of Newcastle)

**IRON AND MYELIN: SHARED MECHANISMS IN RARE AND COMMON BRAIN DISEASE**

**Ly SL, Riveros C, Milward EA.**
*School of Biomedical Sciences and Pharmacy, The University of Newcastle, Callaghan, NSW, Australia.*

Iron is stored in myelin, oligodendrocytes and other unidentified cells. Our work suggests iron/myelin dyshomeostasis contributes to various brain disorders but it is unclear how iron is regulated by oligodendroglia. We studied differential RNASeq expression of 82 iron-related genes across pairwise combinations of oligodendroglial subtypes differentiated by transcriptomic profiles across contrast groups that included newly-formed oligodendrocytes and myelin-forming/mature oligodendrocytes. Compared to newly-formed oligodendrocytes and mature and myelinating oligodendroglial compartments had increased transcripts for the iron regulatory genes transferrin (TFR) and ferritin heavy chain (FTH1), with differential expression ranking in the top 3 of over 20,000 genes examined (fold-change 5.6, \( p=0.0068 \) and 6.0, \( p=0.0197 \) respectively \( n=10-65 \) cells/group). Myelinating oligodendrocyte compartments may be strongly distinguished by an iron-loading phenotype, revealing unexpected relationships between iron homeostasis and myelination. These results provide further evidence that iron dysregulation contributes to a variety of brain diseases.

Anthony Don (University of Sydney)

**LOSS OF MYELIN LIPID HOMEOSTASIS AS A KEY SENSITIZING FACTOR FOR ALZHEIMER’S DISEASE**

**Mona Lei 1,4, Adeena Shafique 1, Timothy A Couttas 1,4, Hua Zhao 1,4, Tim Karl 2,3 and Anthony S Don 1,4.**

1 Prince of Wales Clinical School, University of New South Wales, NSW 2052, Australia; 2 School of Medicine, Western Sydney University, NSW 2560, Australia, 3 Neuroscience Research Australia (NeuRA), NSW 2031, Australia, 4 Centenary Institute and Sydney Medical School, University of Sydney, NSW 2006, Australia

Background: The sphingolipid sphingosine 1-phosphate (S1P) is an essential neuroprotective signalling molecule. It signals through its own family of five G-protein coupled receptors. A loss of S1P is evident in preclinical stages of AD pathology and particularly in brain regions that develop neuronal atrophy. S1P receptors are good pharmacological targets therefore understanding the functions of S1P in normal brain physiology is important in the context of AD therapy.

The enzymes sphingosine kinase 1 and 2 catalyse the synthesis of S1P. SphK2 is the predominant isoform in the brain. We conducted a comprehensive analysis of the role of SphK2 in memory, cognition and preservation of myelin integrity.

Methods: We tested 23 mice SphK2\(-/\) and C57BL/6 mice aged between 10-12 months. Cognitive examination included tests for spatial memory, anxiety, fear memory and motor function. Brain regions underwent biochemical analysis which consisted of lipidomic analysis and western blotting to examine myelin lipids and proteins. Liquid chromatography-mass spectrometry was also used to investigate S1P levels and sphingolipid species.

Results: We observed enhanced anxiety in the SphK2\(-/\) mice compared to the controls using the fear conditioning paradigm but no deficit in fear extinction. We did not observe any deficits in spatial memory in the SphK2\(-/\) mice using the cheeseboard paradigm. A significant loss of myelin lipids and classical myelin protein marker levels in the SphK2\(-/\) mice was identified.
Conclusion: S1P synthesised by SphK2 is required for myelin integrity. The loss of myelin integrity provides a possible explanation for enhanced anxiety in the SphK2−/− mice.

<table>
<thead>
<tr>
<th>Preparing to Prevent and Protect Your Myelin and Precursor Cells in Neurotrauma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melinda Fitzgerald (The University of Western Australia)</td>
</tr>
</tbody>
</table>

**PREVENTING MYELIN ABNORMALITIES AND PRECURSOR CELL LOSS IN NEUROTRAUMA**

Giacci MK¹, Bartlett CA¹, Kilburn MR² and Fitzgerald M³.

1. Experimental and Regenerative Neurosciences, School of Biological Sciences, 2. Centre for Microscopy, Characterisation, and Analysis. The University of Western Australia, Perth WA 6009, Australia. 3. Curtin Health Innovation Research Institute, Curtin University and the Perron Institute for Neurological and Translational Science, Sarich Neuroscience Research Institute, QEII Medical Centre, Nedlands WA 6009, Australia

Following injury to the central nervous system, we have demonstrated that oxidative stress in myelinating oligodendrocytes and their precursor cells is associated with disruptions to the structure of myelin. Simultaneous assessment of cellular subpopulations and structures using nanoscale secondary ion mass spectrometry (NanoSIMS) has revealed oligodendroglia as particularly vulnerable to DNA oxidation, following partial optic nerve transection injury in adult female rats (p<0.001). When 5-ethyl-2′-deoxyuridine (EdU) was used to label cells that proliferated in the first 3 days after partial optic nerve transection, oligodendrocyte precursor cells were shown to be vulnerable regardless of proliferative state, whereas mature oligodendrocytes derived after injury demonstrated increased susceptibility to DNA damage and lipid peroxidation (p ≤ 0.05). Decompacted, abnormal myelin is likely to be a key contributor to chronic functional loss, and rescuing intact, but vulnerable, myelin from oxidative damage is one of the key goals of our work. Current therapeutic strategies we are developing employ combinations of ion channel inhibitors, and the use of nanotechnology to deliver therapeutics to the cells that need it.

**Symposium 3**

**Recent breakthroughs in the research towards the neuronal basis of consciousness**

Joel Pearson (The University of New South Wales)

**FROM HALLUCINATIONS TO THE IMAGINATION: SEEING WHAT’S NOT THERE AND MEASURING IT**

*Abstract still to come.*

Makiko Yamada (National Institute for Quantum and Radiological Science and Technology, Japan)

**THE EMERGENCE OF POSITIVE ILLUSION: INTEGRATING FROM MOLECULES TO NEURAL RESPONSES, TO THE SELF**

*Abstract still to come.*

Olivia Carter (University of Melbourne)

**PUPIL DILATION AS A MEANS TO ASSESS CONSCIOUSNESS AND COGNITION IN NON-RESPONSIVE PATIENTS**

*Abstract still to come.*

Marta Garrido (The University of Queensland)

**FEEDBACK LOOPS IN DETECTING (UN)SEEN CHANGE**

*Abstract still to come.*
Symposium 4
Synapse under the nanoscope

Daniel Choquet (Université Bordeaux, France)

NANOSCALE IMAGING OF THE SYNAPSE

Abstract still to come.

Merja Joensuu (The University of Queensland)

HETEROGENEOUS MOTION STATES OF SYNAPTIC VESICLES REVEALED BY SUBDIFFRACTIONAL TRACKING OF INTERNALIZED MOLECULES

Abstract still to come.

Jürgen Götz (The University of Queensland)

AMYLOID-ß AND TAU CONTROL FYN LATERAL TRAPPING IN NANOCLUSTERS OF DENDRITIC SHAFT AND SPINES RESPECTIVELY

Abstract still to come.

Emma Sierecki (University of New South Wales)

MOLECULAR INSIGHTS INTO A-SYNUCLEIN AGGREGATION GLEANED BY SINGLE MOLECULE DETECTION

Abstract still to come.

Symposium 5
Why do we overeat? Unravelling the neuronal mechanisms underlying food intake

Amy Reichelt (RMIT)

IMPACT OF OBESOGENIC DIETS ON SOCIAL BEHAVIOURS, REWARD NEUROTRANSMISSION AND GUT MICROBIOTA IN ADOLESCENT RATS

Reichelt AC1, Loughman A1, Bernard A1, Abbott KN1, Raipuria M1, Van TTH2, Moore R2
1. School of Health and Biomedical Sciences, RMIT University, Melbourne, Australia 2. School of Science, RMIT University, Melbourne, Australia. 3. School of Psychology, UNSW Sydney, Australia

Across mammalian species, social interaction with peers is considered a rewarding experience. Social behaviour in rats has been shown to be sensitive to environmental and neurochemical factors such as reward signaling in the prefrontal cortex (PFC) and hippocampus. In these experiments we examined the impact of 2 h daily access to a high fat / high sugar (HFHS) diet on social behaviours in adolescent male rats. Social interaction behaviours with a novel control rat were significantly reduced when HFHS consuming rats were withdrawn from the palatable diet, but not following access to the HFHS food. Rats exposed to the HFHS diets also showed impairments at a social memory task. However, HFHS and control rats showed similar odour recognition memory and preference for social odours animals suggesting other aspects of memory and sociability were not impaired.

Analysis of gene expression in the PFC and hippocampus by RT-PCR demonstrated reduced levels of monoamine oxidase A (MAO-A) and catechol-O-methyltransferase (COMT) mRNA in HFHS diet fed rats compared to controls. These enzymes are involved in the synthesis of monoamines including dopamine and serotonin, indicating that the HFHS diet had an impact on reward neurotransmission in these brain regions. We also observed changes in neuroinflammatory (IL6) and neuroplasticity (BDNF) markers in the PFC. 16S rRNA sequencing revealed significant differences in gut microbiota composition measured between the diet groups, particularly with respect to decreased abundance of Bacteroidetes phyla, which has been linked to obesity and mood disorders in humans.
CRACKING NEURONAL CIRCUITS THAT DRIVE SURVIVAL BEHAVIORS USING NOVEL OPTICAL METHODS

Abstract still to come.

BRAIN REWARD SYSTEM ALTERATIONS IN INDIVIDUALS WITH OBESITY

Verdejo-Garcia A1
1Monash Institute of Cognitive and Clinical Neurosciences, Monash University, Australia

Obesity has been traditionally viewed as a problem of energy regulation, and research on its brain underpinnings has been focused on the hypothalamus. However, increases in food availability and attractiveness, as well as changes in lifestyle, have made evident that extra-hypothalamic brain systems including frontal-striatal circuits involved in reward processing, emotion and decision-making are needed to control food intake and weight. I will present evidence from functional brain activation and functional connectivity studies showing that striatal, insula and prefrontal cortical systems involved in reward processing, interoception (perception of bodily changes) and food choices are linked to high-calorie food intake and obesity, and can hinder attempts to control weight.

TRANSPORT OF INSULIN INTO THE CNS AND ITS EFFECTS ON ENERGY BALANCE AND COGNITIVE FUNCTION

Begg DP1
1. School of Psychology, UNSW Sydney, Sydney, NSW, Australia.

Insulin acts within the central nervous system to alter numerous physiological outcomes including energy balance and glucose homeostasis. Insulin is transported into the central nervous system by a saturable receptor-mediated process that is proposed to be dependent on the insulin receptor and is altered by numerous factors including diet-induced obesity. It has previously been observed that the weight sparing effect of detemir insulin, relative to other long-acting insulin formulations, is associated with increased transport into the central nervous system. We hypothesized that the effects of detemir insulin on energy balance would be mediated by an increase in central nervous system insulin signalling. Chronic treatment with detemir insulin resulted in reductions in both food intake and weight gain relative to insulin glargine or normal insulin treatment in mice. Peripheral detemir insulin treatment resulted in reduced food intake, with increased phosphorylated Akt also observed in the arcuate nucleus of the hypothalamus of detemir insulin treated mice, relative to other insulin treatments. High fat diet inhibited the effects of detemir insulin on energy balance and phosphorylated Akt. Furthermore, when specific neuronal and endothelial populations of insulin receptors were knocked out, animals were insensitive to the effects of detemir insulin on energy balance. These data demonstrate that detemir insulin reduces weight gain by acting on the central nervous system to reduce food intake. The inhibition of this effect in high fat diet treated animals indicates that detemir insulin is subject to resistance of insulin transport into the brain.

Symposium 6
New mechanisms and treatments for motor neuron disease

EXPANDING MECHANISMS AND THERAPEUTIC TARGETS FOR NEURODEGENERATIVE DISEASE.

Gitler AD1
1. Department of Genetics, Stanford University, Stanford, California, USA.

My lab studies the mechanisms of human neurodegenerative diseases, including Parkinson’s disease and amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig’s disease). Our approach is to use a combination of
yeast and human genetics to define the genes and cellular pathways that are involved in neurodegeneration. Because many neurodegenerative diseases, including Parkinson’s disease and ALS, are associated with protein aggregation, we have been using a simple model system, the budding yeast, to introduce aggregation-prone human disease proteins into these cells and then perform genomewide modifier screens for genes that can suppress or enhance toxicity. In one example, we identified a yeast gene that modified the toxicity of an ALS protein. We sequenced the human homolog of that gene, ataxin-2, in ALS patients and controls and identified ataxin-2 mutations as a major genetic risk factor for ALS. Because our studies suggested that these mutations increase the activity of ataxin-2, we next performed experiments in mice to inactivate ataxin-2 and found that this provided a profound extension in survival to an ALS mouse model. In recent work we have been performing genomewide modifier screens in human cells using CRISPR/Cas9 for modifiers of ALS and Parkinson’s disease genes. Our overall vision is to define novel mechanisms of human neurodegenerative diseases and then to translate those mechanisms to novel therapeutics to help treat these devastating conditions.

Tracey Dickson (University of Tasmania)

GETTING THE BALANCE RIGHT: TARGETING EXCITATORY DYSFUNCTION IN THE ALS CORTEX

Clark RM, Brizuela M, Blizzard CL, Dickson TC

Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia

In amyotrophic lateral sclerosis (ALS), hyperexcitability of the motor cortex is a prominent event, often preceding motor neuron degeneration. While many factors may be attributed to this pathophysiology, a possible candidate, the interneuron, has largely been overlooked. Our previous research has identified that degeneration in the transgenic G93A SOD1 mouse model of ALS is marked by progressive and dynamic interneuron involvement. However, it remains unclear if these changes represent a primary or secondary disease mechanism. To further explore the underlying mechanisms we utilised a primary culture approach. We found that mutant G93A SOD1 significantly altered the intrinsic firing properties and neuronal morphology of cortical interneurons. We also found a differential vulnerability of bipolar versus multipolar interneurons to disease. Outward potassium currents were significantly increased in bipolar interneurons (n=6, G93A SOD1, 1.4 ± 0.13nA; n=5, C57Bl6, 0.84 ± 0.07nA; P<0.05) and decreased in multipolar interneurons (n=10, G93A SOD1, 1.1 ± 0.2nA; n=13, C57Bl6, 2.0 ± 0.2nA; P<0.05). In addition, the neurite morphology of bipolar interneurons was unaltered while multipolar interneurons had significantly increased neurite complexity observed as increased branch number (n=6, G93A SOD1, 135 ± 13; n=9, WT, 98 ± 10, P < 0.05) and neurite tree path length (n=6, G93A SOD1, 15 ± 1.6μm; n=9, WT, 11 ± 0.7μm, P < 0.05). Our results show that cortical interneurons are innately vulnerable to the human G93A SOD1 mutation and suggest that differential priming of interneurons may be an early step in the initiation of disease.

Peter Crouch (University of Melbourne)

COPPER IN AMYOTROPHIC LATERAL SCLEROSIS: EVIDENCE FOR COPPER MALFUNCTION IN SPORADIC ALS AND EFFICACY OF THE THERAPEUTIC AGENT COPPER-ATSM IN MOUSE MODELS OF FAMILIAL ALS

Hilton JB¹, Kysenius K¹, Mercier SW¹, Roberts BR², Roberts DJ², Rautengarten C⁴, McLean CA⁵, Beckman JS⁶, Donnelly PS⁷, White AR¹, Crouch PJ¹,²

1. Department of Pathology, The University of Melbourne, Victoria, Australia. 2. Florey Institute of Neuroscience and Mental Health, Victoria, Australia. 3. Elemental Bioimaging Facility, University of Technology Sydney, Sydney, Australia. 4. School of Biosciences, The University of Melbourne, Victoria, Australia. 5. Anatomical Pathology, The Alfred Hospital, Victoria, Australia. 6. Linus Pauling Institute, Department of Biochemistry and Biophysics, Oregon State University, United States. 7. School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria, Australia. 8. Mental Health Program, Cell and Molecular Biology, QIMR Berghofer Medical Research Institute, Queensland, Australia.

Copper is an essential micronutrient. It is required by all cells throughout the body because its redox properties confer catalytic activity to diverse and important enzymes, including the antioxidant superoxide dismutase-1 and the mitochondrial respiratory chain complex cytochrome c oxidase. We have shown that endogenous pools of copper become rate-limiting in affected spinal cord tissue from mouse models of familial amyotrophic lateral
sclerosis (ALS), a fatal neurodegenerative disease, and that the copper-containing drug copper-ATSM is remarkably and reproducibly protective in these animals. Based on these pre-clinical outcomes, phase 1 testing of copper-ATSM in Australian ALS patients is now underway. But the preponderant amount of clinical cases of ALS are sporadic, so it is not yet clear whether therapeutic outcomes generated in familial ALS models will translate to the broader clinical context. To this end, we provide evidence for a connection between a therapeutic mechanism of copper-ATSM and human sporadic ALS; we show the anatomical and biochemical distribution of endogenous copper is disrupted in the sporadic ALS spinal cord and that multiple cuproenzymes are affected. We hypothesise that copper malfunction is an important, druggable feature of ALS, including sporadic cases of the disease.

Kelly Williams (Macquarie University)

IDENTICAL TWINS DISCORDANT FOR ALS: INSIGHTS FROM GENOME, TRANSCRIPTOME AND EPIGENOME DATA

Abstract still to come.

Symposium 7
Cytoskeleton-dependent regulation of neuronal network formation

Mike Fainzilber (Weizmann Institute of Science, Israel)

MOTOR-DRIVEN RNA LOCALIZATION IN NEURONAL GROWTH CONTROL

Abstract still to come.

Alla Kostyukova (Washington State University)

TROPOMODULINS - NEW PLAYERS IN DENDRITE FORMATION

Gray KT1,2, Keller CJ1, Suchowerska AK2, Ly T1, Colpan M1, Bland T3, Wayman GA3, Fath T2, Kostyukova AS1
1. Voiland School of Chemical Engineering and Bioengineering, Washington State University, Pullman, WA, USA. 2. School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia. 3. Integrative Physiology and Neuroscience, Washington State University, Pullman, WA, USA.

Tropomodulin (Tmod), a tropomyosin-dependent capping protein for the pointed end of actin filament, is one of the key players in the regulation of actin dynamics. Three Tmod isoforms, Tmod1, Tmod2 and Tmod3, were found in brain cells; Tmod2 had been shown to be essential for spine plasticity. We demonstrated that Tmod1 and Tmod2, but not Tmod3, are positive regulators of dendritic complexity and dendritic spine morphology. Overexpression of Tmod1 in hippocampal neurons increased dendritic branching distal from the cell body and the number of filopodia/thin spines. Overexpression of Tmod2 increased dendritic branching proximal to the cell body and the number of mature dendritic spines. Tmods have two actin-binding sites located in the N-terminal and C-terminal domains and two tropomyosin-binding sites located in the N-terminal domain. Disruption of Tmod1’s tropomyosin-binding sites abolished the overexpression phenotype. In contrast, overexpression of Tmod2 with disrupted tropomyosin-binding sites caused the same phenotype as wild-type overexpression. We overexpressed Tmod1 and Tmod2 mutants with disrupted actin-binding sites and found that the isoforms required both of their actin-binding sites to alter morphology. Proximity ligation assays indicated that the mutated Tmods are shuttled similarly to wild-type Tmods. We designed chimeric Tmods containing the N-terminal domain of Tmod1 and the C-terminal domain of Tmod2 or Tmod3. Overexpression of these constructs indicated that the C-terminal domain is responsible for features that distinguish Tmod3 from other two isoforms. Our results demonstrate that Tmod1 and Tmod2, while both being important in dendritogenesis, differentially utilize their tropomyosin- and actin-binding sites to affect morphology.

Massimo Hilliard (The University of Queensland)
### ROLE OF THE CYTOSKELETON IN AXONAL MAINTENANCE

Abstract still to come.

Yazi Ke (The University of New South Wales)

### DIVERSE ROLES OF ACTIN REGULATION IN ALZHEIMER MICE

Abstract still to come.

---

**Symposium 8**  
**Novel neural circuits and plasticity regulating stress and stressor responses**

Cecilia J. Hillard (Medical College of Wisconsin, USA)

#### ENDOCANNABINOID SIGNALING AND GLUCOCORTICOID EFFECTS IN THE BRAIN

Abstract still to come.

Andrew M. Allen (The University of Melbourne)

#### IDENTIFICATION OF PATHWAYS INVOLVED IN THE AUTONOMIC RESPONSES TO INTEROCEPTIVE STRESSORS

Abstract still to come.

Karl Iremonger (University of Otago)

#### PLASTICITY IN HYPOTHALAMIC STRESS CIRCUITS

Kim J, Gouws J, Iremonger KJ,  
Department of Physiology and Centre for Neuroendocrinology, University of Otago, Dunedin, New Zealand.

Corticotropin-releasing hormone (CRH) neurons control the levels of corticosteroid stress hormones (CORT) in the body. Rhythmic, low levels of CORT are released throughout the day whereas a surge of CORT is rapidly released in response to stress. Despite the essential role of CRH neurons in controlling CORT release, little is known regarding the underlying patterns of activity in this neural population. To study CRH neuron network activity at the single cell level, GCaMP6f Ca\(^{2+}\) imaging was performed in live brain slices. Imaging revealed that the vast majority of CRH neurons were inactive in vitro. CRH neurons transitioned into a repetitive bursting pattern of activity in response to depolarising stimuli, however, these bursts were not synchronised between neurons. To record the activity patterns of the CRH neuron population in vivo, we used GCaMP6s fibre photometry in freely behaving mice. CRH neuron population activity was found to be dynamic under resting, non-stressful conditions. In response to an acute stress, CRH neuron population activity was rapidly elevated, which was sustained throughout the duration of the stressor with a slow decay. Following termination of the stressor, population activity showed burst-like reverberations before slowly returning to baseline. Together these data reveal the activity patterns of the hypothalamic CRH neuron population in vitro and in vivo both under resting conditions and in response to stress stimuli. These data show that burst firing is an intrinsic property of CRH neurons and that this likely contributes to population activity dynamics observed in vivo.

Chris Dayas (University of Newcastle & the Hunter Medical Research Institute)

#### OPTOGENETIC DISSECTION OF NOVEL PATHWAYS CONTROLLING THE HPA AXIS

Abstract still to come.
Symposium 9
Sensory neuroscience and bionics

Sliman Bensmaia (University of Chicago, USA)

**BIOLOGICAL AND BIONIC HANDS: NATURAL NEURAL CODING AND ARTIFICIAL PERCEPTION**

*Abstract still to come.*

David McAlpine (Macquarie University)

**HOW THE BRAIN CREATES A SENSE OF AUDITORY SPACE**

*Abstract still to come.*

Mohit Shivdasani (University of Melbourne)

**BIONIC VISION IN AUSTRALIA – JOURNEY FROM BENCHTOP TO CLINICAL TRIALS AND THE FUTURE**

*Abstract still to come.*

Americo Migliaccio (The University of New South Wales & NeuRA)

**A DEVICE THAT IMPROVES BALANCE AND VISION IN PATIENTS WITH INJURY TO THE BALANCE ORGANS**

*Abstract still to come.*

Symposium 10
Animals in Research

Colin Blakemore (University of London, UK)

**DOING SCIENCE UNDER FIRE**

*Abstract still to come.*

Michael Goldberg (Colombia University College of Physicians and Surgeons, USA)

**THE AWAKE, BEHAVING MONKEY: TRIUMPH FOR SCIENCE, TARGET FOR ANIMAL ACTIVISTS**

*Abstract still to come.*

Jacqueline Phillips (Macquarie University)

**ANIMALS IN NEUROSCIENCE RESEARCH: THE AUSTRALIAN SITUATION**

*Abstract still to come.*

Symposium 11
How brains adapt to the world: A comparison of sensory processing in animal taxa off the beaten track

Kara E. Yopak (University of North Carolina Wilmington, USA)

**IS BIGGER ALWAYS BETTER? DEVELOPING QUANTITATIVE MEASURES OF COGNITIVE ABILITY IN SHARKS AND THEIR RELATIVES**

Yopak, KE
University of North Carolina Wilmington, Dept. of Biology & Marine Biology & the UNCW Center for Marine Science, 5600 Marvin K Moss Lane, Wilmington, NC 28409

Selection for cognitive ability has been proposed as a key factor driving the evolution of larger brains and/or the
brain structures associated with problem solving, social behavior and other cognitively demanding tasks. These brain structures, including the olfactory bulbs, telencephalon, diencephalon, mesencephalon, cerebellum, and medulla, are often subject to different selection pressures, resulting in a significant degree of variation in brain size and complexity across vertebrates. Using a range of neuroanatomical techniques, this talk will explore major evolutionary patterns of brain organization in fishes, with particular emphasis on one of the most basal vertebrate groups, the cartilaginous fishes (which includes sharks, skates, rays and chimaerids), and how the relative development of major brain structures reflect an animal’s ecology, even in phylogenetically unrelated species that share certain lifestyle characteristics. These data pave the way for predicting cognitive function and/or more complex behavioral repertoires in fishes, with implications for how “intelligence” has evolved across vertebrates.

Wen-Sung Chun (The University of Queensland)

**CEPHALOPODS - SOFT-BODIED PREDATORS WITH BIG BRAINS**

Chung WS
Queensland Brain Institute, The University of Queensland, St Lucia, 4072, Queensland, Australia

Cephalopods have been utilised in neuroscientific research for more than 100 years. In particular, the study of the squid giant axon led to the understanding of nerve excitation mechanisms, and the complex behaviour of cephalopods has been a fruitful approach to investigate learning and memory formation. Modern technologies, as used in studies of the vertebrate nervous system, can examine the cephalopod brain at very high resolution by looking at individual genes, molecules and synaptic activity, or investigate it at lower resolution with whole brain imaging. This talk will show our recent work using both classical and modern techniques (histology and MRI/diffusion tensor imaging) to study cephalopods and their visual abilities. The discovery of diverse forms of retinal neurons, a complex neural network, and interconnecting nodes shows that these soft-bodied visual predators have developed complex adaptations in their eyes and brains in response to different habitats and habits.

Yuri Ogawa Kato (Macquarie University)

**VISUAL ADAPTATIONS IN HYMENOPTERANS: POLARIZATION SENSITIVITY IN OCELLI**

Yuri Ogawa
Department of Biological Sciences, Macquarie University, Sydney, NSW, Australia

Acquiring visual information is crucial to make quick decisions while navigating. In addition to the compound eyes, most insects possess two or three dorsal ocelli, which are small single lens eyes situated on top of their heads. Even though the optics of ocelli generate relatively low resolution images, the superior speed and sensitivity of ocelli, compared to the compound eyes, means that they can improve the visual performance of insects by complementing and modulating compound eye functions (Mizunami, 1995). Here, I will discuss the variation in the organisation of the ocellar rhabdoms, their spectral and polarisation sensitivity between walking and flying insects and between day- and night-active insects. I will also discuss the functional significance of the distribution of polarization sensitivities across the visual field by highlighting the information the ocelli are able to extract from the visual environment.

Ximena Nelson (University of Canterbury, NZ)

**CAN SPIDERS GET ‘BORED’?**

Nelson XJ¹, Helton W¹,², Melrose A¹, Humphrey B¹
1. School of Biological Sciences, University of Canterbury, Christchurch, New Zealand. 2. George Mason University, Psychology, Fairfax, Virginia, USA

Jumping spiders (Salticidae) use vision to a level unprecedented in other groups of spiders, and is of considerable interest for understanding how animals with small brains process complex information and mediate visually-based behaviour that has often been compared with that of mammals. One hypothesis is that the peripheral nervous system ‘filters out’ vast amounts of ‘irrelevant’ information. In this talk, I will discuss a series of experiments in which we investigate the idea that the significant loss of response to repeated visual
stimuli is due to sensory habituation, as exemplified by Kandel’s work on the gill withdrawal reflex in *Aplysia*. Modifying spider hunger level, biological relevance of the repeated stimuli, and central nervous system arousal, our results suggest that the observed response decrement is not mediated in the sensory periphery, but instead within the central nervous system. These results once again defy how these tiny animals can be ‘Jack of all trades and masters of all’ with the estimated 500,000 neurons in their brains.

Symposium 12

*Synaptic Dysfunction in Neurodegenerative Diseases*

**Lars M. Ittner (The University of New South Wales)**

**REGULATION OF POST- SYNAPTIC SIGNALLING BY THE ALZHEIMER PROTEIN TAU**

Abstract still to come.

**Emily Handley (University of Tasmania)**

**TDP-43<sup>431ST</sup> EXPRESSION ALTERS SYNAPSE DEVELOPMENT IN PRIMARY CORTICAL NEURONS**

Jiang, TC, Brizuela, M, Handley, E.A, Dawkins, E, Dickson T.C and Blizzard C.A

Amyotrophic lateral sclerosis (ALS) is a multifactorial disease characterised by the progressive death of motor neurons in the central nervous system. Aggregated proteinaceous inclusions, predominately in neurons, characterise ALS pathologically. Transactive response DNA-binding protein 43 (TDP-43) is the most frequently associated protein in these aggregations. Recent research indicates that this RNA binding protein may play an important role at the synapse, however the early pathophysiological dysfunctions causing impairment in synapse are still unknown. We utilised the YFP-TDP-43<sup>431ST</sup> mouse model expressing mutant human TDP-43<sup>431ST</sup> in cortical neurons to investigate post-synapse formation in vitro. Primary cortical neurons, derived from individual E15.5 embryos were grown to 3, 5, 10 and 15 days in vitro (DIV). Our data implicated that TDP-43<sup>431ST</sup> dendrites developed normally - total dendrite length and mean dendrite length significantly (P<0.05) increased between 3 and 15 DIV in both wild-type (WT) and TDP-43<sup>431ST</sup> cultures with no significant differences (P>0.05) in total dendrite length, mean dendrite length, dendrite branching number and branching order WT and TDP-43<sup>431ST</sup> neurons. Spine tracing of YFP positive WT and TDP-43<sup>431ST</sup> cortical neurons at 10 and 15 DIV, and YFP positive WT cortical neurons transfected mCherry tagged TDP-43<sup>WT</sup> and TDP-43<sup>431ST</sup> plasmids, demonstrated a significant decrease in dendritic spine density in the TDP-43<sup>431ST</sup> cortical neurons in comparison to WT controls (P<0.05). Furthermore, electrophysiological analysis indicated that there was a significant (P<0.05) increase in depolarisation threshold in the TDP-43<sup>431ST</sup> cortical neurons in comparison to WT controls. This work will be imperative in the pursuit of identifying novel therapeutic interventions.

**Vladimir Sytnyk (The University of New South Wales)**

**DISRUPTION IN SYNAPTIC ADHESION IN ALZHEIMER’S DISEASE**

Abstract still to come.

**Victor Anggono (The University of Queensland)**

**GLU1SUBNIT UBIQUITINATION MEDIATES AMYLOID-B-INDUCED LOSS OF SURFACE AMPA RECEPTORS.**

Guntupalli S<sup>1</sup>, Jang SE<sup>1</sup>, Zhu T<sup>1</sup>, Huganir RL<sup>2</sup>, Widagdo J<sup>1</sup>, Anggono V<sup>1</sup>.

1.Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Queensland 4072, Australia. 2. Department of Neuroscience and Kavli Neuroscience Discovery Institute, The Johns Hopkins University School of Medicine, Baltimore,
The accumulation of soluble amyloid-β (Aβ) peptides produces profound neuronal changes in the brain during the pathogenesis of Alzheimer's disease. Excessive levels of Aβ disrupt excitatory synaptic transmission by promoting the removal of synaptic AMPA receptors (AMPARs), dendritic spine loss, and synaptic depression. Recently, activity-dependent ubiquitination of the GluA1 subunit has been shown to regulate the intracellular sorting of AMPARs toward late endosomes for degradation. However, whether this ubiquitin signaling pathway mediates Aβ-induced loss of surface AMPARs is unknown. In this study, we demonstrate that acute exposure of cultured neurons to soluble Aβ oligomers induces AMPAR ubiquitination concomitant with the removal of AMPARs from the plasma membrane. Importantly, expression of the GluA1 ubiquitin-deficient mutants inhibited the adverse effects of Aβ on the surface expression of AMPARs in neurons. Furthermore, we revealed the cross-talk between GluA1 ubiquitination and phosphorylation, in particular phosphorylation at Ser-845, which is crucial for AMPAR recycling and is known to be dephosphorylated in the presence of Aβ. Our data showed that the GluA1 ubiquitin-deficient mutant enhances GluA1 phosphorylation on Ser-845. Conversely, the GluA1 S845D phosphomimetic mutant reduced binding with Nedd4-1 and hence the ubiquitination of AMPARs. Importantly, the GluA1 S845D mutant also prevented Aβ-induced removal of surface AMPARs. Taken together, these findings provide the first demonstration of the dynamic cross-modulation of GluA1 ubiquitination and phosphorylation, a process that is perturbed by Aβ, in regulating the membrane sorting decision that ultimately determines the number of AMPARs on the cell surface.
BALANCING THE INNATE IMMUNE SYSTEM IN THE BRAIN

Abstract still to come.

Ilan Gobius (University of Queensland)

ASTROGLIA REGULATE INTERHEMISPHERIC CONNECTIVITY IN THE DEVELOPING BRAIN

Gobius I¹, Morcom L², Suárez R², & Richards LJ¹, ².
¹Queensland Brain Institute, and ²the School of Biomedical Sciences, The University of Queensland, St Lucia, Brisbane, 4072, Australia.

The corpus callosum forms the major interhemispheric connection in the human brain and is unique to eutherian (or placental) mammals. Although ~1:4000 children are born with developmental absence of the corpus callosum, the primary etiology of this condition is poorly understood. We found that midline crossing of callosal axons is dependent upon the prior remodeling and degradation of the intervening interhemispheric fissure. This remodeling event is initiated by astroglia either side of the interhemispheric fissure, which intercalate with one another and degrade the intervening leptomeninges. Callosal axons then preferentially extend over these specialized astroglial cells to cross the midline. A key regulatory step in interhemispheric remodeling is the differentiation of these multipolar astroglia from bipolar radial glia. Using in vivo gain- and loss-of-function models, we have determined multiple molecular pathways that work together to program the development of multipolar astrocytes at the midline and initiate interhemispheric remodeling, including Fgf8 signaling to downstream Nfi transcription factors. Comparative studies also reveal that interhemispheric remodeling is conserved in humans and mice, yet is absent in naturally acallosal mammals such as marsupials and monotremes. Thus, astroglial-mediated interhemispheric remodeling may be associated with the evolutionary origin of the corpus callosum. Furthermore, our findings from human neuroimaging studies reveal that developmental defects in interhemispheric remodeling are likely to be a primary etiology underlying human callosal agenesis. Taken together, our results reveal astroglia as key regulators of interhemispheric connectivity in the developing brain.

Jessica Fletcher (The University of Melbourne)

TRK-ING TOWARDS CENTRAL NERVOUS SYSTEM MYELIN REPAIR

Department of Anatomy and Neuroscience, School of Biomedical Sciences, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville

Multiple Sclerosis is a neurodegenerative disease common in young adults, caused by an autoimmune attack against myelin. Current immunomodulatory treatments are successful in early disease, but do not directly stimulate remyelination, or prevent progressive accumulation of neurologic disability. We have shown that brain-derived neurotrophic factor (BDNF) enhances myelination through TrkB receptors on oligodendrocytes. To test if TrkB activation can promote remyelination, we infused BDNF, and TrkB agonists: TDP-6, LM22A-4 and 7,8-dihydroxyflavone (DHF), into the brain of adult mice demyelinated with 0.2% cuprizone. Following 7-days treatment, all TrkB agonists, but not BDNF, enhanced remyelination above vehicle controls, increasing MBP in the corpus callosum (p<0.001). Each TrkB agonist had a distinct effect on oligodendrocytes, with TDP-6 and LM22A-4 increasing the density of post-mitotic oligodendrocytes (p=0.01) and DHF increasing oligodendrocyte progenitor density (p=0.02). Morphometry revealed TDP-6 and LM22A-4 increased the percentage of remyelinated axons (p=0.04) and increased myelin sheath thickness (p<0.001). BDNF did not alter oligodendrocyte density, or the percentage of remyelinated axons. To elucidate the divergent effects of TrkB agonists, quantification of TrkB phosphorylation is being performed in conjunction with in vitro assays to examine potential signalling bias. To confirm enhanced remyelination is an oligodendrocyte-driven effect, cuprizone-fed conditional knockout mice, where TrkB has been deleted from oligodendrocytes, have been treated with selected TrkB agonists. The enhanced remyelination effect of TDP-6 and LM22A-4, in contrast to the poor response to BDNF, highlights that while native neurotrophins have poor therapeutic properties, selective modulation of TrkB signalling can promote myelin repair in the brain.
Symposium 15
Emerging Strategies for Improving Stroke Outcomes

Brad Broughton (Monash University)

HUMAN AMNION EPITHELIAL CELLS LIMIT BRAIN INJURY AND FUNCTIONAL DEFICIT FOLLOWING ISCHEMIC STROKE

Evans MA1,2, Lim R3, Kwan W4, Bourne JA4, Drummond GR2, Wallace EM3, Sobey CG2, Broughton BRS1.

1Department of Pharmacology, Monash University, Clayton, Victoria, Australia; 2Department of Physiology, Anatomy & Microbiology, La Trobe University, Bundoora, Victoria, Australia; 3The Ritchie Centre, Hudson Institute of Medical Research, Clayton, Victoria, Australia; 4Australian Regenerative Medicine Institute, Monash University, Clayton, Victoria, Australia.

Stem cells derived from human tissue, including embryonic, induced pluripotent, neural, and mesenchymal cells, have been reported to improve outcome in experimental models of ischemic stroke. However, ethical issues, concerns regarding tumorigenicity and problems with deriving sufficient cells may hamper their suitability as a widely available cell therapy for stroke patients. In contrast, placental stem cells, particularly human amnion epithelial cells (hAECs) are abundant, non-immunogenic, non-tumorigenic and pose no ethical challenges. Surprisingly, hAECs have received almost no attention as a cell therapy for ischemic stroke. Therefore, our aim was to test whether hAECs could limit brain damage and improve functional outcome after ischemic stroke.

We tested the efficacy of intravenous injection of 10^6 hAECs in four animal models of cerebral ischemia. We found that hAECs administered acutely (1.5 h) following stroke in mice migrated to the ischemic hemisphere via a CXCR4-dependent mechanism, and reduced brain inflammation, infarct development and functional deficits. Furthermore, if hAEC administration was delayed until 3 d post-stroke, survival and functional recovery, but not infarct volume, was improved. Proof-of-principle evidence of arrested infarct development by intravenous hAECs after stroke was also shown in a marmoset model of stroke. These findings indicate that early post-stroke intravenous delivery of hAECs elicits marked neuroprotection and facilitates mechanisms of repair and recovery whereas delayed post-stroke hAEC treatment improves survival and functional performance. Thus, intravenous administration of hAECs may be an effective clinical therapy for promoting recovery following ischemic stroke.

Renee Turner (University of Adelaide)

TAKE THE PRESSURE DOWN: A NOVEL AGENT FOR THE TREATMENT OF CEREBRAL OEDEMA AND ELEVATED INTRACRANIAL PRESSURE FOLLOWING STROKE.


1. Adelaide Medical School and Adelaide Centre for Neuroscience Research, The University of Adelaide, Adelaide, SA, Australia 2. Sansom Institute for Health Research, University of South Australia, Adelaide, SA, Australia.

Cerebral oedema and elevated intracranial pressure (ICP) are the leading cause of death in the first week following stroke. Despite this, current treatments are limited and fail to address underlying mechanisms, highlighting the need for development of targeted treatments. Recently, neurogenic inflammation and associated release of substance P (SP) following stroke has been linked to the development of profound cerebral oedema. SP elicits its effects by binding the NK1 tachykinin receptor (NK1R), with administration of an NK1R-antagonist ameliorating cerebral oedema following stroke in rodent models. When screening novel agents, it is also essential to use clinically relevant large animal models to improve the likelihood of successful clinical translation. The current study thus examined the efficacy of NK1R-antagonist treatment in reducing cerebral oedema and ICP in an ovine stroke model. Merino sheep (9M;13F) were anaesthetised and subject to 2hrs transient MCAo, then allocated into the following treatment regimes: early treatment (1mg/kg
NK1R-antagonist at 28, 33, 52, 57, 76, 81hrs post-stroke; n=6), delayed treatment (1mg/kg NK1R-antagonist at 124 and 129hrs post-stroke; n=6) saline vehicle (n=6) or sham surgery (n=4). At 6d post stroke ICP was measured for 4hrs, followed by FLAIR MRI to assess cerebral oedema. Following stroke, ICP was significantly decreased following NK1R-antagonist administration in both the early (p<0.01) and late (p<0.0001) treatment regimes compared to vehicle. Profound cerebral oedema was observed in vehicle treated animals at 6d, in keeping with the elevated ICP. These findings provide substantial evidence that NK1R-antagonist treatment is efficacious for the treatment cerebral oedema and elevated ICP following stroke.

Neil Spratt (University of Newcastle)

SHORT DURATION HYPOTHERMIA PREVENTS INTRACRANIAL PRESSURE ELEVATION AND REDUCES INFARCT VOLUME

McLeod DD, Murtha LA, Beard DJ, Logan CL, Hood RJ, Kovacs TM, Garcia-Esperon C, Schmidt B, Spratt NJ

Therapeutic hypothermia is the only proven non-reperfusion neuroprotectant in human brain ischaemia (post cardiac arrest). It is the most extensively tested potential neuroprotectant in animal stroke models, and a phase III trial is currently being conducted in stroke patients. Interestingly, although the vast majority of animal studies have used <6 h of cooling, clinical trials have almost all used cooling intervals of 24-48 hours. Cooling patients is an onerous undertaking and clinical trials have progressed incredibly slowly largely due to feasibility issues, including rebound intracranial pressure (ICP) elevation when rewarming after 24-48 hours. Data from my laboratory demonstrate that 2.5 hours of mild-moderate hypothermia (32.5 °C) administered soon after ischaemic stroke in experimental animals abolishes the dramatic elevation of ICP seen in normothermic controls 24 hours after stroke (7·0 ± 2·8 mmHg in hypothermia-treated and 31·6 ± 9·3 mmHg in normothermic animals, p<0.001) and reduces infarct volume (15·4 ± 11·8 mm³ and 31·3 ± 18·4 mm³ respectively, p<0.05). The complete mechanism of this switch-like, delayed effect of hypothermia is not yet fully elucidated. However, our data indicate that the trigger to ICP elevation requires active cellular processes in injured cells, that there is a triggering molecule in the cerebrospinal fluid of rats after experimental stroke, and that intracranial pressure elevation reduces perfusion of the ischaemic penumbra by reducing flow through collateral vessels. These findings suggest a potential new avenue for the assessment and treatment of ischaemic stroke patients.

Julie Bernhardt (The Florey Institute of Neuroscience and Mental Health)

EMERGING REHABILITATION INTERVENTIONS FOR PEOPLE WITH STROKE

Abstract still to come.

Symposium 16

Chronic neuroinflammatory mechanisms and their role in Parkinson’s disease progression

Antony Cooper (Garvan Institute of Medical Research)

DYSREGULATED MIRNA EXPRESSION IN PARKINSON’S DISEASE RESULTS IN IMPAIRED ENDOCYTOSIS, MITOCHONDRIAL DYSFUNCTION AND LYPOSOME DYSHOMEOSTASIS.

Duly AMP1, Guennewig B1, Cooper A1,2.
1. The Garvan Institute of Medical Research, Sydney, Australia. 2. St Vincent’s Clinical School, Faculty of Medicine, and School of Biotechnology and Biomolecular Sciences, Faculty of Science, The University of New South Wales, Sydney, Australia

Parkinson’s disease (PD) is a progressive neurodegenerative disorder with ~85% of patients classified as idiopathic (iPD). To identify pathways rendered dysfunctional early in the disease, and thus with enhanced therapeutic potential, small RNA sequencing was performed on patient brain
regions yet to display significant pathology. The up-regulation of miR-142-3p in iPD patients led to the identification of several miR-142-3p target genes, correspondingly down-regulated in patients, that include SYNJ1 (a familial PD gene), WASL and MOB4, all of which are associated with synaptic vesicle endocytosis. Elevated miR-142-3p expression *in vitro* impaired endocytosis, implicating that iPD, like familial PD, may involve endocytic defects. This work also revealed an endocytosis-associated inhibition of early stages of autophagy, potentially explaining autophagy impairments observed in post-mortem iPD brain tissue.

SERAC1, another miR-142-3p target gene, was also down-regulated in idiopathic patients. Elevated miR-142-3p expression induced decreased SERAC1 expression, resulting in altered lipid metabolism and producing (1) mitochondrial dysfunction and elevated ROS production (2) lysosomal dyshomeostasis. Finally, the miR-132/212 cluster, whose reduced expression results in impaired neuronal function, was also identified as a target of miR-142-3p, and was down-regulated *in vitro* and in patients.

Dysregulation of endocytosis, autophagy, mitochondrial and lysosomal homeostasis are all features observed in PD; and elevated miR-142-3p expression may provide a common mechanism contributing their perturbation in iPD. Targeted reduction of miR-142-3p expression in patients could be a potential therapeutic target for the treatment PD.

Nicolas Dzamko (University of Sydney)

**ALPHA-SYNUCLEIN AND LRRK2, IT TAKES TLR2 TO TANGO**

Abstract still to come.

Juliet Taylor (The University of Melbourne)

**MODULATION OF NEUROINFLAMMATION IN PARKINSON’S DISEASE: ROLE OF THE TYPE-I INTERFERONS**

Taylor JM
Department of Pharmacology & Therapeutics, University of Melbourne, Australia

The type-I interferons (IFNs) are master regulators of the neuroinflammatory process, however their contribution to the progression of Parkinson’s disease (PD) is still largely unknown. We recently demonstrated a key role for the type-I IFNs in mediating the neuroinflammatory response and nigral cell death in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) mouse model of PD. Our current studies are focused on characterising the CNS and peripheral cell types that contribute to the type-I IFN-mediated neuroinflammatory response seen in PD. Using a bone marrow chimera approach, the type-I IFN receptor, IFNAR1 was ablated in either the brain or the peripheral haematopoietic cell compartment of C57Bl/6 mice. Mice (n=12/group) were administered MPTP (4x15mg/kg, 2h intervals) and brains harvested at day-1, -3, -10 and -21 post-MPTP. QPCR analysis confirmed that CNS cells were the major source of type-I IFN and pro-inflammatory cytokine production in the brain at day-3 post-MPTP (n=6, p<0.001). These mice also displayed increased microglial activation (Iba-1+ cells) in the substantia nigra (SN). Tyrosine Hydroxylase (TH) quantification confirmed attenuated loss of SN neurons in the brains of mice which lacked CNS expression of IFNAR1 (n=10, p<0.0001), however some neuroprotection was also seen in mice which lacked IFNAR1 only in peripheral haematopoietic cells (n=10, p<0.01). This suggested the type-I IFN mediated peripheral immune response was also a contributor to the MPTP-induced nigral cell death. Our data identifies type-I IFN signalling as a potential therapeutic target for the treatment of PD.

1Main et al., 2016 Glia.
2Main et al., 2017 J Neurochem.

Richard Gordon (The University of Queensland)

**TARGETING THE NLRP3 INFLAMMOSOME IN PD USING ORALLY ACTIVE SMALL MOLECULE DRUGS**
Symposium 17
Evolution and development of neocortical circuits

Ken Ashwell (University of New South Wales)

WHAT MAKES THE MONOTREME CEREBRAL CORTEX DIFFERENT? IS IT JUST LIFE IN THE DEVELOPMENTAL SLOW LANE?

Ashwell KW
Department of Anatomy, School of Medical Sciences, University of New South Wales, 2052 NSW Australia.

The monotremes and therians have followed separate evolutionary paths for as much as 150 million years. Expansion of the cerebral cortex probably occurred independently in therians and monotremes, producing both the large gyrified cortex in the tachyglossids and the remarkable electrosensory field in the ornithorhynchids. Despite this, cellular, neurochemical and synaptic features of the monotreme cortex are remarkably similar to those in eutherians. This suggests that the precursors of neuromorphological and chemoarchitectural archetypes were present in the cerebral cortex of the last common ancestor of therians and monotremes. The two-zone pattern of cortical neurogenesis, often considered a hallmark of the advanced eutherian isocortex of primates and carnivores, appears to also be present in monotremes, albeit to a more limited extent than in primates or carnivores. Findings in the platypus also suggest that there may be a region serving the role of isocortical subplate, at least beneath the developing electrosensory cortex. Nevertheless, isocortical growth in developing monotremes is slow and measured. Cortical thickness growth is at least 50% slower in developing monotremes than in therians. The proliferative compartments of the cortex remain in evidence to a much greater body size and cortical thickness, suggesting a slow but prolonged cortical neurogenesis. Nutrient utilization may be slower in the developing cortex of monotremes than eutherians and this appears to be reflected in both lower aortic outflow and reduced cerebral arterial inflow compared to eutherians.

Charles Watson
University of Western Australia and Neuroscience Research Australia

DEVELOPMENT OF THE CLAUSTRUM AND INSULAR CORTEX – LESSONS FROM GENE EXPRESSION

The origins of the developing claustrum, the insular cortex, and the endopiriform nuclei have been much debated the literature, but gene expression studies have recently clarified the situation. First of all, the claustrum is a pallial rather than a subpallial structure. The cells of the claustrum primordium, which express N\textit{r}4\textit{a}2, are formed among the subplate cells of the lateral pallium at the site of the future insular cortex. The insular cortex cells, which are born later and which are \textit{N}\textit{r}4\textit{a}2-negative, migrate through the developing claustrum toward the pial surface to form layers 2 to 6a of the insular cortex. The cells of the dorsal endopiriform nucleus are formed in the deep part of the claustrum primordium in the lateral pallium, and so are \textit{N}\textit{r}4\textit{a}2-positive like the claustrum cells. At E14.5 in the mouse, these cells migrate ventrally to reach the ventral pallium deep to the piriform cortex. On the other hand, the ventral endopiriform nucleus is formed by radially migrating \textit{N}\textit{r}4\textit{a}2-negative cells in the ventral pallium; it is therefore developmentally distinct from the \textit{N}\textit{r}4\textit{a}2-postive dorsal endopiriform nucleus, which is a derivative of the lateral pallium. The dorsal endopiriform nucleus is therefore a ventral extension of the claustrum, which can be called the endopiriform claustrum. These findings support the recognition of the claustrum/insula complex as forming the lateral pallium, distinct from the remaining dorsal pallium.

David Reser (Monash University)

CYTOARCHITECTONIC PARCELATION OF THE MARMOSET CLAUSTRUM.

Pham, Atapour N, Watkins, Worthy, and Reser DH
There has been a surge of interest in the structure and function of the mammalian claustrum in recent years, due in part to widely publicized theories surrounding a potential role for the claustrum in initiation or maintenance of consciousness. Most experimental work in the claustrum has been in rodent species, although recent studies from our lab and others have indicated that substantive differences in claustral-cortical connectivity exist between rodents and primates. In this study, we examined the cyto- and myelo-architecture of the claustrum of a small primate, the common marmoset (Callithrix jacchus), to determine whether the primate claustrum contains internal anatomical structures or compartments which could inform understanding of its role in brain function. Nissl, NeuN, parvalbumin, calbindin, and myelin-stained sections from 4 adult marmosets were studied using light microscopy and serial reconstruction to identify potential internal compartments. Our findings suggest that there is an internal organization of the marmoset claustrum, including at least 2 major subdivisions, which correspond to the endopiriform and insular claustrum nuclei reported in other species. Continuing efforts are underway to relate these findings to the pattern of cortical connectivity revealed by tracer studies from multiple laboratories internationally.

Rodrigo Suárez (The University of Queensland)

**EVOLUTIONARY CONSERVATION OF AN INTERHEMISPHERIC CONNECTOME IN THE MAMMALIAN CORTEX**

Suárez R¹, Paolino A¹, Kozulin P¹, Morcom LR¹, Fenlon LR¹, Richards LJ¹,²
1. Queensland Brain Institute, The University of Queensland, Brisbane, Australia. 2. School of Biomedical Sciences, The University of Queensland, Brisbane, Australia.

The brain of mammals differs from that of all other vertebrates by having a six-layered cerebral cortex, massively interconnected within and between hemispheres. In monotremes and marsupials, the left and right cortices are connected through the anterior commissure, whereas eutherians evolved a new route, the corpus callosum, which constitutes the largest axon tract in the human brain. While the corpus callosum conveys a pattern of interhemispheric connectivity broadly shared by rodents and primates, it is not yet clear whether such organisation arose as a consequence of callosal evolution, or instead it reflects a more ancient feature of mammalian brain organisation. Here we show that dunnarts, an Australian marsupial, share with eutherians features of an interhemispheric cortical connectome that strongly suggest a pre-callosal origin. Interhemispheric circuit mapping in dunnarts revealed not only connections between homotopic regions of each hemisphere, but also the presence of bilaterally hyper-connected hubs along the dorso-medial (cingulate, motor) and ventro-lateral (claustrum, insula) borders of the cortex, resembling the callosal connectome of eutherians. Our results reveal a shared interhemispheric connectome that likely originated before the split of modern mammalian lineages, and suggest that callosal origin via axon re-routing in early eutherian ancestors involved conservation of such a connectome.
A distinguishing feature of anthropoid primates is the capacity for precise visual guidance in grasping movements. The ability to preshape the hand to accommodate the size of grasped objects is thought to rely upon visual input to the posterior parietal cortex, and is linked closely with theories of primate evolution. It has recently been suggested that early postnatal visual experience in primates is mediated by a developmental visual pathway, in which the middle temporal (MT) area receives retinal input through a transient relay in the thalamic pulvinar. Here we demonstrate that this transient pathway is critical for the normal development of prehension in the marmoset monkey. Small early-life excitotoxic lesions to a subdivision of the inferior pulvinar subnucleus, PIm, led to permanent deficits in grasping behaviour in the adult, and this was reflected in abnormal anatomical development of cortical areas throughout the dorsal stream.

Elizabeth Zavitz (Monash University)

HOW IS MOTION INFORMATION TRANSFORMED BETWEEN V1 AND MT?

Abstract still to come.

Anthony Burkitt (University of Melbourne)

A COMPUTATIONAL NEURAL NETWORK MODEL OF PATTERN AND COMPONENT MOTION IN AREA MT

Abstract still to come.

Symposium 19

Epigenetic mediators of brain function and dysfunction

John Mattick (The Garvan Institute of Medical Research)

ROLES OF REGULATORY RNA IN BRAIN DEVELOPMENT, FUNCTION AND DISEASE

Abstract still to come.

Timothy Bredy (The University of Queensland)

DNA MODIFICATION AND EXPERIENCE-DEPENDENT PLASTICITY: MOVING BEYOND 5MC

Abstract still to come.

Ian Maze (Icahn School of Medicine at Mount Sinai, USA)

CHROMATIN DYSREGULATION IN DOWN SYNDROME AND OTHER BRAIN DISORDERS

Abstract still to come.

Terence Pang (The Florey Institute of Neuroscience and Mental Health, University of Melbourne)

EPIGENETIC MECHANISMS MEDIATING TRANSGENERATIONAL PATERNAL TRANSMISSION OF ACQUIRED TRAITS

My research examines how paternal environments and lifestyle factors modify offspring behaviour (anxiety and stress-response), cognition (memory) and physiology. I have specific interest in the regulation of the hypothalamic-pituitary-adrenal axis and its direct modification of behavioural phenotypes, having also conducted research on transgenic mouse models of neurological conditions (e.g. Huntington’s disease) and acquired conditions (e.g. alcohol withdrawal). I have >1000 career citations, with 3 papers with 100+ cites (H-index 16). A major focus of my work is
elucidating the transgenerational response to paternal stress. Specifically my work investigates the impact of preconception low-dose stress on F1 and F2 offspring behaviour, cognition, and the molecular and physiological aspects of stress response.

Symposium 20
Knowing where you’re going: comparative perspectives on a core problem

Jochen Zeil (Australian National University)

Navigating Brains: Acquiring and Using Views for Homing

Zeil J1, Kócsi Z2, Murray T1, Stürzl W2
1. Research School of Biology, Australian National University, Canberra, Australia. 2. German Aerospace Center (DLR), Institute of Robotics and Mechatronics, Wessling, Germany.

The ability to navigate is fundamentally important for life on earth. Vision, in particular, provides robust information on routes and places and this information can (and must) be quantified in order to understand the neural basis of navigational knowledge. Animals need to acquire navigational knowledge, most importantly for tasks at the local scale of their daily lives, and insects such as ants, bees and wasps allow us to study this process of active acquisition and subsequent use of navigational information in detail under the complex natural conditions in which animals operate. A particularly provocative fact is that these small insects with their comparatively low-resolution vision systems and compact brains are so competent at finding their way around the world. They do offer us the unique opportunity, however, to track their movements and gaze directions in detail, to reconstruct what information they have available under natural operating conditions, to investigate their behaviour in reconstructed reality arenas and increasingly also to unravel the neural machinery that supports their competence. I will discuss what insects tell us about ‘where we should be going’ with research into the neural basis of animal navigation.

Karin Nordström (Flinders University)

Hoverfly Vision in Naturalistic Surrounds

Nordström K.
Centre for Neuroscience, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia

Despite being equipped with low-resolution eyes and tiny brains, many insects show exquisite abilities to detect and use visual information. For example, the optic flow generated by a flying insect can be used to maintain a straight flight course, or to avoid obstacles. Many insects, including hoverflies, which is our main research animal, are also amazingly good at pursuing small moving targets even in highly complex surrounds. Hoverflies are recognized, as the name implies, by their ability to hover nearly stationary mid-air, for prolonged periods of time. The males are very territorial, and aggressively pursue conspecific males who enter their territories, whereas females are pursued for mating. Females, however, can be quite aggressive when fighting over flowers from which they feed. Such field data show that not only visual, but also olfactory cues, are involved in hoverfly choice of which flowers to feed from. We find that the observed behaviors are associated with neural adaptations that work together to optimize the amount and type of information acquired from the visual input. For example, in the insect brain we find some neurons tuned to the detection of optic flow, and others tuned to the visualization of target motion. Using a range of behavioral techniques, together with electrophysiology and modeling, we investigate the underlying mechanisms that allow a relatively simple brain to provide appropriate behavioral responses to complex stimuli. Remarkably, hoverfly visual mechanisms share many features with visual processing in vertebrates, and our findings therefore have relevance even for human vision.

Adam Morris (Monash University)

A Stable Visual World in Primate Cortex During Eye Movements

Morris, AP1 & Krekelberg, B2
1. Neuroscience Program, Biomedicine Discovery Institute, Department of Physiology, Monash University, Clayton, Victoria, Australia. 2. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark,
Sensory systems inform the brain about the state of the outside world. Most sensory events, however, are caused not by changes in our environment but rather by our own actions! Consider what happens as we walk: our arms brush against our sides, our footsteps “clop” against the sidewalk, and our eyes dart around to see where we are going, altering how the world projects onto the retina. How does the brain make sense of this mixed sensory signal, and in the case of vision, how does it transform unstable retinal images into a sense of a stable visual world? We show that neural representations in primary visual cortex (V1) are not only visual; they contain a real-time “eye tracker” that signal the momentary direction of gaze. We recorded the activity of neurons in V1 using high-density multi-electrode arrays while the animal tracked a moving target on a computer display. Using decoding methods, we show that neural activity can be used to track the animal’s gaze regardless of whether the animal is fixating, exploring, or tracking a moving target. We show that this signal allows V1 neurons to encode the location of objects and the animal’s own heading direction even during eye movements. Further, in related work we showed that the cortex has access to time-lagged variants of the eye-tracking signal, including predictive and delayed signals. Taken together, our results provide a neural basis for the stability of vision and goal-directed behaviour in the face of ongoing eye movement.

Frannie Kamhi (Macquarie University)

NEURAL ADAPTATIONS IN ANTS FOR NAVIGATING IN LOW LIGHT ENVIRONMENTS

J. Frances Kamhi, Zachary Sheehan, Ajay Narendra
Department of Biological Sciences, Macquarie University, Sydney, NSW, 2109, Australia

Successful navigation is crucial for finding food, home, and mates and avoiding predators. Vision is an important modality for navigation in most animals. Visual navigation and the required sensory adaptations have been studied mostly in animals active in brightly lit environments; however, a large number of animals are active in low light conditions where accessing visual navigational information is difficult. To determine how the brain has adapted to navigating in specific temporal niches, we focused on two closely related Australian bull ants, the nocturnal *Myrmecia midas* and diurnal *Myrmecia gulosa* and identified the patterns of investment in sensory integration regions. The nocturnal ants have significantly larger mushroom bodies, higher-order sensory integration regions, which may be necessary to obtain sufficient visual information in dim light conditions. However, it is not clear how navigational information is processed in the mushroom bodies. Both of these species predominately rely on visual landmarks for navigation; these landmarks may be learned when the ants first leave the nest to explore their surroundings. We hypothesized that the mushroom body alpha lobe, a region specifically implicated in long-term memory formation, is involved in processing terrestrial landmark information. In ants that were motivated to return home, we pharmacologically inhibited activity in the alpha lobe. In this talk we will show how inhibiting alpha lobe activity affects the ability of ants to visually navigate in their natural environment.
Maureen Hagan (Monash University)

EXAMINING HOW DISTRIBUTED NETWORKS COMMUNICATE THROUGH HIERARCHICAL PROCESSING IN THE VISUAL SYSTEM

Abstract still to come.
### Subsection 6: Imaging workshops (Session 1-2)

#### Imaging Workshop I

<table>
<thead>
<tr>
<th>Speaker</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daniel Choquet (University of Bordeaux, France)</td>
<td><strong>NANOSCALE IMAGING OF SYNAPSE ORGANIZATION TO UNDERSTAND FUNCTION</strong></td>
</tr>
<tr>
<td>Roland Brandt (University of Osnabrück, Germany)</td>
<td><strong>WHAT CAN WE LEARN ABOUT THE STRUCTURE AND FUNCTION OF STRESS GRANULES BY ADVANCED IMAGING APPLICATION IN LIVING CELLS?</strong></td>
</tr>
<tr>
<td>Fred Meunier (University of Queensland, Brisbane)</td>
<td><strong>NANOSCALE ORGANIZATION OF THE EXOCYTIC MACHINERY</strong></td>
</tr>
<tr>
<td>Jean-Baptiste Sibarita (University of Bordeaux, France)</td>
<td><strong>MULTIDIMENSIONAL QUANTITATIVE SINGLE MOLECULE IMAGING OF BIOLOGICAL STRUCTURE</strong></td>
</tr>
<tr>
<td>Ruth Redman (Carl Zeiss Microscopy GmbH, Singapore)</td>
<td><strong>2D AND 3D LIGHT AND ELECTRON CORRELATIVE MICROSCOPY STUDY OF NEURONAL SPINE FORMATION</strong></td>
</tr>
</tbody>
</table>

#### Imaging Workshop II

<table>
<thead>
<tr>
<th>Speaker</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Junichi Nabekura (National Institute for Physiological Sciences, Japan)</td>
<td><strong>IN VIVO IMAGING; FROM SYNAPSES TO NEURONAL CIRCUITS</strong></td>
</tr>
<tr>
<td>Sandra Fok (University of New South Wales)</td>
<td><strong>TISSUE CLEARING TECHNIQUES</strong></td>
</tr>
<tr>
<td>John Van Horn (USC Mark and Mary Stevens Neuroimaging and Informatics Institute)</td>
<td><strong>ANALYSIS OF WHITE MATTER CONNECTIVITY AFFIRMS THE CLAUSRUM AS A MEMBER OF THE RICH CLUB NETWORK OF THE HUMAN BRAIN.</strong></td>
</tr>
</tbody>
</table>

---

**Van Horn JD, Bhattra A, Irimia A, and Torgerson C**

Laboratory of Neuroimaging, Mark & Mary Stevens Neuroimaging and Informatics Institute, Keck School of Medicine of USC, University of Southern California, Los Angeles, CA USA

The claustrum has broad connectivity to most (sub)cortical regions\(^1, 2\), yet its function is poorly understood. Here, we investigate the relationship between the claustrum and the 'rich-club' (RC) network of the human brain. Structural magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) volumes were acquired from N=100 healthy adults\(^3\); ages: 18.6-61.1yrs; \(\mu\pm\sigma: 32.7\pm11.6yrs\). T1-MPRAGE volumes were acquired (TR=20ms, TE=88ms, flip angle=90°, voxel size=1mm\(^3\)) and 68-direction
DTI data were also obtained (TR=9.4s, TE=88ms, flip angle=90°, voxel size=8mm³). Image processing (FSL), tissue classification (FreeSurfer), tractography (TrackVis) and connectivity analyses were performed as detailed elsewhere (2-4). The claustrum was identified and segmented using in-house methods (2). Network analysis was implemented using the Brain Connectivity Toolbox (5, 6). RC membership of brain structures was based on previous reports (3). The claustrum was strongly connected to all regions comprising the RC. Specifically, the number of WM bundles (μ±σ) per cm³ between the claustrum and RC members was: 8.4±2.3 (paracent. gyr. and sulc.), 6.2±2.1 (sup. par. gyr.), 2.5±0.9 (mid. fr. gyr.), 3.0±1.9 (sup. fr. gyr.), 7.2±2.6 (precuneus), 4.5±1.1 (precent. gyr.), 3.7±1.4 (inf. precent. sulc.) and 4.1±2.1 (sup. precent. sulc.). It was also found that the removal of the claustrum from the network model led to statistically significant changes in the nodal betweenness centrality of RC nodes; e.g. left (t=2.01, df=98, p<0.04) and right (t=1.97, p<0.04) precent. gyri.; the left (t=2.17, p<0.03) and right (t=2.10, p<0.03) mid. fr. gyri. This study strongly supports the claustrum as a part of the human brain’s RC. Future research will investigate the role of the claustrum in cognitive operations which involve the RC network.

Merja Joensuu (Queensland Brain Institute, The University of Queensland)

VISUALIZING ENDOCYTIC RECYCLING AND TRAFFICKING IN LIVE NEURONS BY SUBDIFFRACTIONAL TRACKING OF INTERNALIZED MOLECULES

Joensuu M1,2,3, Martínez-Mármol R1,2, Padmanabhan P1,2, Glass NR4, Durisic N2, Pelekanos M1,2, Mollazade M1,2, Balistreri G5, Amor R1,2, Cooper-White JT6,7,8, Parton RG9,10, Goodhill GJ1,2,11 and Meunier FA1,2
1 Clem Jones Centre for Ageing Dementia Research, The University of Queensland, Australia. 2 Queensland Brain Institute, The University of Queensland, Australia. 3 Minerva Foundation Institute for Medical Research, Finland. 4 Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Australia. 5 Division of General Microbiology, Department of Biosciences, University of Helsinki, Finland. 6 School of Chemical Engineering, The University of Queensland, Australia. 7 Materials Science and Engineering Division, CSIRO, Australia. 8 UQ Centre for Stem Cell Ageing and Regenerative Engineering, The University of Queensland, Australia. 9 Institute for Molecular Bioscience, The University of Queensland, Australia. 10 Centre for Microscopy and Microanalysis, The University of Queensland, Australia. 11 School of Mathematics and Physics, The University of Queensland, Australia.

While the resolution of conventional optical microscopy is diffraction-limited, isolated light-emitting molecules can be localized within a far smaller volume using nanoscopic methods. One of the main limitations of the current super-resolution technologies for direct visualization of synaptic vesicles (SVs) in presynapses is their limited ability to track multiple vesicles simultaneously. To address this, we implemented a novel pulse-chase technique based on the subdiffractional tracking of internalized molecules (sdTIM). The technique allowed us to image anti-green fluorescent protein Atto647N-tagged nanobodies trapped in SVs from live hippocampal nerve terminals expressing vesicle-associated membrane protein 2 (VAMP2)–pHluorin (Joensuu et al., JCB 2016). We have further extended our protocol for sequential dual-colour super-resolution imaging of Atto565-tagged nanobodies and Alexa647-tagged cholera toxin subunit-B internalized in SVs and signalling endosomes, respectively, undergoing long-range axonal retrograde transport with 30-50 nm localization precision (Joensuu et al., Nat. Protocols in press). Our results showed that, once internalized, VAMP2-pHluorin/Atto647N-tagged nanobodies exhibited a markedly lower mobility than on the plasma membrane, an effect that was reversed upon restimulation in presynapses but not in neighbouring axons. Combining sdTIM with Bayesian model selection applied to hidden Markov modelling, allows determining both (i) the number of active transport and diffusive states underlying a particular particle trajectory, and (ii) when transitions between these states occur. Our results showed that SVs oscillate between diffusive states, or a combination of diffusive and transport states with opposite directionality. sdTIM technique offers a transferable approach to track additional subdiffractional endocytic structures in live neurons and other cell types.

Sangwon Yoo (The University of Melbourne)

IDENTIFYING NEUROPATHOLOGICAL DEFICITS IN THE CUPRIZONE MODEL OF Demyelination by Utilising a Novel Imaging Technique

Yoo S1, Gonsalvez D1, Craig GA1, Wood RJ1, Murray S1,2, Xiao J1,2
The capacity to detect and quantify changes to compact myelin in pre-clinical models of demyelinating disease such as Multiple Sclerosis (MS) is critical to the development of new therapies that ultimately target myelin regeneration. The Electron Microscopy (EM) has long been the only experimental tool with the resolution to evaluate adaptive changes to myelin structure. However, demyelination in model systems and in human disease is never uniform, therefore sampling enough tissue to capture disease related changes using EM is a real challenge and in many instances virtually impossible.

Myelin is a structure made by oligodendrocyte membranes that wrap axons in multiple layers. These layers are compacted together with myelin proteins, and in this structure, myelin is optically reflective. However, in pathological conditions where compact structure of myelin is damaged, its reflective property is reduced. We employed a novel imaging technique, Spectral Confocal Reflectance (SCoRe) microscopy that can quantify the optical reflection from compact myelin at a high resolution. Through utilising the murine cuprizone model of demyelination, we found that SCoRe imaging can detect a significant reduction \((p=0.0281)\) in optical signal within the entire midline corpus callosum as early as 4-week post cuprizone challenge, indicating that myelin structure is pathologically altered.

Together, we show that SCoRe imaging is a novel and high throughput method that can sensitively detect and quantify changes to compact myelin \textit{in vivo}, paving the way for assessing the therapeutic influence that novel therapeutics exert upon the extent of myelin repair in the context of MS.

Mark Hackett (Curtin University)

**MULTI-MODAL IMAGING TECHNIQUES TO STUDY THE CHEMICAL BIOLOGY OF BRAIN DISEASE**

Hollings A1,3, Fimognari N1,4, Tidy RJ2,3,4, Lam V1,5, Takechi R1,5, Mamo JC1,5, Hackett MJ1,3

1. Curtin Health Innovation Research Institute, Curtin University, Bentley, Western Australia 6102, Australia
2. Curtin Institute for Functional Molecules and Interfaces, Curtin University, Bentley Western Australia 6845, Australia
3. Department of Chemistry, Curtin University, GPOBox U1987, Bentley Western Australia 6845, Australia
4. School of Biomedical Sciences, Curtin University, Bentley, Western Australia 6102, Australia
5. School of Public Health, Curtin University, Bentley, Western Australia 6102, Australia

Numerous methods of microscopy that probe cellular structure and chemical composition are available to the modern neuroscientist. However, there has long being an unfilled niche for techniques capable of direct biochemical imaging and elemental mapping at the cellular or sub-cellular level.

Conventional microscopies reveal important information about cellular and sub-cellular structure, as well as the distribution of “stainable” targets, such as individual proteins. However, many biochemical parameters cannot be studied with these techniques. For example, no imaging method exists for small and mobile molecules such as taurine, or diffusible ions such as \(K^+\) and \(Cl^-\). There has been much improvement in the development of fluorescence probes for \(Ca^{2+}\) and labile metal pools (Fe, Cu, Zn). However, although compatible with cell culture studies, the probes are often incompatible with animal models. In addition, direct imaging of markers of oxidative stress, such as lipid oxidation, altered thiol redox and protein aggregation, is notoriously difficult.

This presentation will discuss advances in recent direct spectroscopic imaging techniques such as Fourier transform infrared spectroscopic imaging (FTIRI), X-ray fluorescence microscopy (XFM), and X-ray absorption spectroscopy (XAS). These techniques allow direct imaging of important biochemical parameters such as: lactate, lipids, and protein aggregates (FTIRI)\(^{1,2}\); thiol redox and taurine (XAS)\(^{2,3,4}\); \(Cl^-\), \(K^+\), \(Ca^{2+}\), Fe, Cu, Zn (XFM)\(^{5,6}\). Applications of these techniques has revealed statistically significant biochemical and elemental alterations \((p < 0.05, \text{animal groups } n > 5)\), which occur within the hippocampus during memory loss following cerebral ischemia and during ageing induced dementia, in rodent models.
Subsection 7: Orals sessions (Oral 1-15)

Oral 1
Cognition and behavior I

Malinda Tantirigama (The Australian National University)

CELLULAR EVENTS CRITICAL FOR ODOR HABITUATION IN THE MOUSE CORTEX IN VIVO

Olfaction employs habituation to de-emphasize static or repetitive odour inputs in order to process novel, potentially more important odours. Piriform cortex (PC) is the first cortical destination of odour information but little is known about how habituation to an odour manifests in the PC circuitry. We applied repetitive odor stimuli and simultaneously measured the responses of up to 250 neurons in the PC of anaesthetized mice using 2-photon calcium imaging. A given odor excited a unique ensemble pattern of principal neurons in layer 2. With each reapplication of the odor, neurons participating in the ensemble were dropped or replaced, but the total number of excited cells declined, indicative of habituation. Reinstatement of the responses occurred over a recovery period of >60 min. The habituated state was absent when a novel odor was presented and a different ensemble of neurons was excited; thus, habituation is odor-specific. Habituation is not inherited from the upstream mitral/tufted (M/T) cells in the olfactory bulb because M/T cells did not change their odor responses upon repeated exposure. However, imaging activity in somatostatin-expressing (SOM+) interneurons in the PC revealed an upregulation of their activity. Local superfusion of the NMDA channel blocker MK801 into PC blocked both the upregulation of SOM+ interneuron activity and the habituation of layer 2 principal cells. In summary, we find long-lasting odor-specific NMDA receptor-dependent changes to odor representation in the PC that are accompanied by upregulated inhibitory activity, suggesting a novel mechanism for the habituation of odor responses in the PC.

Christina Perry (Florey Institute of Neuroscience and Mental Health)

CHRONIC ALCOHOL PRODUCES SPECIFIC COGNITIVE DEFICIT

Chronic alcoholism is associated with cognitive effects that range from mild impairment to profound and irreversible dementia. Even where mild, these deficits are clinically relevant because they impede the process of behavioural change during therapy. Despite this, addiction therapy frequently fails to account for the cognitive deficits that may be present, and there is poor understanding regarding the mechanisms that underlies this decline. In this project we established a rodent model of chronic alcoholism to measure the cognitive effects and underlying neural changes. Rats had intermittent access to ethanol, or an isocaloric solution, in their home cage under voluntary 2-bottle choice conditions. After 6 months, the animals were divided into two groups, matched by consumption. One group underwent a battery of cognitive tasks using touchscreen technology. The others were perfused and their brains retained for volumetric analysis. Rats consumed on average 6 g/kg/session over the 6 month period. Ethanol-exposed and control rats showed equivalent acquisition of pairwise discrimination, however ethanol rats performed fewer trials (p<.05), and with lower accuracy (p < .05) when the contingencies were reversed, indicating reduced behavioural flexibility. In addition, when tested in a 5-choice serial reaction time task ethanol-exposed rats showed increased attentional bias towards a reward associated over a neutral cue (p < .05). Importantly, the cognitive changes observed - decreased behavioural flexibility and specific attentional bias - resemble those seen in human alcoholics. Going forward we will use this model to describe emerging neuropathology in order to elucidate the mechanism(s) for alcohol-induced cognitive decline.

James Peak (University of New South Wales)
PROJECTIONS FROM THE DORSOMEDIAL STRIATUM TO THE SUBSTANTIA NIGRA ARE IMPORTANT FOR GOAL-DIRECTED LEARNING

James Peak, Genevra Hart & Bernard Balleine

School of Psychology, University of New South Wales

It is now well accepted that the posterior region of dorsomedial striatum (pDMS) is necessary for learning goal-directed instrumental actions; however the role of the pDMS output pathways in such learning remains unknown. In a series of experiments, we aimed to examine the role of these output pathways, the striatonigral and striatopallidal projections, in the acquisition of goal-directed learning. Our first experiment used double retrograde labelling in combination with an activity marker (zif-268) to measure activity of striatonigral and striatopallidal projection neurons following instrumental or yoked, non-contingent training. Immunofluorescence quantification revealed significantly higher zif-268 expression in the pDMS following instrumental training in striatonigral but not striatopallidal neurons. In a second experiment, we examined whether striatonigral projection neurons were functionally required for goal-directed learning. We used a two-virus approach; retrograde AAV-Cre was infused bilaterally into the substantia nigra, and Cre-dependent hM4Di DREADDs was infused bilaterally into the pDMS to specifically infect striatonigral projection neurons. Rats were trained on two lever press actions for distinct outcomes and prior to each training session, striatonigral neurons were silenced using a systemic injection of CNO (control rats received vehicle). We then assessed goal-directed learning in the hM4 and control groups using an outcome devaluation test in which the ability of the rats to choose appropriately after one or other outcome was devalued was measured. We found that silencing striatonigral neurons during training generated insensitivity to devaluation on test suggesting that activity in striatonigral projection neurons in the pDMS is important for goal-directed learning.

Khalid Khan (Kuwait University)

EFFECTS OF INTRAVENTRICULAR INFUSION OF QUINOLINIC ACID ON SPATIAL LEARNING AND MEMORY IN YOUNG RATS

Khan KM1, Rao MS1, Rahman A2, Guillemin GJ3

1Department of Anatomy, Faculty of Medicine, 2Department of Food Science & Nutrition, College of Life Sciences; Kuwait University, 3MND and Neurodegenerative Diseases Research Center, Macquarie University, Australia.

Quinolinic acid (QA), metabolite of kynurenine pathway of tryptophan metabolism, is a known neurotoxicant. QA toxicity affects hippocampal, striatal and cortical neurons. QA induces tau phosphorylation and reduces the expression of serine/threonine phosphatases. These effects are associated with age-related memory loss. We investigated the effects of intraventricular infusion of QA on spatial learning and memory in young Wistar rats. QA (9 mM) was infused into right lateral ventricle of 21-day old rats for 7 days using osmotic pumps. Rats infused with the same volume of normal saline served as vehicle control (VC). Learning and memory was assessed by Morris Water Maze test on postnatal day 30 (PND30) and PND45. Two-way repeated measure ANOVA analysis revealed that learning was significantly impaired in QA-infused rats compared to VC group (p < 0.05) at PND30 but not at PND45. Short-term memory(STM) tested 2 days after the last learning session showed significant memory deficits in QA-infused rats compared to VC at both PND30 and PND45 (p < 0.05 and 0.01, respectively), whereas long-term memory (LTM) tested after 10 days showed no significant difference between the groups either at PND30 or PND45 (p > 0.05). These results show that intraventricular infusion of QA causes learning and STM impairment, but has no significant effect on LTM. It is tempting to speculate that the difference in STM and LTM results is due to time-dependent clearance of QA.

Nathan Holmes (University of New South Wales)

THE MOLECULAR EVENTS UNDERLYING CONSOLIDATION OF NEWLY FORMED AND UPDATED FEAR MEMORIES IN THE BASOLATERAL AMYGDALA

Lay BPP1, Westbrook RF1, Glanzman DL2, Holmes NM1
Consolidation of fear memories requires an array of intracellular signalling processes within the basolateral complex of the amygdala (BLA). These processes include activation of protein kinase pathways, transcription of specific genes, and importantly, synthesis of proteins that stabilize the fear memory through structural changes in BLA neurons. New information can be integrated into that memory by reactivating the fear memory with reminder cues (memory updating). However, little is known about the molecular events that mediate the consolidation of an updated fear memory. In this study, rats were exposed to pairings of a stimulus (e.g., a tone) and foot-shock to produce an auditory fear memory. Next, rats received pairings of a visual stimulus (e.g., a light) and the fear-eliciting tone in order to incorporate the new visual information into the reactivated fear memory. Immediately after these pairings, rats received an intra-BLA infusion of drug or vehicle. Finally, they were tested for fear (freezing) to the light. The results show for the first time that there are both commonalities and differences in the molecular events involved in the consolidation of newly formed and updated fear memories. Consolidation of both types of memory requires neuronal activity in the BLA, activation of the CaMKII/CaMKIV signalling pathways, DNA methylation and gene transcription. However, whereas consolidation of a newly formed fear memory in the BLA requires activation of ERK/MAPK and PKA/PKC signalling pathways as well as de novo protein synthesis, updating that memory to incorporate new information does not require these processes for its consolidation.

Mihaela Iordanova (Concordia University)

DOPAMINE TRANSIENTS IN THE VENTRAL TEGMENTAL AREA REDUCE PREDICTION ERROR ABOUT AVERSIVE OUTCOMES

1Department of Psychology, Center for Studies in Behavioural Neurobiology/Groupe de Recherche en Neurobiologie Comportementale, Concordia University, Montreal, Quebec, Canada

Learning depends on our ability to predict the future. If our predictions are correct i.e. there is no prediction error, then no further learning is necessary. In the lab, this can be modelled using the blocking paradigm. In blocking, the presence of a good predictor for an outcome prevents learning about other novel cues and the same outcome. Dopamine (DA) in the Ventral Tegmental Area (VTA) has been implicated in prediction error about rewarding events such that the greater the DA the greater the prediction error and thus the greater the increments in learning. However, reduction in DA transmission at VTA target sites (nucleus accumbens and amygdala) have shown a similar effect i.e. increase in prediction error and increase in learning but about aversive outcomes. Here we sought to determine the role of VTA DA in prediction error in fear. We used the Th:cre rats line in conjunction with cre-dependent channelrhodopsin viral vector to show that inducing a dopamine transient at time of an expected footshock in a blocking paradigm augmented the blocking effect i.e. further decreased prediction error and retarded learning about the novel cue. Further, by stimulating nucleus accumbens terminals, we show that this effect is regulated by the VTA-nucleus accumbens pathway. These data show that VTA DA transients have an opposing effect in fear to that in reward, and suggest a possible valence-specific prediction error mechanism.

Leigh Walker (Florey Institute of Neuroscience and Mental Health)

PATTERN AND PHENOTYPE OF NEURAL ACTIVATION FOLLOWING YOHIMBINE-INDUCED REINSTATEMENT OF ALCOHOL SEEKING

Walker LC1, Kastman HE1, Krstew E1, Gundlach AL1, Lawrence AJ1

1. Florey Institute of Neuroscience and Mental Health, Melbourne, Australia

Alcohol use disorders are chronic relapsing disorders that constitute one of the leading causes of preventable deaths worldwide. Although it is widely reported that stress triggers relapse, the relationship between stress and addiction is complex, and current therapeutics fail to address this problem. Neuropeptides, many of which are
implicated in anxiety and addictive behaviours, may provide more targeted pharmacotherapeutic treatment strategies, with limited adverse side effects. The α2-adrenoceptor antagonist, yohimbine, precipitates relapse-like behaviour in rodents, however the pattern of neural activation and neurochemical phenotype of activated cells following yohimbine-induced reinstatement is unknown. Therefore, in alcohol preferring (iP) rats, we examined Fos immunoreactivity following yohimbine-induced reinstatement of alcohol seeking, home-cage yohimbine administration (1 mg/kg), home-cage vehicle administration (1 ml/kg) and in age matched alcohol naïve controls. Both home-cage yohimbine administration and yohimbine-induced reinstatement of alcohol seeking significantly increased neuronal activation in the extended amygdala and prefrontal cortex compared to vehicle and alcohol naïve controls. Consequently, we examined the potential role of several neuropeptides, including corticotropin releasing factor (CRF), dynorphin (DYN) and cocaine and amphetamine regulated transcript (CART), using dual immunohistochemistry in the central nucleus of the amygdala (CeA). Yohimbine-induced reinstatement of alcohol seeking increased activation of CRF, DYN and CART cells within the CeA; CART neurons being the most sensitive. Collectively, these results identify several brain regions where yohimbine may act to precipitate reinstatement of alcohol seeking and implicate CRF, DYN and CART neuropeptide systems within the CeA in this behaviour.

Wei Wei (Queensland Brain Institute)

DNA REPAIR REGULATES TEMPORAL CODING OF GENE TRANSCRIPTION REQUIRED FOR MEMORY CONSOLIDATION

Wei W, Li X, Marshall PR, Leighton LJ, Zajaczkowski EL, Bredy TW

Cognitive Neuroepigenetics Laboratory, Queensland Brain Institute, The University of Queensland, St Lucia, QLD 4072, Australia

The formation of memory requires the coordinated action of learning-induced gene expression programs that align temporally with encoding and consolidation. The growth arrest and DNA damage inducible 45 (Gadd45) family, of which there are three members, are key players in the DNA damage repair pathway. Here we explore the phenomena of DNA repair in the regulation of gene transcription required for memory consolidation and how it might relate to temporal coding of gene programs. We found that viral-mediated knockdown of Gadd45g, but not Gadd45a or Gadd45b, impaired fear memory consolidation through a temporally-specific regulation of immediate early gene expression (IEG) after fear training. This included the regulation of a previously uncharacterised IEG, cysteine rich angiogenic inducer 61 (Cyr61). Importantly, Cyr61 was subject to DNA double strand break and repair by Gadd45g and, intriguingly, following Gadd45g-induced DNA repair we observed a second induction wave of Cyr61 mRNA expression, which appears to be associated with a late phase of fear memory consolidation.
## Oral 2

### Motor neuron disease I

**INCREASED PHOSPHORYLATED AND INSOLUBLE TAU IN INDUCED PLURIPOTENT STEM CELL- DERIVED MOTOR NEURONS FROM AMYOTROPHIC LATERAL SCLEROSIS PATIENTS**

Lezanne Ooi (University of Wollongong)

| Stevens CH¹, Guthrie NJ¹, Do-Ha D¹, Baez M¹, Balez R¹, Cabral-da-Silva MC¹, Engel M¹, Yang S², Rowe D², Blair I², Buskila Y³, Ooi L¹ |
| 1. Illawarra Health and Medical Research Institute, School of Biological Sciences, Faculty of Science Medicine and Health, University of Wollongong, NSW, Australia. 2. Centre for MND Research, Faculty of Medicine and Health Sciences, Macquarie University, NSW, Australia. 3. School of Medicine, Western Sydney University, Penrith, NSW, Australia. 4. The MARCS Institute, Western Sydney University, Penrith, NSW, Australia. |

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterised by the progressive degeneration of motor neurons in the brain and spinal cord, leading to widespread muscle wasting and weakness. A significant proportion of ALS patients show evidence of cognitive impairment and fulfil criteria for frontotemporal dementia (FTD). Around 45% of FTD patients show inclusions in neurons containing hyperphosphorylated tau. However, despite the known overlap between ALS and FTD only limited research has addressed the role of the FTD-associated protein tau in ALS. Therefore we used biochemical and electrophysiological tools to analyse motor neuron function, along with the phosphorylation state and solubility of tau in motor neurons from induced pluripotent stem cells (iPSCs) from ALS patients and healthy controls. Importantly, motor neurons in vitro developed electrophysiological profiles consistent with cholinergic neurons and expressed mature forms of tau. We found that oxidative stress led to an increase in phosphorylated and insoluble tau and the development of ALS-relevant cellular phenotypes specifically in ALS motor neurons. Immunohistochemical analysis of post mortem human spinal cord tissue confirmed the presence of phosphorylated tau deposits in the motor neurons of clinically pure ALS cases, which were absent in age-and gender-matched healthy controls. Together our data suggests that tau has a pathogenic role in ALS motor neurons and highlights that iPSC-derived motor neurons can be used for investigating tau-based therapeutic avenues in ALS.

**USING ALS PATIENT SKIN FIBROBLASTS FOR THE DISCOVERY AND ASSESSMENT OF RAPID DIAGNOSTIC AND PROGNOSTIC BIOMARKERS**

Shu Yang (Macquarie University)

| Yang S¹, Galper J¹, Zhang KY¹, Baez M², Fifita JA¹, Deva AK³, Ooi L², Yerbury JJ², Rowe D¹, Nicholson GA¹, Ooi L¹, Blair IP² |
| 1. Centre for MND Research, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW 2109, Australia. 2. School of Biological Sciences, Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, NSW 2522, Australia. 3. Surgical Infection Research Group, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW 2109 Australia. 4. Northcott Neuroscience Laboratory, ANZAC Research Institute, Sydney, NSW 2139, Australia. 5. Sydney Medical School, University of Sydney, NSW 2006, Australia. 6. Molecular Medicine Laboratory, Concord Hospital, NSW 2139, Australia. |

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterised by the progressive degeneration of brain and spinal cord motor neurons. Increasing evidence shows that the ubiquitin-proteasome system (UPS) plays an important role in MND pathogenesis, including the identification of mutations in the E3 ubiquitin ligase gene CCNF. The aim of this project was to determine whether UPS dysfunction could be detected in readily available skin fibroblasts from ALS patients to serve as a biomarker to assist short-term diagnosis and prognosis of ALS. We first investigated whether ALS patients fibroblasts share biochemical abnormalities seen in affected neuronal cells. Using immunofluorescence staining, we found that UPS inhibition led to a significant accumulation of ubiquitinated TDP-43 inclusions, a hallmark pathology of ALS, in ALS fibroblasts compared to...
control. Second, we utilised a flow cytometry based UPS reporter assay-GFP assay, in these fibroblasts to investigate whether UPS function was inhibited. We consistently saw significant accumulation of GFP, indicating UPS dysfunction, in familial ALS patients with mutations in CCNF, TARDBP, UBQLN2 and C9orf72. Using the same assay, we also found that there was a significant increase in GFP accumulation between CCNF patient fibroblasts collected at an earlier than a later disease state, suggesting that UPS dysfunction may be used to monitor disease progression. Altogether, we have demonstrated that UPS dysfunction may be measured as a potential biomarker from a subset of ALS patient skin fibroblasts. This may prove useful for pathogenic studies and have short-term diagnostic and prognostic utility for this rapidly progressive disease.

Merryn Brettle (University of New South Wales)

NOVEL MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS WITH A PROFILIN 1 MUTATION


Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease. Sporadic and familial forms of disease present with similar clinical symptoms and histopathology. Understanding the underlying pathogenesis of the disease is essential for the development of treatments. Mutations in the actin-associated protein, profilin 1, have been identified as a rare cause of familial ALS. We have developed a novel mouse model to elucidate the role that PFN1 plays in ALS. Expression of V5-tagged PFN1 is targeted to α-motor neurons in the spinal cord.

Initial data shows V5-PFN1 expression in the anterior horn of the neural tube starting from embryonic stages in transgenic mice. Motor testing shows that transgenic mice have motor deficits on RotaRod commencing at 1 months of age. This novel mouse model of PFN1 will provide a potential tool to understand the role that PFN1 plays in the pathogenesis of ALS and could be used for testing future ALS therapeutics.

Jacinta Conroy (The University of Queensland)

A PROTECTIVE ROLE FOR COMPLEMENT C3AR ACTIVATION IN SOD1G93A MICE

1. School of Biomedical Sciences, the University of Queensland, QLD. 2. Queensland Brain Institute, the University of Queensland, QLD. 3. University of Queensland Centre for Clinical Research, the University of Queensland, QLD.

The complement system is an integral component of innate immunity and has been implicated in the pathogenesis of motor neuron disease (MND). Complement is activated in human MND and in mouse models, and its activation generates the bioactive fragment C3a, which can modulate the inflammatory response by binding to its receptor, C3aR. However, the contribution of C3aR to disease progression in MND is still unknown.

The current study aimed to determine the effects of upstream factors such as C3a. Ideally be targeted towards downstream C5a inhibition, in order to avoid blocking any endogenous protective effects of upstream factors such as C3a.

Marta Vidal (Macquarie University)
IDENTIFICATION OF A NOVEL EXTRACELLULAR ISOFORM OF FUSED IN SARCOMA (FUS)

1 Department of Biomedical Sciences, Macquarie University, North Ryde, New South Wales, Australia. 2 Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Bundoora, Victoria, Australia

The FUS gene is mutated in 4% of familial cases of Amyotrophic lateral sclerosis (ALS) and 1% of sporadic cases. Importantly, mutations in FUS account for a particularly aggressive, juvenile form of the disease. Previous bioinformatics analyses predicted the existence of a novel extracellular FUS isoform, resulting from a combination of a frame-shift splicing event and the production of an alternative start codon (Wilson et al. 2014). The outcome is a protein with a different N-terminus from its canonical counterpart, but with a shared C-terminus. Most of ALS mutations, clustered at the C-terminus, could be present in the isoform. This study aimed to confirm the existence of the predicted novel isoform of FUS, to characterise it and to examine its role in ALS. In human neuronal cell lines and in human primary neurons, we detected the expression of the novel FUS isoform at mRNA level. Expression of the isoform at protein level was confirmed by western blotting and mass spectrometry. As predicted, the isoform was detected in the media of SH-SY5Y cells confirming its extracellular localization. Expression of GFP and HA-tagged constructs encoding the isoform in SH-SY5Y cells revealed that the protein was found extracellularly and PNGaseF assay showed it is N-glycosylate. Our findings confirm the expression of a novel isoform of FUS, which has a unique extracellular localization. This implies it possesses a novel physiological function distinct from the canonical isoform. These results provide a framework for future investigations into the role of the new isoform in ALS.

Prachi Mehta (Macquarie University)

APOLIPOPROTEIN D (APOD) AS A POTENTIAL NEW BLOOD PROTEIN BIOMARKER FOR MOTOR NEURON DISEASE (MND)

Mehta P1, Krisp C2, Le S1, Hedl T1, Lee A1, Molloy M2 and Walker AK1

1. Centre for MND Research, Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Australia. 2. Australian Proteome Analysis Facility (APAF), Macquarie University, Australia.

Motor neuron disease (MND) is a devastating neurodegenerative disease and approximately 97% of MND cases are characterised pathologically by the presence of TAR DNA-binding protein of 43 kDa (TDP-43) inclusions in the brain and spinal cord. Diagnosis and monitoring of MND is largely based on clinical examination and exclusion of other disorders. Therefore, there is an undeniable need for prognostic biomarkers to identify disease and rate of disease progression. To identify new biomarkers of disease, we used a thoroughly-characterised mouse model of MND (rNLS TDP-43 mice) which recapitulates the hallmark cytoplasmic TDP-43 pathology of MND with a disease-reminiscent phenotype including progressive motor impairment and early death. In this mouse model, we performed advanced quantitative label-free SWATH mass spectrometry analysis of serum from pre-symptomatic (2 weeks) and symptomatic (4 & 6 weeks) TDP-43 mice and litter-matched controls (n=3-4/group). We identified >200 quantifiable protein targets including apolipoprotein D (apoD), which showed a statistically significant increase by >1.5-fold in serum from the TDP-43 mice compared to control mice, at both 4 and 6 weeks of disease progression. Immunoblotting on mouse brain (cortex) tissues using two commercially-available antibodies specific for mouse apoD revealed similar increased levels of apoD protein along with accumulation of TDP-43 in diseased mice compared to controls. Future studies will investigate changes in apoD protein in human MND samples. These findings suggest apoD as a potential blood-based biomarker for MND, and indicate that changes in apoD protein levels in the blood reflect underlying pathological changes occurring in the brain in disease.

Anna King (University of Tasmania)

REGULATION OF THE CYTOSKELETON BY ALS/ FTD PROTEIN TDP-43 AND IMPLICATIONS FOR NEURODEGENERATION

Wicking Dementia Research and Education Centre, University of Tasmania. 2. Central Science Laboratory, University of Tasmania
TDP-43 is implicated in amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD), which are pathologically characterized by axonal degeneration. Links between loss of TDP-43 function and axonal pathology are unclear. **Objective:** We have investigated the role of TDP-43 in regulating the formation and maintenance of neurites in *in vitro* and *in vivo* models. **Methods:** Animal use was approved by the University of Tasmania animal ethics committee. Primary cortical neurons, were derived from transgenic mice expressing human wildtype (WT) TDP-43 as well as from WT mice. To examine the effect of TDP-43 *in vivo*, AAV2 virus was used to introduce human WT-TDP-43 and TDP-43 with a mutation in the nuclear localization signal (ΔNLS) into retinal ganglion cells (RGCs) and the effect on axons examined histologically. **Results:** Over-expression of TDP-43 in cultured neurons resulted in significantly (p<0.05) more branching in the neurons and significantly (p<0.05) altered growth cone morphology at 3 days. Label-free quantitative proteomic analysis, followed by functional classification of significantly modulated proteins (*t*-test, FDR<1%) revealed that actin-binding proteins were among the most down regulated proteins (DAVID enrichment score 4.1). RGC expression of ΔNLS-TDP-43, but not WT-TDP-43 resulted in a significant (p<0.05, n=10) loss of visual acuity at 6 weeks post injection. Preliminary studies using electron microscopy suggested that altered TDP-43 induced axonal pathology including swollen axon structures filled with organelles. **Conclusion:** These data suggest that TDP-43 pathology could result in cytoskeletal changes and neurite dysfunction leading to synaptic disconnection. Targeting the cytoskeleton may be a therapeutic target for ALS/FTD.

Jennifer Fifita (Macquarie University)

**MULTIDISCIPLINARY STRATEGIES FOR DISCOVERY OF NOVEL MND GENES**

1. Centre for MND Research, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, New South Wales, Australia

Amyotrophic lateral sclerosis, also known as motor neuron disease (MND), is a fatal neurodegenerative disease that is caused by the progressive death of motor neurons. Ten percent of cases are familial (FALS), while 90% are considered sporadic (SALS). To date, the only known causes of MND are gene mutations, though 40% of familial cases remain unsolved. We aim to identify novel familial MND gene mutations using a multi-disciplinary approach including genetics, *in silico* and *in vitro* analyses. Whole-exome sequencing was completed on three individuals from an MND family with an unknown gene mutation. Custom bioinformatics analysis of the data resulted in the identification of five candidate gene mutations. These five candidates were then scored using various *in silico* predictive analyses, including protein predictions and conservation, and gene natural variation, identifying two candidates with strong pathogenic potential. A novel *in vitro* analysis pipeline was then designed to determine the functional consequences of these candidates. This included toxicity studies, and testing for known aspects of MND pathology, including protein aggregation and interaction with TDP-43. The top two candidates from *in silico* analysis were found to be significantly more toxic than their wild-type proteins. In addition, one candidate was found to co-aggregate with TDP-43, have a significantly higher level of accumulation in insoluble fractions and led to ubiquitin proteasome system dysfunction. The pathogenic basis of MND remains poorly understood. The identification of novel MND genes increases our knowledge of disease biology and provides tools for the development of disease models and therapeutic discovery.
INFLAMMATORY CYTOKINES ARE ELEVATED IN THE MIDBRAIN IN SCHIZOPHRENIA AND FOLLOWING MATERNAL IMMUNE ACTIVATION

Tertia Purves-Tyson¹,², Ulrike Weber³, Juliet Richetto³, Debora A Rothmond¹,² Kate Naude¹,² Urs Meyer³ and Cynthia Shannon Weickert¹,²

1. Schizophrenia Research Laboratory, Neuroscience Research Australia, Sydney, Australia 2. School of Psychiatry, University of New South Wales, Sydney, Australia 3. Institute of Pharmacology and Toxicology, University of Zurich-Vetsuisse, Zurich, Switzerland.

Pro-inflammatory cytokine transcripts are elevated in post-mortem cortex of some people with schizophrenia. Dopamine dysregulation contributes to cognitive deficits and psychosis, yet post-mortem midbrain cytokine transcripts have not been examined. Maternal immune activation (MIA) with a viral mimic in rodents yields behavioural disturbances in adult offspring reminiscent of schizophrenia. We hypothesise that changes in immune-related pathways can be identified in adult MIA-offspring midbrain. We tested if the elevation of inflammatory markers occurs in the midbrain of a subset of people with schizophrenia and in a subset of adult mouse MIA-offspring.

The human cohort comprised 28/29 schizophrenia/control cases. The mouse cohort (32control/32 MIA-offspring) was from adult offspring (12 weeks) of dams (C57Bl6/N) given a tail injection of 5mg/kg poly(I:C) or vehicle on gestational day 17. Interleukin (IL)1β, IL6, IL18, TNFα, SERPINA3 (human and mouse) and IL6 signal transducer (IL6ST) and IL8 (human only) mRNAs were measured by qRT-PCR. Two-step clustering of gene expression followed by chi-squared analysis revealed inflammatory groups based on diagnosis or treatment. Human: control/low inflammation, schizophrenia/low inflammation, schizophrenia/high inflammation (n=29/15/13); mouse: vehicle/low inflammation, vehicle/high inflammation, poly(I:C)/low inflammation, poly(I:C)/high inflammation (n=29/3/19/13).

Most cytokine transcripts (except IL18 and IL8) were increased in the schizophrenia/high compared to control/low and schizophrenia/low groups, and in poly(I:C)/high compared to poly(I:C)/low and vehicle/low mouse groups (all p<0.05). Increases in cytokines extend to midbrain regions in schizophrenia and MIA-offspring.

In conclusion, increased cytokine transcripts in the midbrain in some people with schizophrenia may indicate a neuroinflammatory process consistent with an early developmental immune-activating signal.

CHARACTERIZATION OF THE FUNCTIONAL ROLE OF SEZ6L2, AN AUTISM CANDIDATE GENE, IN MOTOR, SOCIAL AND COGNITIVE BEHAVIOURS

Wilson YM¹, Perera A¹, Takeshima H², Gunnersen JM¹.

1. Anatomy and Neuroscience Department, University of Melbourne, Parkville, VIC, Australia. 2. Kyoto University, Kyoto, Japan

Evidence suggests that members of the Seizure-related gene 6 (Sez6) protein family play critical roles in synapse development and maintenance. In humans, it has been hypothesised that one Sez6 family member, SEZ6L2, contributes to the aetiology of autism spectrum disorders (ASD). This is based on observations that the SEZ6L2 gene is one of ~30 genes found in a recurrent copy number variation (CNV) on chromosome 16, which results in deletion or duplication of this region, that is associated with ~1% of cases of ASD. Given our interest in the Sez6 protein family and the clinical observations regarding the 16p11.2 CNV, we aim to understand more about the functions of Sez6L2. The specific aim of this project is to characterise the functional role of Sez6L2.
by analysing motor, affective and cognitive behaviours in Sez6L2 null mice (Sez6L2 KO). We analysed cohorts of littermates (10-20/genotype/sex) from early postnatal time points through to adult in a range of behavioural assays relevant to ASD. Male sez6L2 KO pups show enhanced neuromotor development while adult Sez6L2 KO males exhibit social interaction and fear memory deficits. We also observed that stress responsive behaviour became more prominent with age. Thus, the Sez6L2 KO mice exhibit a complex, multi-modal change in their behavioural profile across a variety of cognitive, affective and motor domains. Our results suggest that Sez6L2 plays a role in sex-related differences in motor development, fear memory and social interaction.

Monica Langiu (Florey Institute of Neuroscience & Mental Health)

GPR88 MODULATES COGNITIVE BEHAVIOUR RELEVANT TO PSYCHIATRIC DISORDERS

Langiu M1,2, Stewart GD1, Langmead CJ1* and Nithianantharajah J2*

1. Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, VIC, Australia.
2. The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, VIC Australia.
*Authors contributed equally.

GPR88 is an orphan G protein-coupled receptor expressed throughout the brain with high levels in the striatum, where it is localised in medium spiny neurons expressing D1 and D2 dopamine receptors. Human polymorphisms of the GPR88 gene have been linked to major psychosis in bipolar disorder and schizophrenia, and Gpr88 expression is regulated by antidepressant and mood-stabilizer treatments in both rodent models and humans. Mice lacking the Gpr88 gene (Gpr88Cre/Cre) exhibit locomotor hyperactivity, deficits in sensorimotor gating, reduced motor-coordination and impaired cue-based learning, however there is a lack of understanding on the role of Gpr88 in complex cognitive functions relevant to psychiatric disorders. To address this, we evaluated Gpr88Cre/Cre mice in a battery of tests using the rodent touchscreen system. Our results show that lack of Gpr88 alters distinct cognitive behaviours, including visual and visuo-spatial learning and memory. These findings highlight the important role of Gpr88 in modulating cognitive behaviour relevant to psychiatric disorders.

Iain Perkes (University of New South Wales)

PAVLOVIAN-TO- INSTRUMENTAL TRANSFER IMPAIRMENT IN PEOPLE WITH OBSESSIVE-COMPULSIVE DISORDER: COMPULSION-CORRELATED ORBITOFRONTAL CORTEX HYPERACTIVITY AND CORTICAL DISCONNECTION

Perkes IE1-6, Morris RW1-5,6, QUIL S1, Hazell PL2-3, Balleine B W1-6.

1. Brain & Mind Centre, The University of Sydney, NSW, Australia. 2. Department of Psychiatry, The University of Sydney, NSW, Australia. 3. Sydney Local Health District, NSW, Australia. 4. New South Wales Institute of Psychiatry, NSW, Australia. 5. Australian Research Council, Centre for Cognition and its Disorders. 6. School of Psychology, UNSW, NSW, Australia.

Obsessive-compulsive disorder (OCD) is common, disabling, and starts in childhood. Seventeen years divide symptoms from treatment. Understanding pathophysiology will enable development of more specific diagnostic methods to deliver targeted treatment earlier. Sights and sounds predicting effective handwashing seem not to control obsession-prompted compulsive handwashing in people with OCD. The lateral orbitofrontal cortex (OFC) integrates sensory input in healthy decision-making. OCD imaging studies repeatedly implicate the OFC. Decision-neuroscience can interrogate OCD pathophysiology. Here we present the first use of pavlovian-to-instrumental transfer (PIT) to investigate OCD. We partnered PIT with task-related functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI).

We found adolescents (n=21) with OCD, compared to controls (n=20), had impaired use of
pavlovian (predictive) stimuli to target instrumental actions to earn rewards; vigour of actions was intact. Impaired use of pavlovian stimuli to guide action was associated with bilateral lateral OFC hyperactivity. Synchronous rostral OFC hyperactivity positively correlated with OCD compulsions (R=0.59). DTI analysis seeded from hyperactive regions identified negative correlation between lateral OFC hyperactivity and tract strength to the precentral gyrus.

The application of general appetitive paradigms allowed interrogation of a core underpinning phenotype rather than symptom-specific behaviour. Compulsion correlation with OFC hyperactivity and associated altered white matter tractography implicates disconnection and aberrant decision-making as core OCD deficits. It may be that impaired integration of predictive information to guide choice leads to doubt and incapacity to quell inbuilt harm avoidance systems – obsessions and compulsions – the hallmarks of OCD.

Ben Gu (The Florey Institute of Neuroscience and Mental Health)

MULTIPLE PHAGOCYTOSIS RELATED FACTORS UNDERPIN THE PATHOGENESIS OF AGE-RELATED MACULAR DEGENERATION

Gu BJ1, Huang X1, Avula PK1, Caruso E2, Vessey KA3, Fowler C3, Masters CL1, Wiley JS1, Fletcher EL3, Guymer RH1

1. The Florey Institute, Parkville, Victoria; 2. Centre for Eye Research Australia, East Melbourne, Victoria; 3. Department of Anatomy, The University of Melbourne, Victoria, Australia

Age-related macular degeneration (AMD) is characterized by the deposition of debris, where the accumulation of by-products exceeds the normal clearance capacity of the retina, which may due to impaired phagocytosis. To investigate the significance of phagocytosis and related molecules in AMD pathogenesis and progress, AMD patients were recruited and compared with matched healthy controls. Using a newly developed real-time tri-colour flow cytometry method, the phagocytic function of peripheral blood monocyte subsets (non-classic, intermediate and classic) was determined. Cell surface expression of a range of blood cell markers was also assessed. Phagocytic ability was impaired in all three subsets of monocytes in both early and late AMD participants, coupled with a decreased surface expression of phagocytosis related molecules CD11b and CD11c. This deficit could be restored in vitro by glatiramer acetate (Copaxone ®) treatment. Moreover, the neutrophil/monocyte ratio was decreased, and monocyte surface expression of P2X7 and CD33 was significantly increased in AMD, particularly in late stages of disease. Our findings indicate, for the first time, that defective systemic phagocytosis is involved in pathogenic mechanisms underlying AMD. Assessing peripheral monocyte phagocytic function and phagocytosis related leukocyte surface biomarkers may provide additional insights into this disease.

Hannah Loke (Hudson Institute of Medical Research)

REGULATION AND FUNCTION OF THE Y-CHROMOSOME GENE, SRY, IN AN ANIMAL MODEL OF ATTENTION-DEFICIT HYPERACTIVITY DISORDER (ADHD)

Loke H1,2, Pinares-Garcia P1,3, Harley VR1,2,3, Lee J1,2,3.

1. Hudson Institute of Medical Research, Clayton, VIC, Australia. 2. Department of Molecular and Translational Science, Monash University, Clayton, VIC, Australia. 3. Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia

ADHD is a common neurodevelopmental disorder affecting over 5% of children worldwide. The male-sex is a significant risk factor, as boys are three times more likely to develop ADHD than girls. Emerging evidence indicates that sex-chromosome genes may influence the male-bias in ADHD. We previously showed that the Y-chromosome gene, Sry, is expressed in brain regions associated with ADHD symptoms (e.g. prefrontal cortex, hippocampus), and regulates dopamine.
transmission in males. We propose that dysregulation of Sry expression could underlie male susceptibility to ADHD. Here, we investigated the regulation and function of Sry in the spontaneously-hypertensive rat (SHR) model of ADHD. Male SHRs were more hyperactive than male Wistar-Kyoto rats (WKY, wild-type strain), exhibiting greater velocity (p<0.001) and distance travelled (p<0.001). The hyperactive behaviour in SHRs were associated with reduced Sry mRNA expression in the prefrontal cortex (p<0.05 and p<0.001, 4 and 12 weeks old), hypothalamus (p<0.05, p<0.00001) and thalamus (p<0.0001, p<0.05) compared to WKYs. Sry mRNA expression was also reduced in the striatum, substantia nigra (SN) and hippocampus of 12 week old SHRs (p<0.05). Reducing Sry expression in male WKYs, via intracerebroventricular Sry antisense oligonucleotide (ASO) infusions, impaired performance in the novel object recognition test (p<0.05 vs. sense-control) and spontaneous alternation test (p<0.01 vs. sense-control), to levels similar to those in male SHRs. Collectively, our results indicate that Sry dysregulation may contribute to the male-bias in ADHD and that Sry may be a novel therapeutic target for ADHD in males.

Jay Shukla (The Florey Institute of Neuroscience and Mental Health/ University of Melbourne)

BRAIN REGION SPECIFIC CHANGES IN METALS AND PROTEINS IN A MOUSE MODEL OF MULTIPLE SYSTEM ATROPHY

Shukla JS, McAllum EJ, McColl G, Finkelstein DI

The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria, Australia.

Multiple System Atrophy (MSA) is a rare, atypical parkinsonian disorder that is characterized by progressive neurodegeneration predominantly in nigrostriatal and olivopontocerebellar regions. The presence of protein aggregates primarily composed of α-synuclein in oligodendrocytes is the pathological hallmark of MSA, classifying it as a synucleinopathy. Regional differences in the distributions of metal ions like iron, copper and zinc have been observed in synucleinopathies and other neurodegenerative diseases and abnormally high iron deposition has been found in putamen and substantia nigra in MSA. We hypothesize that perturbations in metal metabolism is an underlying event in the pathogenesis of MSA.

This study aims to explore regional changes in the metal ion content and metalloproteome in the brains of mouse model of MSA.

Using Inductively Couple Plasma Mass Spectrometry (ICPMS) and Size Exclusion Chromatography-ICPMS, we found regional differences in the brain iron and copper content in 8-month-old MSA mice overexpressing human-α-synuclein in oligodendrocytes. The brain iron content was elevated with age. In the cerebellum, iron was increased in MSA mice compared with non-transgenic littermates, and a copper-binding protein with a molecular weight consistent with ceruloplasmin had a significantly decreased copper content. In addition, ferritin-associated iron was decreased in the caudate putamen in MSA mice. These findings indicate that decreased iron storage by ferritin and insufficient export of iron from the cells may increase the labile iron pool. Preliminary evidence suggests that ceruloplasmin dysfunction may be the cause of this dysregulation.

Yan Li (The Fourth Military Medical University)

NDRG2 DEFICIENCY LEADS TO ATTENTION DEFICITS AND HYPERACTIVE BEHAVIORS

Yan Li¹,², Anqi Yin¹, Xin Sun³, Ming Zhang¹, and Lize Xiong¹.

¹. Department of Anesthesiology, Xijing Hospital, the Fourth Military Medical University, Xi’an, Shaanxi 710032, China. ². Institute of Neuroscience, the Fourth Military Medical University, Xi’an, Shaanxi 710032, China. ³. Department of Pediatrics, Xijing Hospital, the Fourth Military Medical University, Xi’an, Shaanxi 710032, China
Attention-deficit/hyperactivity disorder (ADHD) is a prevalent psychiatric disorder in children. Although an excitatory/inhibitory imbalance has been proposed, the mechanisms underlying this highly heterogeneous disease remain largely unknown. Here, we report a novel role for N-myc downstream-regulated gene 2 (NDRG2) in the development of ADHD in both mice and humans. NDRG2 knockout (Ndrg2−/−) mice exhibited ADHD-like symptoms characterized by attention deficits, hyperactivity, impulsivity, and impaired memory. Furthermore, interstitial glutamate levels and excitatory transmission were significantly increased in the brains of Ndrg2−/− mice due to reduced astroglial glutamate clearance. We then developed an NDRG2 peptide to rescue astroglial glutamate clearance and reduce excitatory glutamate transmission. The ADHD-like hyperactivity in the Ndrg2−/− mice was also rescued by the NDRG2 peptide treatment, while no effect was observed with routine methylphenidate treatment. In addition, we found that children who were heterozygous for rs1998848, a single-nucleotide polymorphism (SNP) in NDRG2, had a higher risk of ADHD than children who were homozygous for rs1998848. Our results indicate that NDRG2 deficiency leads to ADHD phenotypes and that impaired astroglial glutamate clearance underlies the resultant behavioral abnormalities, which is distinct from the dopamine deficit hypothesis for ADHD.
**Oral 4**  
*Parkinson’s disease*

**GLUCOCEREBROSIDASE ACTIVITY IN PERIPHERAL MONONUCLEAR CELLS FROM PARKINSON’S DISEASE PATIENTS**

Atashrazm F.², Hammond D.¹, Kwok J.², Lewis S.¹, Dzamko N.², Halliday G.M.²

1. Brain and Mind Centre, Central Clinical School, University of Sydney, Australia.  
2. Neuroscience Research Australia, University of New South Wales, Australia.

Heterozygous mutations in *GBA1*, the gene that encodes the enzyme glucocerebrosidase (GBA), are the most common genetic risk factors for non-familial Parkinson’s disease (PD). Decreased GBA activity and protein levels have been found in the brain of PD patients, and this has been linked to the pathological accumulation of the hallmark PD protein alpha-synuclein. GBA is also highly expressed in peripheral immune cells, particularly monocytes. Thus, to determine if reduced GBA activity can also be detected in peripheral immune cells from PD patients we generated a fluorescent flow cytometry-based method and measured GBA activity in twenty PD patients and twenty matched healthy controls. We recruited patients that were less than eight years since diagnosis and simultaneously measured GBA activity in CD14+ monocytes, CD19+ B-cells and CD3+ T-cells. GBA protein levels were also measured in immunomagnetically isolated monocytes and lymphocytes. Following multivariate analysis, co-varying for age and gender, a significant 30% reduction in GBA activity was seen in monocytes from PD patients compared to controls (p<0.05). In contrast, no significant difference between the two groups was seen for GBA activity in either T-cells or B-cells. There was also no significant difference in GBA protein levels between the two groups in either the monocytes or lymphocytes. These initial results suggest that GBA activity may be reduced in monocytes in PD patients, and this may occur early in disease pathogenesis. If so, monocyte GBA activity may have utility as a peripheral Parkinson’s disease biomarker.

**ACCUMULATION OF ENDOGENOUS ALPHA-SYNCULEIN FOLLOWING TREATMENT OF NEURONS WITH ALPHA-SYNCULEIN FIBRILS**

Gao J.¹, Perera G.¹, Halliday G.M.¹, Dzamko N.²

1. Faculty of Medicine, Central Clinical School, University of Sydney

Accumulation of the alpha-synuclein protein is a defining neuropathological feature of Parkinson’s disease (PD), and likely contributes to neuronal dysfunction and clinical symptoms. The alpha-synuclein protein invades vulnerable neurons in the PD brain in a predictable, staged pattern. Recent evidence suggests that this propagation has some characteristics similar to the propagation of the prion protein, with distinct toxic alpha-synuclein species triggering the pathological conversion of normal endogenous alpha-synuclein in neighbouring cells. Subsequently there is much interest in determining the mechanism by which this occurs. We show that alpha-synuclein fibrils, but not monomeric alpha-synuclein, can induce the accumulation of endogenous alpha-synuclein in SHSY5Y cells, as well as in primary human neurons differentiated from induced pluripotent stem cells. Alpha-synuclein accumulation peaked at 6 days post treatment with fibrils and the 6-day conditioned media was able to induce alpha-synuclein pathology in new cells. We also generated alpha-synuclein knockout SHSY5Y cells using CRISPR/Cas9, and confirmed that endogenous alpha-synuclein is required for fibril induced alpha-synuclein pathology. These results increase the understanding of how alpha-synuclein fibrils trigger PD-related pathology and provide a convenient model for the screening of potential therapeutics to prevent alpha-synuclein accumulation.
Yujing Gao (Murdoch Childrens Research Institute)

GENERATION AND CHARACTERISATION OF NOVEL STEM CELL AND MOUSE MODELS TO INVESTIGATE THE MOLECULAR BASIS OF RAB39B-MEDIATED PARKINSON’S DISEASE

Gao Y1,2, Wilson GR1,2, Stephenson SEM1,2, Wilson M1, Dottori M3, Thomas PQ4, Lockhart PJ1,2.
1. Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, Melbourne, Australia. 2. Department of Paediatrics, University of Melbourne, Melbourne, Australia. 3. Centre for Neural Engineering, University of Melbourne, Melbourne, Australia. 4. School of Biological Sciences, The University of Adelaide, Adelaide, Australia.

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra and presence of α-synuclein (αSN) aggregates. We previously demonstrated loss of function mutations in Ras Analog in Brain 39B (RAB39B) cause early onset PD, with dysregulation of αSN observed in vitro and in vivo. Our aim is to investigate the function of RAB39B and its role in αSN regulation.

We utilised CRISPR/CAS9 genome editing to generate isogenic pluripotent stem cell (PSC) lines with deletion of RAB39B, in addition to PSC from RAB39B deletion patient fibroblasts. We confirmed RAB39B deletion by western blot analyses in knockout (KO) cells and successfully differentiated cortical and dopaminergic neurons. Mature knockout neurons showed a significant increase in steady-state αSN. CRISPR/CAS9-mediated KO of Rab39b in C57BL/6 mouse was also performed. Behavioural analysis of a ~13 month old KO cohort identified motor phenotypes suggestive of nigrostriatal disturbance. In a tail suspension test, we observed sustained hindlimb claspung and on the balance beam, KO mice performed significantly worse than littermate controls, slipping four times as often (WT: 1.9±0.7, n=10 Vs KO: 7.6±2.0, n=10; P=0.009, mean±SEM). Similarly, there was a significant deficit recorded by accelerating rotarod (WT: 169±17 seconds, n=7 Vs KO: 83±21 seconds, n=6; P=0.009, mean±SEM).

In conclusion, RAB39B–mediated PD involves a perturbation of αSN homeostasis. We have generated unique models that recapitulate aspects of the human disease; these will be useful tools to determine the neuropathological mechanisms underlying RAB39B-mediated PD and the therapeutic potential of RAB39B.

Jin Sung Park (Kolling Institute)

THERAPEUTIC POTENTIAL OF NIX-MEDIATED MITOPHagy IN PINK1/PARKIN-RELATED PARKINSON’S DISEASE

Jin-Sung Park, Brianada Koentjoro and Carolyn M. Sue

Department of Neurogenetics, Kolling Institute, Royal North Shore Hospital and the University of Sydney, St. Leonards, NSW 2065, Australia

Mitochondrial dysfunction has been suggested as a key pathogenic mechanism in Parkinson’s disease (PD) due to its common association with both sporadic and familial forms. Among the PD-related genes, loss-of-function mutations in Parkin (PARK2) cause autosomal recessive early-onset PD with a high penetrance by impairing mitophagy and mitochondrial function. Previously, we identified a rare case of an asymptomatic homozygous Parkin mutation carrier (MC), who had not developed PD in her seventies despite the complete loss of Parkin, while the proband (a compound heterozygote) developed early-onset Parkin-related PD. When mitochondrial function and mitophagy were assessed in patient-derived fibroblasts, MC cells, unlike the proband cells, showed well-preserved mitochondrial function and intact mitophagy upon exposure to the mitophagy inducer CCCP. In MC cells, we discovered that Nix, but not PINK1, is involved in the Parkin-independent mitophagy. Genetic and pharmacological induction of Nix expression in cell lines derived from Parkin or PINK1-related PD patients, restored mitophagy and improved mitochondrial energy production, while Nix knockdown abolished the observed Parkin-independent mitophagy. Our findings indicate that Nix compensates for the loss of Parkin in MC, preventing the development of PD by maintaining normal mitochondrial quality control and function, and
induction of Nix expression can improve mitochondrial function by restoring mitophagy in Parkin and PINK1-related PD. Taken together, these findings suggest Nix as a novel therapeutic target for neuroprotective treatment in PINK1/Parkin-related PD.

Eduardo Albornoz Balmaceda (University of Queensland)

THE INFLAMMASOME COMPONENT ASC PLAYS A PATHOLOGICAL ROLE IN PARKINSON’S DISEASE

ALBORNOZ E. A.1, GORDON R.1, ROBERTSON A. B.2, STACEY K. J.3, COOPER M.2, WOODRUFF T.M.1

1.School of Biomedical Sciences, The University of Queensland, Brisbane, Australia. 2. Institute for Molecular Biosciences, The University of Queensland, Brisbane, Australia. 3. School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia

Neuroinflammation is a hallmark of Parkinson’s disease (PD), and is triggered by the activation of the innate immune response. Inflammasomes are key protein complexes of innate immunity that can be activated by protein aggregates such as α-synuclein (Syn). Inflammasome activation induces the release of IL-1β through the assembly of the inflammasome complex, involving ASC proteins and the recruitment of caspase-1 enzymes. Interestingly, ASC functions not only as an adaptor protein in the inflammasome, but also has extracellular and prionoid activities that can propagate inflammation. This study aimed to determine the role of ASC in propagating Syn pathology and PD neurodegeneration. We initially assessed ASC expression in post mortem human PD brains and serum from PD patients. We found that ASC was upregulated in the substantia nigra of PD brains, and in serum from PD patients compared to healthy controls. Next, we examined the effects of ASC deficiency in two mouse models of PD, induced through the striatal injection of Syn fibrils or the dopaminergic toxin, 6-OHDA. Strikingly, ASC−/− mice shown a significant improvement in amphetamine-induced rotations in the 6-OHDA model, and improvements in balance-beam and wire-hang tests in Syn-injected mice compared to wild-type mice. In primary microglia, Syn induced microglial inflammasome activation inducing extracellular release of ASC specks, in the absence of cell death (pyroptosis). Collectively, our results suggest that ASC-mediated inflammasome formation and its extracellular release could be involved in the propagation of Syn and dopaminergic neurotoxicity in PD, making it an ideal therapeutic target to mitigate disease progression.

Bolek Zapiec (Max Planck Research Unit for Neurogenetics)

THREE-DIMENSIONAL RECONSTRUCTIONS REVEAL A VENTRAL GLOMERULAR DEFICIT IN PARKINSON’S OLFACTORY BULB

Zapiec B., Dieriks BV, Tan S., Faull RLM, Mombaerts P, and Curtis MA

1 Max Planck Research Unit for Neurogenetics, Frankfurt, Germany
2 Department of Anatomy and Medical Imaging and Centre for Brain Research, Faculty of Medical and Health Science, University of Auckland, Auckland, New Zealand

Olfactory dysfunction is common in Parkinson’s disease (PD) and is an early symptom, but its pathogenesis remains poorly understood. We report a quantitative approach to describe the human olfactory bulb, and statistical comparisons between olfactory bulbs from normal and PD cases. We subjected horizontal 10 µm sections of olfactory bulbs from six normal and five PD cases to fluorescence immunohistochemistry. We scanned the stained sections with a fluorescence slide scanner, segmented the glomeruli, and generated three-dimensional reconstructions of whole olfactory bulbs. We document the occurrence of atypical glomerular morphologies and glomerular-like structures deep in the olfactory bulb, both in normal and PD cases. We define a novel parameter: the global glomerular voxel volume (GGVV), which is the total volume of all voxels that are classified immunohistochemically as glomerular. We find that the GGVV of olfactory bulbs from PD cases is half of that from normal cases. Moreover, the distribution of glomerular voxels along the dorsal-ventral dimension of the olfactory bulb is significantly altered in PD cases: whereas most
glomerular voxels reside within the ventral half of the olfactory bulb from normal cases, glomerular voxels are more evenly spread among the ventral and dorsal half-bulbs from PD cases. These observations indicate a preferentially ventral glomerular deficit in PD, which is consistent with the olfactory vector hypothesis for its pathogenesis. The distribution of α-synuclein also correlates with that of glomerular voxels. Our quantitative approach will help our understanding of the human olfactory bulb and its alterations in PD.
Oral 5
Glia

Jana Vukovic - University of Queensland

DEPLETION OF MICROGLIA IMPROVES SPATIAL LEARNING AND PROMOTES HIPPOCAMPAL NEUROGENESIS FOLLOWING TRAUMATIC BRAIN INJURY

Emily F. Willis,1 Justine Y. Yu,1 Min Chen,3 Marc J. Ruitenberg,1 and Jana Vukovic1,2
1School of Biomedical Sciences, 2Queensland Brain Institute, 3Centre for Advanced Imaging, The University of Queensland, Australia

Traumatic brain injury (TBI) is a leading cause of ongoing disability, including cognitive deficits. Microglia have been implicated in driving secondary injury and contributing to learning and memory deficits after TBI. A direct causal role in these processes is, however, yet to be demonstrated. To address this, we conditionally depleted microglia in Cx3cr1CreERT2 x IDTR transgenic mice and subsequently examined the consequences of a moderate unilateral controlled cortical impact on spatial learning. We found that microglial depletion (>85%) significantly improved reversal learning in the Morris water maze (P<0.05) and Y-maze (P<0.001) task after TBI. Having previously established a role for hippocampal neurogenesis in learning and memory (Vukovic et al., 2013), we next explored the neurogenic response to TBI in the presence or absence of microglia. We found that microglia initially supported the survival of immature doublecortin (DCX)+ neurons, as their depletion reduced the number of DCX+ cells 6 hours post-TBI (28.5%, P<0.05). Over time, however, microglia appeared to become neurotoxic as their depletion increased both numbers of EdU+/Tbr2+ proliferating neuronal progenitors (47.3% increase, P=0.016) and newly generated BrdU+/DCX+ cells (53% increase, BrdU injected 1-3 days post-injury; P=0.04). Increased tissue preservation was also apparent in these animals for the whole hippocampus (48%), dentate gyrus (34%) and CA1 (60%; P<0.05). Moreover, microglial depletion resulted in significantly reduced IL-1β protein expression in the ipsilateral hippocampus 12 days after TBI (P=0.039, 57.68%). Collectively, our results suggest that microglia causally contribute to suppression of hippocampal neurogenesis and impair spatial learning abilities after TBI.

Laura Morcom (Queensland Brain Institute)

DCC SIGNALLING REGULATES GLIAL CELL MORPHOLOGY TO INITIATE GLIAL-MEDIATED INTERHEMISPHERIC MIDLINE REMODELLING AND CORPUS CALLOSUM FORMATION.

Morcom L1, Gobius I1, Douglass A1,2, Donahoo ALS1, Marsh APL3,4, Edwards1,5, TJ, Levenger RJ4,6,7, Lockhart PJ3,4, & Richards LJ1,8.

1Queensland Brain Institute, the University of Queensland, Brisbane, Australia.
2Current Address: Max Planck Institute of Neurobiology, Am Klopferspitz 18, 82152 Martinsried, Germany.
3Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, Royal Children’s Hospital, Parkville, Victoria 3052, Australia.
4Department of Paediatrics, the University of Melbourne, Parkville, Victoria 3052, Australia.
5Faculty of Medicine, the University of Queensland, Brisbane, Australia.
6Neuroscience Research Group, Murdoch Childrens Research Institute, Parkville, Victoria.
7Department of Neurology, the University of Melbourne, Royal Children’s Hospital, Parkville, Victoria 3052, Australia.
8The School of Biomedical Sciences, the University of Queensland, Brisbane, Australia.

During brain development, commissural axons must cross the midline between the brain hemispheres to reach their contralateral targets and establish interhemispheric communication. We recently identified that astroglial-mediated remodelling of the interhemispheric fissure of the forebrain midline is a novel process that permits the formation of the largest commissure in mammals, the corpus callosum. Defects in this process are
associated with congenital absence of the corpus callosum in humans and mice. Individuals with mutations in the axon guidance receptor DCC fail to form a corpus callosum and show aberrant retention of the interhemispheric fissure. Given these results, we investigated the role of DCC in interhemispheric fissure remodelling. Dcc and Dcc ligands Ntn1 and Draxin, are expressed by the midline zipper glia, which are astrogial cells that initiate midline remodelling and act as a bridging substrate for callosal axons to cross into the contralateral hemisphere. In glial cell culture, DCC promotes process extension and cell elongation by modulating the actin cytoskeleton. In Dcc and Ntn1 mouse mutants, midline zipper glia show defects in process extension and somal translocation to the pial surface of the interhemispheric fissure and are unable to initiate interhemispheric fissure remodelling. Together our results reveal a novel role for axon guidance genes in regulating glial cell morphology. Our results strongly suggest that defects in astroglial-mediated interhemispheric fissure remodelling are likely to underlie congenital absence of the corpus callosum associated with human DCC mutations.

Anthony Boghdadi - Monash University

A NOVEL TRANSIENT GLIAL INTERACTION FOLLOWING ISCHAEMIC STROKE IN THE MARMOSET

Australian Regenerative Medicine Institute, Monash University, Clayton, VIC, Australia.

Ischaemic stroke affecting the primary visual cortex (V1) results in permanent cortical blindness. The myelin-associated inhibitor (MAI) ligand, neurite outgrowth inhibitor-A (NogoA) and receptor, paired immunoglobulin-like receptor-B (PirB), are major inhibitors of regeneration. This study profiles NogoA/PirB at specific acute time points following ischaemic stroke to the adult marmoset V1.

Injections of 0.5µL endothelin-1 (1mg/mL) over 4-sites surrounding the posterior cerebral artery of operculum V1 in adult marmosets (>1 year; n=20) were used to induce ischaemia. Control, 1-day post injury (dpi), 7dpi and 21dpi brains were snap-frozen (n=8) or fixed (n=12) for downstream analysis. Western blot, immunohistochemistry and in vitro techniques were used to analyse the ligand-receptor pair.

Elevated NogoA/PirB expression was observed in marmoset V1 (peri-infarct) 7-21dpi. NogoA labelled a distinct population of GFAP+ reactive astrocytes surrounding the lesion site at 7dpi. PirB labelled two subpopulations of Iba1+ macrophages (microglia + infiltrating macrophages) with different morphologies, separated by the population of NogoA+ astrocytes. NogoA expression on reactive astrocytes was downregulated by 21dpi, corresponding to deeper infiltration of PirB+ macrophages into the cortex. Analysis of key downstream effectors, such as POSH/Shroom3, was strongly suggestive of an active signalling pathway. In vitro assays using human microglia plated on Nogo-66 (NEP1-40)-coated stripes confirmed NogoA/PirB-induced repulsion.

These findings provide evidence of a novel NogoA/PirB-dependent interaction where astrocytes transiently repel infiltrating inflammatory cells in the acute stages following ischemic stroke in primates. This previously undescribed NogoA-dependent interaction may associate the ligand with additional roles, such as mediating the inflammatory response following CNS injury.

Junhua Xiao - University of Melbourne

TRKB SIGNALING IN NEURONS REGULATES OLIGODENDROGLIAL DEVELOPMENT AND CNS MYELINATION

Fatemeh Daemi1, Emma Hoffmann1, Rhiannon Wood1, Melissa Biemond1, Tim Aumann2, Julie Pasquet1, David Gonsalvez1, Jessica Fletcher1, Agnes Wong1, Simon Murray1 and Junhua Xiao1
1 Neurotrophin and Myelin Laboratory, Department of Anatomy and Neuroscience, Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Parkville, Victoria, 3010
2. **Midbrain Dopamine Plasticity Laboratory, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, 3010**

Coordinated neuron-glial cell signalling is critical for the development and maintenance of a healthy nervous system function. Communication between neurons and oligodendrocytes are central to normal myelin formation during both development and in adult. This has profound implications for life-long plasticity and learning. However, the molecular cues that instruct myelin wrapping around CNS axons have yet to be identified. Brain-derived neurotrophin factor (BDNF) has been implicated in controlling CNS myelination via its tropomyosin related kinase B (TrkB) receptors, however the precise cellular and molecular mechanisms by which BDNF/TrkB signalling regulates CNS myelination remains unclear. TrkB is expressed by both neurons and oligodendrocytes. We have previously identified that TrkB signalling in oligodendroglial cells to exert specific influence upon myelin wrapping. Here we investigated the influence that TrkB signalling in neurons exerts upon oligodendrocyte and myelination via generating a neuronal specific TrkB knockout mouse (TrkBflflNFLCre, TrkB cKO). We found that neuronal deletion of TrkB significantly altered the number of oligodendroglia lineage cells in key white matter tracts throughout development and in youth adulthood in vivo. Importantly, the initiation of myelination and myelin thickness was significantly reduced in TrkB cKO mice compared to littermate controls without altering either axonal number or calibre. Together, our results suggest that TrkB signalling in neurons exerts crucial and direct effects upon oligodendroglial development and subsequent myelin formation, identifying TrkB as a novel neuronal signal that controls glial cell functions in the CNS in vivo.

Georgina Craig - The University of Melbourne

**NEW INSIGHTS INTO THE MODE OF OLIGODENDROCYTE PRODUCTION THROUGHOUT DEVELOPMENT**

Craig GA1, Gonsalvez DG1, Yoo SW4, Wood RJ1, Murray SS1,2, Xiao J1,2
1. Department of Anatomy and Neuroscience, School of Biomedical Sciences, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne. 2. The Florey Institute of Neuroscience and Mental Health Research, The University of Melbourne.

Dysmyelination is a common feature of many juvenile and adolescent neurological disorders. Surprisingly, however, the normal process of central nervous system (CNS) myelination during development is poorly understood. One of the barriers to studying developmental myelination has been the inability to obtain temporally-sensitive information on in vivo cell cycle dynamics of oligodendrocyte precursor cells (OPCs) using traditional cumulative labelling approaches. To overcome this problem, we have developed a short, double-S phase labelling protocol to determine cell cycle lengths of OPCs throughout normal CNS development (from postnatal day 5 (P5) to P90). Combining this approach with design-based stereology, we have identified a mode of OPC proliferation and production that is contrasting to previous inferences made about OPC production using traditional cumulative labelling methods. Namely, we have found that aging does not appear to alter an OPC’s capacity for fast cell division. Instead, it seems oligodendrocyte production is regulated by the proliferative fraction (PF) of OPCs, which peaks early in development and then rapidly decreases in an exponential manner. Furthermore, it has been found that while adjacent CNS tracts exhibit identical OPC cell cycle lengths (~40 hours in corpus callosum and cortical grey), the initial-state PF values identified at peak proliferation significantly vary (p = 0.0043**; Corpus Callosum (PF ± SEM): ~0.43 ± 0.012, n=3; Cortical Grey: ~0.32 ± 0.015, n=3). To our knowledge, this is the first study which will comprehensively model OPC production in various CNS regions throughout postnatal development.

Lulu Xing - Monash University

**NEURAL PROGENITOR CELLS CONTRIBUTE TO REGENERATION OF OLIGODENDROCYTE PROGENITOR CELLS AFTER PHARMACOGENETIC ABLATION IN THE ADULT MOUSE BRAIN**
Xing YL, Chuang BHA, Mitew S, Kilpatrick TJ, and Merson TD.

1. Australian Regenerative Medicine Institute, Monash University, Clayton, Australia. 2. Melbourne Neuroscience Institute, University of Melbourne, Parkville, Australia.

Adult oligodendrocyte progenitor cells (OPCs) expressing the platelet-derived growth factor receptor alpha (PdgfRα) are known primarily for their role in the generation of new myelin-forming oligodendrocytes in both the healthy and demyelinated central nervous system (CNS). Using the cuprizone model of demyelination, we recently demonstrated that, in addition to OPCs, Nestin+ neural progenitor cells (NPCs) residing within the adult subventricular zone (SVZ), also contribute significantly to remyelination of the corpus callosum. In this model, oligodendrogenic NPCs were recruited principally to regions proximal to the SVZ. To determine whether oligodendrogenic NPCs have the capacity to migrate more broadly throughout the CNS, we ablated adult OPCs using a highly efficient pharmacogenetic approach. These mice exhibited a complete loss of OPCs throughout the entire brain for up to 10 days. Strikingly, from this time-point onwards, PdgfRα+ cells gradually reappeared, arising first in regions adjacent to the SVZ and progressively repopulating the brain. Combined pharmacogenetic ablation and genetic fate-mapping of OPCs revealed that the vast majority of new PdgfRα+ cells did not derive from non-ablated OPCs. These data are consistent with the view that the ablation of OPCs in the normally myelinated adult brain mobilises oligodendrogenic NPCs to efficiently regenerate the entire PdgfRα+ cell population. Our data lend credence to the notion that adult NPCs have the capacity to migrate extensively throughout the CNS and that strategies targeted towards mobilising NPCs could promote remyelination of demyelinated lesions located large distances from the SVZ.

Sarah-Jane Leigh - University of New South Wales

ORAL MINOCYCLINE HYDROCHLORIDE REVERSES HIPPOCAMPAL-DEPENDENT COGNITIVE IMPAIRMENT ASSOCIATED WITH CAFETERIA DIET AFTER THE ONSET OF OBESITY

Sarah-Jane Leigh, Fred Westbrook and Margaret J. Morris

1 School of Medical Sciences, UNSW Sydney, Australia
2 School of Psychology, UNSW Sydney, Australia

While a large body of literature indicates that obesogenic diets are associated with cognitive impairments in people and rodents, the underlying mechanisms remain controversial. One potential mechanism is increased inflammatory signalling. The anti-inflammatory minocycline hydrochloride (mino) has been routinely used to depress microglial activity. We used a rodent model to test the hypothesis that blocking inflammatory signalling both peripherally and centrally with mino would alleviate the cognitive deficits induced by a diet rich in saturated fat and refined carbohydrate. Male adult rats were fed either regular chow or a cafeteria diet, consisting of regular
chow, various cakes, biscuits, savoury foods and a 10% sucrose solution for four weeks. Half the rats in each dietary condition were treated orally daily for the next two weeks with vehicle (syrup) while the remainder received mino (40mg/kg/day). The four groups performed equally well on the perirhinal-dependent object recognition memory task. However, they differed in their performances on the hippocampal-dependent place recognition task. Specifically, vehicle treated rats fed cafeteria diet performed worse in the place recognition task than those fed chow receiving vehicle or mino and, critically, rats fed the cafeteria diet and treated with mino. These results confirm a diet-induced impairment in a hippocampal-dependent form of cognition. Importantly, that impairment is reversed by ingestion of the anti-inflammatory drug mino, indicating a role for inflammatory signalling in the impairment. However, since mino also has antibiotic activity, further investigation into the role of the gut-brain axis in diet-induced cognitive impairments is underway.

Jaisalmer De Frutos - Universidad Autónoma de Madrid

DOES BILINGUALISM AFFECT FUNCTIONAL CONNECTIVITY IN HIGHLY EDUCATED OLDER ADULTS?

Bilingualism has been said to improve cognition, particularly executive function, and even delay the onset of Alzheimer’s disease. These claims have generated a great deal of controversy since such results have not always been replicated. However bilingualism is a complex trait and thus many have specified a list of confounding factors that could potentially account for the above-mentioned effects. One such factor is educational attainment. In this study, we wanted to investigate whether bilingualism leaves a neurophysiological trace even when people are highly educated. With this purpose, we conducted a magnetoencephalographic study with a group of healthy older adults all of which had enrolled in postsecondary studies. The sample included 22 late bilinguals (9 men) and 16 monolinguals (5 men). We estimated functional connectivity using phase-locking value in the theta, alpha, beta1 and beta2 bands in 4 minutes eyes closed resting state recordings. We found five clusters in which bilinguals exhibited significantly greater functional connectivity than monolinguals. These clusters included brain regions typically implicated in language processing (such as the bilateral lingual gyri, the left supramarginal gyrus, and other superior and inferior occipital regions) as well as regions in the default mode network (such as the left posterior cingulate cortex and the left precuneus). Our results highlight that using two languages shapes brain connectivity beyond the effect of educational level. Whether this results in a higher degree of cognitive reserve is a topic that requires future research work.

George Kalatzis - University of Technology Sydney

EXPLORING COGNITIVE FUNCTION IN DIABETES AND NON-DIABETES SAMPLES USING ELECTROENCEPHALOGRAPHY (EEG) AND PSYCHOMETRIC ASSESSMENT: A COMPARATIVE STUDY

Kalatzis G1, Lees T1, Nassif N1, Zaslavski C1,2, Lal S1

1. Chronic Disease Solutions Team, University of Technology, Sydney, Australia. 2. Traditional Chinese Medicine Clinic, University of Technology, Sydney, Australia.

Increasing evidence indicates diabetes mellitus—both type 1 and type 2—is associated with accelerated central nervous system decay; however, little is known about the cognitive complications of diabetes mellitus, particularly the neurophysiological changes. Also unclear are the
Australasian Neuroscience Society Annual Scientific Meeting 2017
International Convention Centre, Sydney, December 3rd – 6th 2017

precise cognitive domains detrimentally affected by diabetes or the relationship between blood glucose concentration and cognitive deficits. In the present ongoing comparative investigation, cognitive function is being assessed in non-diabetes (control) (n=48) and in those with diabetes mellitus (Type 1 or Type 2) (n=19) using 32-channel electroencephalography (EEG) and neuropsychometric batteries. Global cognitive performance is being examined using the Mini-Mental State Examination (MMSE); domain-specific cognitive performance using the Cognistat. Blood glucose levels following a 2-hour fast are also being determined. Preliminary analysis of electrophysiological and neurocognitive data reveal subjects with diabetes demonstrate slightly altered brain activity and worse overall cognitive performance in specific cognitive domains. Specifically, subjects with diabetes exhibit significantly worse performance in the construction domain (p= 0.005) compared to controls. Correlation analysis additionally revealed a significant negative association between pre-study blood glucose and the similarity domain (p=0.002). Present electroencephalographic and psychometric data indicate that patients with diabetes mellitus show early changes in brain electrical activity and perhaps worse overall cognitive performance. Data obtained highlight the importance of short-term and long-term glycaemic control, and raise the possibility that EEG could be used to non-invasively monitor changes in neuronal activity long before irreversible deficits in cognition have manifested, potentially delaying progression to dementia.

Kathryn Baker - School of Psychology, University of New South Wales

**A HIGH-FAT HIGH-SUGAR DIET-INDUCED IMPAIRMENT OF FEAR INHIBITION AND PLACE-RECOGNITION MEMORY IN ADOLESCENT RATS**

*Williams-Spooner M, Richardson R, Baker KD.*
*School of Psychology, UNSW Sydney, NSW, Australia.

High-fat high-sugar (HFHS) diets are associated with increased prevalence of anxiety disorders and these disorders often emerge in adolescence. However, little is known about the consequences of HFHS diets during adolescence on fear inhibition. The present experiments investigated the effect of a HFHS diet lasting 10 or 21 days on fear extinction in adolescent and adult rats. Male rats received HFHS pellets (2 h/day; Specialty Feeds, SF04-025) in addition to a chow diet for 10 or 21 days during adolescence or adulthood. Controls received a chow-only diet. Rats were tested for extinction retention as well as object-recognition and place-recognition memory. We found that rats exposed to 21, but not 10, days of a HFHS diet in adolescence had higher levels of freezing during a second extinction session compared to controls, indicating impaired extinction retention. An equivalent 21 days HFHS diet in adulthood did not induce extinction deficits. In addition, rats given a HFHS diet in adolescence exhibited impaired hippocampal-dependent place-recognition memory but equivalent perirhinal-dependent object-recognition memory compared to controls. Importantly, the extinction and place-recognition memory deficits were reversible by switching animals to a standard diet. However, the neural mechanisms of these deficits remain elusive as the HFHS diet did not affect parvalbumin neuron counts in this study in the medial prefrontal cortex or hippocampus. These results indicate that adolescence is a sensitive developmental period for HFHS diet-induced impairments of fear inhibition and place-recognition memory compared to adulthood.

Rebecca Norris - Florey Institute

**MICE LACKING THE SYNAPTIC PROTEIN NEUROLIGIN-3 SHOW ALTERED COGNITION IN A BATTERY OF TOUCHSCREEN TESTS**

*Norris RHC, Hannan AJ, Brose N, Nithianantharajah J.*
*1.The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Victoria, Australia. 2.Max Planck Institute for Experimental Medicine, Göttingen, Germany.

*Neuroligins are a family of postsynaptic cell adhesion molecules that form trans-synaptic complexes critical for synapse formation, maturation and function. Human mutations in neuroligin genes have*
been reported in *neurodevelopmental* disorders including schizophrenia and autism spectrum disorders, where cognitive dysfunction is a core symptom. Specifically, human mutations in the x-linked neuroligin-3 gene (NLGN3) have been found in autism spectrum disorders. Male Nlgn3 null mutant mice (Nlgn3^{-/-}) show region, circuit and cell-type specific alterations in excitatory and inhibitory synaptic transmission. To comprehensively examine the impact of Nlgn3 loss on cognitive behaviour, we assessed male Nlgn3^{-/-} mice on a battery of cognitive tasks using the rodent touchscreen system. The tasks we selected dissociate different components of cognition including attention, working memory, pattern separation, visual discrimination and cognitive flexibility. We found that Nlgn3^{-/-} mice display significant alterations in distinct cognitive functions, including pronounced changes in sustained attention and behavioural flexibility. Our results show that Nlgn3 is differentially involved in discrete aspects of cognitive processing, which has strong relevance for understanding the impact of Nlgn3 mutations in *human disorders.*

Adam Walker - Monash University

**ASPIRIN BLOCKS CANCER-INDUCED COGNITIVE IMPAIRMENT**

1. Drug Discovery Biology Theme, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria 3052, Australia. 2. Division of Cancer Surgery, Peter MacCallum Cancer Centre, East Melbourne, Victoria 3002, Australia. 3. Cousins Center for PNI, UCLA Semel Institute, Jonsson Comprehensive Cancer Center, and UCLA AIDS Institute, University of California Los Angeles, Los Angeles, CA 90095, USA

Neuroinflammation induces cognitive impairment in patients with a range of clinical diseases, yet is understudied in cancer patients despite its high prevalence in this population. Instead chemotherapy is typically cited as the cause of memory, learning and concentration difficulties that frequently occur in cancer patients. Here, we show that a solid tumour alone is sufficient to induce memory loss prior to treatment in mice through neuroinflammation. We demonstrate that an anti-inflammatory drug (aspirin) blocks tumor-induced cognitive impairment. We injected luciferase-tagged syngenic mammary adenocarcinoma cells into BALB/c female mice, and assessed episodic memory. Tumor-bearing mice had significantly poorer episodic memory than non-tumor-bearing mice. This was independent of cancer-induced sickness behaviour, which was only observed during the later stage of cancer progression with high metastatic burden. Proinflammatory cytokines were elevated in the brains of tumor-bearing mice. Oral treatment with low-dose aspirin completely blocked tumor-induced episodic memory impairment without affecting tumor-induced sickness or tumor growth. These data demonstrate that proven anti-inflammatories may be given to patients at diagnosis to alleviate cancer-associated cognitive impairment.

**Oral 7**

_Sensory systems_

Jason Ivanusic - The University of Melbourne

**PIEZO2 CONTRIBUTES TO ALTERED EXCITABILITY OF AΔ BONE MARROW NOCICEPTORS**

Nencini S, Thai J and Ivanusic J

*Department of Anatomy and Neuroscience, The University of Melbourne*

Piezo2 is a newly discovered mechanically gated ion-channel that has received significant attention because of its remarkable structure. Piezo2 has a well-defined role in innocuous mechanical sensitivity, but very recently it has also been suggested to play a role in mechanically-induced pain. Here we have explored the role of Piezo2 in bone pain. We have used immunohistochemistry to identify Piezo2 expression in nerve terminal endings in the bone marrow. We have combined immunohistochemistry with retrograde tracing to show that Piezo2 is expressed in the soma of the majority of small myelinated (Aδ) bone marrow nociceptors. We have also used an *in vivo* electrophysiological bone-nerve preparation to record the activity of single Aδ-fibre bone marrow nociceptors.
nociceptors in response to a sustained noxious mechanical stimulus (increased intra-osseous pressure) after knockdown of Piezo2 expression in the DRG with intrathecal injections of Piezo2 antisense oligodeoxynucleotides. There were no differences in the number of units responding to the pressure stimulus in Piezo2 knockdown animals compared to mismatch control animals. However units with a phasic-tonic response to mechanical activation appeared to lose the tonic part of their response after Piezo2 knockdown. Units recorded from Piezo2 knockdown animals also took much longer to recover from stimulus-evoked fatigue than those recorded from control animals that received mismatch oligodeoxynucleotides. Together, these findings suggest that Piezo2 contributes to altered excitability of Aδ bone marrow nociceptors and that it might constitute a channel to target for therapeutic benefit in painful conditions driven by mechanical disturbance in bone.

Ehsan Kheradpezhouh - Australian National University

TRPA1 MODULATION OF INFORMATION PROCESSING IN MICE SOMATOSENSORY CORTEX

Ehsan Kheradpezhouh1,2, Ehsan Arabzadeh1,2
Eccles Institute of Neuroscience, John Curtin School of Medical Research, Australian National University, Acton, Australia. 2. The Australian Research Council Centre of Excellence for Integrative Brain Research, Australian National University Node, Acton, Australia.

Transient Receptor Potential Ankyrin 1 (TRPA1) is a non-selective cation channel broadly expressed throughout the body including in the nervous system. We recently demonstrated the expression and functional activation of TRPA1 in rodent cortex through immunostaining, whole-cell electrophysiology, Ca2+ imaging and photoswitching. In order to better understand the role of TRPA1 in sensory processing, here we quantify how TRPA1 activation modulates the coding of sensory stimuli in the vibrissal primary somatosensory (vS1) cortex. We performed in-vivo juxtacellular recordings from the mouse vS1 cortex under local infusion of artificial cerebrospinal fluid (ACSF), TRPA1 agonist (AITC) or antagonist (HC-030031) while applying brief vibration stimuli to the contralateral whiskers at 6 intensities (0-200 µm). Under ACSF infusion, there was an increase in firing rate with stimulus velocity, which is typical of vS1 neurons. Application of AITC and HC-030031 produced significant modulation of activity in neurons both in their baseline firing rate and their input-output functions. AITC increased the baseline activity of neurons, their maximum evoked responses, and their response range (the range of firing rate in response to the whole stimulus set). The neuronal response functions returned to their original values after replacing AITC by HC-030031. These modulations were absent in the TRPA1 Knockout (KO) mice. Overall, these results confirm our earlier in-vitro experiments by demonstrating the presence of TRPA1 in cortical neurons and suggest a physiological role for TRPA1 in sensory processing.

Alexandria Driessen - University of Melbourne

AIRWAY SENSORY INPUTS TO THE PARATRIGEMINAL NUCLEUS REGULATE COUGH AND THE PERCEPTION OF NOXIOUS AIRWAY IRRITATIONS

Driessen AK1, McGovern AE1, Farrell MJ2, Mazzone SB1
1 Department of Anatomy and Neuroscience, The University of Melbourne, Australia
2 Biomedicine Discovery Institute and Department of Medical Imaging and Radiation Sciences, Monash University, Australia

Respiratory sensations conveyed by airway afferents contribute significantly to morbidity in pulmonary diseases. Using viral tracing, we identified an unknown airway sensory circuit involving the paratrigeminal nucleus (Pa5), a medullary region implicated in somatosensory processing. We therefore hypothesised that the Pa5 might be important for the conscious perception of respiratory sensations. Retrograde neuronal tracing in guinea-pigs demonstrated selective inputs from the jugular vagal ganglia to the Pa5, 31.26±7.81% of which expressed the neuropeptide substance P. The Pa5 was selectively lesioned using substance P-saporin and 5 weeks later changes in cough and
sensorimotor behaviours (a surrogate measure of conscious perception) induced by aerosolised challenges of vehicle and increasing doses (0.3, 1 and 3mg/ml) of Bradykinin (BK) were assessed in conscious animals using whole body plethysmography. BK-evoked cough was decreased in lesioned animals compared to controls (e.g. 1mg/ml BK 8.3±5.5 vs 0.9±0.9 coughs; P<0.05) and this was associated with a significant reduction in the total duration of sensorimotor behaviours (461±89.87 seconds vs 242±36.7 seconds; P<0.05) across challenges. In urethane anaesthetized Pa5 lesioned guinea-pigs, apnoea evoked by electrical stimulation of the larynx was similarly reduced compared to controls (maximum change in respiratory rate 44.67±6.41 vs 33.6±6.77 breaths/min), whereas reflex mechanoreceptor-dependent coughing was unchanged (8/9 Pa5 lesioned animals responded). These data are the first to implicate the Pa5 in the relay of information concerning the conscious perception of airway irritations and in turn have identified a potential novel alternate therapeutic target for alleviating difficult to treat respiratory sensations that are characteristic of pulmonary disease.

Gilles Vanwalleghem - The University of Queensland

A WHOLE-BRAIN ANALYSIS OF WATER-FLOW RESPONSES IN LARVAL ZEBRAFISH

1. The School of Biomedical Science, The University of Queensland, Brisbane, Australia

As a transparent animal and with powerful light-based tools to monitor and manipulate the brain, the larval zebrafish offers a perfect window into functioning neural circuits. We focus on the lateral line that allows fish to detect the movement of water. How the brain processes water flow to drive behaviours such as hunting or rheotaxis is unknown. We have used genetically encoded calcium sensors and SPIM to map the brain-wide processing of lateral line information. To apply water flow stimuli, we developed a custom microfluidics device capable of delivering a range of flow rates in both forward and reverse directions. Using this device, we have delivered a stimulus train that allows us to distinguish neural responses based on the direction, velocity, and duration of water flow. We observed eight functional response profiles, which can be divided between neurons that responded at the onset of the stimulus, for the duration of the stimulus, or in a steadily increasing fashion throughout the stimulus (“integrators”). Each functional profile could be subdivided per their direction selectivity, but some onset and on neurons responded to both directions. Surprisingly, we did not find evidence for encoding of the flow speed, which leads us to suspect it may be temporally encoded using the topographic map. These results will form the basis for a neural model of flow processing, and set the stage for studies of how water flow is integrated with visual and vestibular processing to give the larva a comprehensive representation of space.

Conrad Lee - Australian National University

NEURONAL CORRELATES OF SENSORY PRIORITIZATION IN RATS

Lee C.C.Y 1,2, Diamond M 3, Clifford C.W.G 4, Arabzadeh, E 1,2.

1. John Curtin School of Medical Research, Australian National University, Canberra, Australian Capital Territory, Australia. 2. ARC Centre of Excellence for Integrative Brain Function. 3. Tactile Perception and Learning Lab, International School for Advanced Studies (SISSA), Trieste, Italy. 4. School of Psychology, University of New South Wales, Sydney, New South Wales, Australia.

Operating with finite quantity of processing resources, an animal would benefit from prioritising the sensory signals expected to provide key information, for example to warn prey of predators. To establish sensory prioritisation across modalities, rats (n=6) were trained in a detection paradigm in which the likelihood of a stimulus was manipulated across the modalities of vision and whisker touch. When a stimulus was presented in a high-likelihood context, detection performance increased and was faster compared with the same stimulus presented in the low-likelihood context.
Simultaneous recordings of single-unit activity (n=31) from the vibrissal area of the primary somatosensory cortex revealed signatures of sensory prioritization in the form of (i) increased multiplicative gain and (ii) enhanced encoding of the stimulus. To investigate temporal cueing we trained rats (n=8) in a two-alternative forced-choice variant of the task in which a vibration stimulus was presented at one of two spatial locations (left or right). The vibration stimulus was presented at 1 of 10 delays. In a subset of trials, an auditory cue preceded the onset of the target vibration stimulus by 150ms. This provided temporal information but no spatial information of the target vibration stimulus. The auditory cue enhanced detection of the vibration stimulus: discrimination sensitivity increased and reaction time decreased. Recording of single-unit activity (n=24) in the vibrissal area revealed an enhanced signal to noise ratio in the presence of the auditory cue. Altogether these results establish the rat as an alternative model organism to primates for studying attention.

Naotsugu Tsuchiya - Monash University

ISOFLURANE REDUCES LOW-FREQUENCY FEEDBACK, BUT LEAVES HIGH-FREQUENCY FEEDFORWARD INTACT, IN THE FRUIT FLY BRAIN

Hierarchically organized brains communicate through feedforward and feedback pathways. In mammals, feedforward and feedback are mediated by higher and lower frequencies during wakefulness. Feedback is preferentially impaired by general anesthetics. This suggests feedback serves critical functions in waking brains. The brain of Drosophila melanogaster (fruit fly) is also hierarchically organized, but the presence of feedback in these brains is not established. Here we studied feedback in the fruit fly brain, by simultaneously recording local field potentials (LFPs) from low-order peripheral structures and higher-order central structures. Directed connectivity analysis revealed that low frequencies (0.1-5Hz) mediated feedback from the center to the periphery, while higher frequencies (10-45Hz) mediated feedforward in the opposite direction. Further, isoflurane anesthesia preferentially reduced feedback. Our results imply that similar spectral characteristics of feedforward and feedback may be a signature of hierarchically organized brains and that general anesthetics may induce unresponsiveness by targeting the mechanisms that support feedback.

Oral 8

Motor neuron disease II

John Lee - University of Queensland

THE PATHOGENIC ROLE OF COMPLEMENT C5A RECEPTOR, CSAR1 IN THE SOD1G93A MOUSE MODEL OF MOTOR NEURONE DISEASE

Lee JD1-2, Kumar K3, Fung JNT1, Ruitenberg MJ1-3,4, Noakes PG1,3, Woodruff TM1
1. School of Biomedical Sciences, the University of Queensland, QLD. 2. Centre for Clinical Research, the University of Queensland, QLD. 3. Queensland Brain Institute, the University of Queensland, QLD. 4. Trauma, Critical Care and Recovery, Brisbane Diamantina Health Partners, The University of Queensland, QLD.

Motor neuron disease (MND) is a fatal and rapidly progressive disease without effective treatment. The complement system is upregulated in MND, with recent studies indicating that the activation product C5a may accelerate disease progression via its receptor, C5aR1. This study examined the pathological role of C5aR1 in SOD1G93A mice, by pharmacological inhibition, and in SOD1G93A mice lacking C5aR1. The selective and orally active C5aR1 antagonist, PMX205, was administered to SOD1G93A mice via their drinking water, both pre- and post-disease onset. C5aR1 deficient mice were also backcrossed to SOD1G93A mice to generate SOD1G93A mice lacking C5aR1. The effect of C5aR1 genetic ablation and/or pharmacological inhibition using PMX205 on disease progression of SOD1G93A mice was determined using body weight, hind limb grip strength, survival time and blood analysis. SOD1G93A mice treated with PMX205 prior to disease onset, and SOD1G93A C5aR1 deficient mice, both had significantly improved hind-limb grip strengths, slower disease progression and
extended survival, compared with vehicle treated or control SOD1<sup>G93A</sup> mice. These improvements in
the PMX205-treated group and SOD1<sup>G93A</sup> mice lacking C5aR1 were associated with reductions in
pro-inflammatory monocytes and granulocytes, and increases in T-helper lymphocytes in the
peripheral blood and a reduction in pro-inflammatory cytokines in the lumbar spinal cord.

Importantly, PMX205 treatment beginning several weeks following disease onset also had an
attenuating effect on disease progression, significantly extending survival. These results confirm that
C5aR1 plays a pathogenic role in SOD1<sup>G93A</sup> mice, further validating the C5a-
C5aR1 signalling axis as a
potential therapeutic target to slow disease progression in MND.

Maxinne Watchon - The University of Sydney

TREATMENT WITH SODIUM VALPROATE IMPROVES THE MOTOR BEHAVIOUR OF TRANSGENIC SPINOCErellAR ATAXIA-3 ZEBRAFISH

Maxinne Watchon<sup>1,2,3</sup>, Kristy Yuan<sup>2</sup>, Albert Lee<sup>2</sup>, Hannah Saddull<sup>2</sup>, Alana De Luca<sup>2</sup>, Nicholas J. Cole<sup>2</sup>,
Roger S. Chung<sup>2</sup>, Garth A. Nicholson<sup>1,2,3</sup>, Angela S. Laird<sup>2</sup>.

<sup>1</sup>Sydney Medical School, University of Sydney  <sup>2</sup>Department of Biomedical Sciences, Faculty of
Medicine and Health Sciences, Macquarie University  <sup>3</sup>ANZAC Research Institute, Concord Repatriation
Hospital, Sydney

Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease, is an autosomal
dominant neurodegenerative disease causing a loss of muscle control and coordination. Recognized
as the most common form of spinocerebellar ataxia out of thirty spinocerebellar ataxias, this disease
currently has no effective treatment or cure. ATXN3 is the causative gene with an expanded CAG
trinucleotide repeat region encoding for the polyglutamine (polyQ) region within the ataxin-3
protein. In healthy subjects the ATXN3 gene contains 1-40 CAG repeats whilst SCA3 patients contain
40-80 CAG repeats.

Mutant expanded polyQ proteins, such as polyQ expanded ataxin-3, have been shown to repress
transcription by inhibiting histone acetylation<sup>1</sup>. Several studies have shown impairment in
transcription regulation via hypoacetylation of histones 3/4 within other models of SCA3. By
studying our transgenic zebrafish model of SCA3 we have found decreased levels of acetylated
histones 3 and 4 in zebrafish expressing mutant human ataxin-3 compared to those expressing
wild-type ataxin-3 and non-transgenic zebrafish. Histone deactylases (HDACs) commonly remove
acetyl groups from histones to repress transcription, so inhibiting HDACs may prevent the
hypoacetylation found within the mutant-ataxin-3 zebrafish. We have found that treating our
transgenic zebrafish with the HDAC inhibitor compound, sodium valproate, results in a significant
increase in levels of acetylated histones 3 and 4 compared to vehicle treated mutant-ataxin-3
zebrafish (P<). Sodium valproate treatment also prevents the motor impairment seen previously in
mutant-ataxin-3 fish. HDAC inhibition shows a beneficial effect in our SCA3 zebrafish and may
provide a new avenue for the treatment of SCA3.

Serene Gwee - Macquarie University

AURORA KINASE B (AURKB) IS INVOLVED IN THE MODULATING THE NEURONAL DEVELOPMENT OF ZEBRAFISH SPINAL MOTOR NEURONS

Serene S. L. Gwee, Rowan Radford, Marco Morsch, Andrew Badrock, Nicholas J. Cole, Roger S. Chung

Department of Biomedical Science, Faculty of Medicine and Health Science, Macquarie University,
NSW 2109, Australia

Protein kinases are utilized by cells during cell division and development, cytokinesis, and for the
maintenance of metabolic homeostasis. Given the well-established role of Aurora kinase B (AurkB)
in cellular division and cancer development, we have made a surprising recent discovery – that
AurkB has a critical role in axonal outgrowth and regeneration of neurons. We have recently
identified AurkB in axonal regeneration (Ng et al., 2012) in an in vitro condition, but the role of
AurkB in neurons within an *in vivo* condition has yet to be investigated. Here, we used a zebrafish model to determine whether AurkB modulates the axonal development of spinal motor neurons through pharmacological inhibition and genetic manipulation. Through pharmacological inhibition, we observed an impairment of axonal outgrowth and aberrant axonal branching of developing spinal motor neurons. Additional genetic overexpression of AurkB (i.e. wild-type and kinase-inactive mutant) also demonstrated a significant difference in the axonal length, where the kinase-inactive mutant led to a dominant negative effect that significantly reduced of axonal length at 24 hours post fertilisation (hpf) and the overexpression of wild-type AurkB caused a significantly longer axonal length at 72hpf. Furthermore, pharmacological inhibition of AurkB activity also resulted in a delay within the axonal regeneration process following a laser-mediated injury to the spinal motor axon. Collectively, our findings suggest that AurkB plays a vital role in modulating neuronal development, axonal outgrowth and axonal regeneration.

Mary-Louise Rogers - Flinders University

**URINARY P75 NEUROTYPHIN RECEPTOR EXTRACELLULAR DOMAIN: A BIOMARKER RELEVANT TO MND THERAPY DEVELOPMENT**

Rogers ML1, Shepheard S2, Wuu J3, Andersen PJ4, Schultz D5, Michael Benatar6
1MND&NR Lab, Flinders University, Australia; 2SciTrAN, Sheffield UK, 3Department of Neurology, University of Miami, USA; 4Umeå University, Umeå, Sweden; 5MND Clinic Flinders Medical Centre, Australia.

There is an urgent need for validated prognostic, disease progression and pharmacodynamic biomarkers that might aid motor neuron disease (MND) therapy development. We evaluated urinary neurotrophin receptor p75 extracellular domain (p75ECDD) levels (by ELISA) as an MND biomarker with potential application specifically to predicting prognosis; quantifying disease progression and potential pharmacodynamic effect; and potentially quantifying pre-symptomatic disease in healthy individuals at genetic risk for developing (psMND). This involved n=45 healthy controls and n=54 people with MND (1), n=31 of whom were sampled 2-6 times over a 2-year period, which confirmed our previous results. In addition, we examined n=68 psMND, n=10 of whom have MND, with samples from before and after diagnosis for n=5. Urinary p75ECDD is higher in MND patients compared to controls (p<0.0001), and correlates with ALSFRS-R at baseline (r=-0.44, p=0.008) and across all study visits (r=-0.36, p<0.0001). p75ECDD increased as disease progressed at an average rate of 0.19ng/mg creatinine per month (p<0.0001). In multivariate prognostic analysis, bulbar onset (hazard ratio (HR)=3.0, p=0.0035), faster rate of ∆FRS (HR=4.4, p<0.0001), and higher baseline p75ECDD (HR=1.3, p=0.0004) predict worse survival. Analysis of p75ECDD in the psMND population is ongoing with results to be included in final presentation. Urinary p75ECDD is currently the only biological-fluid-based biomarker of disease progression, and has potential for use in MND clinical trials. Ongoing studies will indicate when in the natural history of MND p75ECDD first begins to increase and potential for predicting the onset of disease.

Martina Pigoni - German Center for Neurodegenerative Diseases

**SEIZURE PROTEIN 6 AND ITS HOMOLOG SEIZURE 6-LIKE ARE MAIN SUBSTRATES OF BACE1: VALIDATION AND FUNCTION**

Pigoni M1,2, Wanngren J1,2, Gunnersen JM1,4, De Strooper B5,6,7, Müller SA1,2, Lichtenthaler SF1,2,8,9.
1. German Center for Neurodegenerative Diseases (DZNE), Munich, Germany. 2. Neuroproteomics, Klinikum rechts der Isar, Technische Universität München, Munich, Germany. 3. Department of Anatomy and Neuroscience, University of Melbourne, Victoria, Australia. 4. The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Victoria, Australia. 5. VIB Center for the Biology of Disease, Leuven, Belgium. 6. Center for Human Genetics, and Leuven Institute for Neurodegenerative Diseases (LIND), University of Leuven (KU

Objectives: The beta-secretase BACE1 is the rate limiting enzyme in amyloid beta generation and a major drug target in Alzheimer's disease. However, therapeutic BACE1 inhibition may cause unwanted side effects due the loss of cleavage of additional BACE1 substrates besides APP. Different proteomic studies have identified numerous membrane proteins as potential BACE1 substrate candidates, but most of them have not yet been validated nor functionally characterized. Two of these substrate candidate are Seizure protein 6 (SEZ6) and its homolog SEZ6L. Here we validate both proteins as BACE1 substrates in vitro and in vivo and start the functional characterization.

Methods: Biochemical and proteomic analysis of primary neurons, brain tissue and mouse CSF.

Results: We demonstrate that SEZ6 and SEZ6L are exclusive BACE1 substrates in primary neurons and in brains of BACE1-deficient mice. Additionally, we demonstrate that the BACE1-generated soluble ectodomains of both proteins (sSEZ6 and sSEZ6L) are strongly reduced in the CSF of BACE-deficient mice. Using immuno histochemistry both proteins were found to be widely expressed in the brain. For the functional investigation we used cell surface proteomics and found that SEZ6 controls the levels of certain ion channels at the neuronal surface.

Conclusions: SEZ6 and SEZ6L are physiological BACE1 substrates in vitro and in vivo. sSEZ6 and sSEZ6L levels in CSF appear as suitable markers to monitor BACE1 inhibition in mice. Finally, our results suggest that SEZ6 controls synaptic transmission and that its loss of cleavage may contribute to the phenotypes observed in BACE1-deficient mice.

Vidya Krishnan - The University of Western Australia

LOSS OF VGLUT1 EXCITATORY TERMINALS ON MOTOR NEURONS IN LUMBAR VENTRAL HORN OF 27 MONTH OLD MALE C57BL/6J MICE

Krishnan VS1, Shavlakadze T1, Grounds MD1, Hodgetts SI1,2 and Harvey AR1,2
1. School of Human Sciences, the University of Western Australia, Western Australia. 2. Perron Institute for Neurological and Translational Science, Nedlands, Western Australia

Age related changes in lumbar spinal cord were analysed in young (3 months) versus old (27 months) C57BL/6J male mice, in the context of known age related loss of skeletal muscle mass and function (sarcopenia). The connectivity of presumed α motor neurons was compared in immunostained transverse sections of lumbar spinal cord using the pre-synaptic markers vesicular glutamate transporter-1 (VGLUT1) and vesicular GABA transporter (VGAT). Previous reports suggest that VGLUT1 positive terminals derive from primary 1a afferents that innervate muscle spindles. We also used glial fibrillary acidic protein (GFAP) and Iba1 immunostaining to monitor changes in astrocytes and microglia respectively, and p62 as an autophagy marker. In aged mice there was a significant (p<0.05, t test) age related decrease in the number (mean of 14.08±1.35 versus 21.4±1.14) and percentage synaptic coverage (mean of 5.68±0.26 versus 10.9±1.5) of VGLUT1 excitatory terminals on the soma of βIII-tubulin immunostained α motor neurons. Importantly, the number of intrinsic VGAT positive inhibitory synaptic contacts did not change with age (means of 16.75 in 27 month and 18.75 in 3 month old mice). Qualitatively we found marked activation of microglia and astrocytes in the grey matter of 27 month old lumbar spinal cords. These data were obtained from aged mice with sarcopenia. Reduced 1a afferent drive of α motor neurons is likely to further impair sensorimotor control of hind limb skeletal muscle function in these animals. It remains to be determined if this synaptic loss is a consequence of, or preceded, age-related changes in peripheral muscles.
MULTI-ETHNIC ANALYSES OF EXOME DATA FOR INSIGHT INTO THE ETIOLOGY OF ALS

Gene discovery has provided remarkable biological insights into the complex, late onset and rapidly progressive neurodegenerative disease, Amyotrophic Lateral Sclerosis. Causative mutations in a number of genes have been identified in ALS, which has the ability to enhance patient diagnosis, prognosis and treatment.

We used whole exome sequencing (WES) (Illumina HiSeq 2500) to identify ALS patients harbouring likely pathogenic mutations in an Australian (n=111 cases) and a Chinese (n=610 cases, 460 controls) clinic-based population. By using publically available databases (controls and ALS), we designed a filter to assess evidence for rare damaging mutations at the gene level and the gene set level (burden of rare variants in cases versus controls).

We identified 4/120 Australian (4%) patients carrying a rare genetic variant previously reported to cause ALS (in SOD1, TARDBP). Similarly, 5% of Chinese patients (27/610) were identified with a rare ALS variant (in DCTN1, FUS, SOD1, TARDBP). In both cohorts, there were a number of previously reported, but relatively common (>0.01) and rare (MAF <0.00005) unknown significance variants identified. Combining rare variant counts in Chinese with those from the largest European WES study resulted in three genes surpassing genome-wide significance: TBK1 ($p=8.3x10^{-12}$), SOD1 ($p=8.9x10^{-9}$) and NEK1 ($p=1.1x10^{-9}$).

We demonstrate the clinical diagnostic capability of WES, which detected a causal variant in ~4-5% ALS cases, irrespective of ethnicity. Given the rare-variant contribution to ALS, large sample sizes are needed to combine evidence with common-variant (risk) contributions to fully elucidate the genetic etiology of ALS.

WHOLE TRANSCRIPTOME ANALYSIS (RNA-SEQ) REVEALS DISTINCT GENE AND ISOFORM EXPRESSION PROFILES AND ALTERNATIVE SPLICING DEFECTS IN C9ORF72-RELATED AND SPORADIC FRONTOTEMPORAL LOBAR DEGENERATION (FTLD-TDP)

FTLD is an umbrella term that defines a spectrum of heterogeneous neurodegenerative disorders characterized by the progressive deterioration of the frontal and anterior temporal lobes with preservation of the posterior areas of the brain. A growing body of evidence now points to the disruption of RNA metabolism as a major player in FTLD pathogenesis, especially in those cases where the RNA binding protein TDP-43 aggregates into pathological inclusions, and in cases with intronic c9orf72 repeat expansions where non-coding RNA must be involved. In this study, we used strand-specific RNA-Seq technology to investigate changes in the transcriptome profile of two brain regions with different pathological abnormalities in FTLD-TDP cases (N=10, 4 with c9orf72 repeat expansions) and controls (N=6) collected with consent by the NSW Brain Banks (following institutional ethics and tissue approvals). The regional RNA levels of genes and isoforms were determined and results indicated different expression profiles between c9orf72-related (c9FTLD) and sporadic FTLD-TDP (sFTLD) cases. Major differences in alternative splicing regulation highlighted defects in RNA processing between FTLD cases and controls, and further indicated that the two...
disease groups impact on RNA processing through distinct transcriptome changes to reach the same disease outcome. This work shows that different sets of dysfunctional RNA binding proteins occur in FTLD with and without c9orf72 repeat expansions, data that assists with explaining the distinct transcriptome profiles in the two disease groups, but also explains why a similar disease outcome can occur.

Magdalena Przybyla - University of New South Wales

IDENTIFICATION OF MODIFIER GENES THAT PROTECT AGAINST TAU INDUCED DISEASES

Accumulation of tau protein is a feature of several neurodegenerative diseases including Alzheimer’s disease (AD) and frontotemporal lobar degeneration (FTLD-tau). Furthermore, tau pathology correlates with cognitive decline and neurodegeneration in these diseases. However, very little is known about mechanisms that protect from tau-induced neurodegeneration. Recent studies of familial FTLD-tau and AD suggest that genetic modifiers may provide a new avenue for prevention of neurodegenerative disorders. However, the identification of modifier genes using conventional methods e.g. human studies has been time consuming, costly and difficult. In this study, we used two unique resources to identify modifier gene(s) that protect against tauopathies. First, a new genetic resource called Collaborative Cross (CC); a large panel of recombinant inbred mice, enabling high genetic diversity, rapid mapping and gene identification of multifactorial traits, as occurring in AD and FTLD-tau. The second resource is our established TAUS8/2 transgenic mouse, expressing a human FTLD-tau mutation. Those mice develop early-onset disinhibition and muscle degeneration, features found in FTLD-tau and AD patients. We crossed 50 CC strains onto TAUS8/2 transgenic mice and assessed functional deficits. One strain showed protection against two traits- disinhibition and weight/muscle loss. To date we have backcrossed and analyzed over 300 mice and maintained both protected traits. Furthermore, by using the mapping power of CC, we have targeted a novel gene on chromosome 8, linked to the protected phenotype. Next, we will introduce the identified polymorphism into a susceptible background and show that this new line is protected against TAUS8/2 functional deficits.

Paul Lockhart - MCRIA

NOVEL METHOD TO IDENTIFY PATHOGENIC REPEAT EXPANSIONS IN EXOME AND GENOME SEQUENCE DATASETS- ENHANCING THE CLINICAL UTILITY OF NEXT GENERATION SEQUENCING

Repeat expansions (RE) cause over twenty neurogenetic disorders of major clinical significance which can present with heterogenous, overlapping clinical phenotypes. Ataxias are the most common of these including spinocerebellar ataxias (SCAs) 1, 2, 3, 6, 7, 8, 10, 12, 17, 36 and Friedreich ataxia. RE also underlie FragileX syndrome and Huntington diseases. To date, technical issues have prevented bioinformatic identification of RE in whole exome or whole genome sequencing (WES and WGS) datasets. Therefore, single gene or small panel PCR-based methods are currently employed for diagnosis of SCAs, but can be slow and costly, with a current diagnostic yield of ~20%.

We developed a novel tool called exSTRa (expanded STR algorithm) to identify RE using either WES or WGS and analysed cohorts of individuals with eight different known RE disorders. Results were assessed by comparing to the known disease status, and performance was also compared to a recently published genotyping-based method. Repeat expansions were successfully identified in seven of eight disorders, with very high predictive capabilities (median area under the curve (AUC) of 0.9) and a median specificity and sensitivity of 0.99 and 0.75 respectively. These results were achieved regardless of whether the library preparation was PCR-free or not. A single affordable front-line test that is able to comprehensively detect the genetic basis of human disease is the ultimate goal of diagnostics for genomic medicine. Our method represents a significant step forward in fully exploiting the clinical utility of NGS datasets and facilitating the cost effective implementation of precision medicine.

Timothy Lynagh - University of Copenhagen
EVOLUTION OF ACID-SENSING ION CHANNELS

Lynagh T, Colding JM, Pless SA
Center for Biopharmaceuticals, Department of Drug Design and Pharmacology, University of Copenhagen, Denmark

Recent evidence shows that protons are neurotransmitters, released at glutamatergic synapses where they activate acid-sensing ion channels (ASICs), a small family of excitatory sodium channels. Synaptic activation of ASICs contributes to learning and plasticity, and ASICs are also implicated in pain and neuroinflammation. Previously, it was concluded that proton-sensing arose with bony fishes around 450 million years ago (Mya), suggesting that earlier chordate neural systems lacked this part of signaling machinery. We revisited the question of ASIC evolution, with phylogenetic analysis of a broader dataset and with functional, electrophysiological experiments. Our phylogenetic analysis of recent chordate genomic data suggest that proton-sensing emerged before cephalochordates and tunicates split from the vertebrate lineage. Furthermore, experiments showed robust proton-gated sodium current through tunicate ASICs, much like their vertebrate homologues. These data suggest that ASICs emerged more than 550 Mya and provide new insight into the molecular mechanism of proton sensing that should inform future neurobiological studies and genomic analyses.

Mark Graham - Children’s Medical Research Institute

PROFILING PRESYNAPTIC TERMINAL DEPOLARISATION AND POST-STIMULUS USING PHOSPHOPROTEOMICS

Protein phosphorylation and dephosphorylation are crucial fast signalling mechanisms following the depolarisation of presynaptic nerve terminal membrane. The calcium influx associated with depolarisation activates calcium binding proteins involved in synaptic vesicle fusion, but also initiates a phospho-signalling cascade. The extent of signalling and the biological processes regulated are not well described. Furthermore, the relationship between global phospho-signalling and neurotransmitter release is not well-described. This is despite knowledge of protein kinases and phosphatases that influence presynaptic plasticity and are expected to modulate neurotransmitter release in the short and long term. We have used KCl depolarisation of isolated presynaptic nerve terminals to profile phospho-signalling. This profile included a time course of the post-stimulus period, which has not been examined in detail, and has allowed new insight on the longer-term influence of phosphorylation in this system. Quantitative analysis of 1,917 significantly up-/down-regulated phosphorylation sites has allowed a detailed assessment of the signalling resulting from KCl across time. The post-stimulus signalling has been correlated with a change in glutamate release. We have shown that the strength of stimulus scales with the presynaptic phospho-signalling response for individual phosphorylation sites and have identified key presynaptic proteins, signal integrators and protein kinases/phosphatases as targets and mediators of depolarisation-dependent phospho-signalling.

Oral 10
Dementia and aging

Michael Lardelli - The University of Adelaide

AGED VERTEBRATE BRAINS SHOW A CONSERVED FAILURE TO RESPOND TO HYPOXIA – A METABOLIC FOUNDATION FOR ALZHEIMER’S DISEASE?

Newman M, Moussavi Nik SH, Lardelli M
University of Adelaide, School of Biological Sciences, Centre for Molecular Pathology

The Alzheimer’s disease (AD) brain is hypometabolic showing reduced glucose and oxygen use. Energy is
the fundamental determinant of cellular function but are energy metabolism changes the cause instead of just a consequence of AD? Hypoxia is implicated in many phenomena associated with AD such as increased Amyloidβ production. Therefore, we tested the effects of hypoxia on two quite distinct models of dominant, early onset fAD-like mutations in the zebrafish’s endogenous PSEN1 orthologous gene. Remarkably, we saw that – in a normoxic environment - the brains of young adult mutant fish and older wild type fish show moderate upregulation of hypoxia response genes (thus young fAD-like mutant brains appear prematurely aged by this measure). Nevertheless, under environmental hypoxia, both fish types could raise their hypoxic response further to increase anaerobic glycolysis (lactic acid production) to provide energy. In contrast, older fAD-like mutant brains were unable to make this response to hypoxia. They appeared incapable of upregulating anaerobic glycolysis. This difference in responsiveness of aged fAD-like mutant brains is apparently due to an inability to stabilise the central regulatory protein HIF1A. Intriguingly, a similar failure to stabilise HIF1A protein was previously observed in aged rat brains (Ndubuizu et al 2009 doi: 10.1152/ajpregu.90829.2008) while human AD brains show significantly reduced HIF1A protein levels (Liu et al. 2008 doi: 10.1016/j.febslet.2007.12.035) suggesting that this is a conserved characteristic of vertebrate brains and may be a fundamental characteristic of AD. We are currently making detailed ‘omics analyses of our fAD-like mutants to investigate this remarkable phenomenon.

Arne Ittner - University of New South Wales

AMYLOID-B TOXICITY IN ALZHEIMER’S MICE IS INHIBITED BY SITE-SPECIFIC PHOSPHORYLATION OF TAU

Amyloid-β (Aβ) toxicity in Alzheimer’s disease (AD) is considered to be mediated by phosphorylated tau protein. In contrast to previous assumptions on tau phosphorylation, we found that, at least in early disease, site-specific phosphorylation of tau inhibited Aβ toxicity. This specific tau phosphorylation was mediated by the neuronal p38 mitogen-activated protein kinase p38y and interfered with postsynaptic excitotoxic signaling complexes engaged by Aβ. Accordingly, depletion of p38y exacerbated neuronal circuit aberrations, cognitive deficits, and premature lethality in a mouse model of AD, whereas increasing the activity of p38y abolished these deficits. Furthermore, mimicking site-specific tau phosphorylation alleviated Aβ-induced neuronal death and offered protection from excitotoxicity. Consistently, newly generated CRISPR-engineered mice expressing phospho-mimicking tau were protected from acute excitotoxicity. Our work provides insights into postsynaptic processes in AD pathogenesis and challenges a purely pathogenic role of tau phosphorylation in neuronal toxicity.

Rachelle Balez - Illawarra Health and Medical Research Institute

ALTERED DISTRIBUTION AND NEUROPROTECTIVE EFFECT OF ALPHA-TOCOPHEROL IN SPORADIC ALZHEIMER’S DISEASE INDUCED PLURIPOTENT STEM CELL DERIVED NEURONS

Alpha-tocopherol (α-toc) is a potent lipid-soluble antioxidant. There are conflicting reports from clinical trials regarding the protective effect of α-toc against the early stages of cognitive decline in sporadic Alzheimer’s disease (sAD). This may be due in part to limited knowledge regarding the distribution and molecular alterations associated with the protective action of α-toc in human neurons. The aim of this research was to investigate the distribution of α-toc in the lipid membrane of induced pluripotent stem cell (iPSC) derived neurons from sAD and non-AD (healthy control) donors and determine the effect of α-toc treatment on neurite length, as well as markers of oxidative and nitrosative stress. Simultaneous tandem time-of-flight secondary ion mass spectrometry imaging indicated that the distribution of α-toc was restricted to the soma in sAD neurons, in contrast to control neurons, where it was localised in both the neurites and soma. Further, sAD neurons had significantly elevated levels of peroxidation, a marker of oxidative stress, and nitrite, a marker of nitrosative stress, in conjunction with significantly shorter neurites. Treatment with α-toc for 7 days in vitro ameliorated levels of peroxidation and nitrite in sAD neurons, and significantly increased the length of neurites. This suggests that the restricted distribution of α-toc to the soma of sAD neurons could increase the susceptibility of neurites to oxidative injury, while modulation of α-toc levels is able to restore some protection. Collectively our results imply that α-toc may be neuroprotective against aspects of sAD pathogenesis.

Pratishtha Chatterjee - Macquarie University
KYNURENINE PATHWAY METABOLITES, AS POTENTIAL BLOOD MARKERS FOR THE DIAGNOSIS OF PRECLINICAL ALZHEIMER’S DISEASE

Chatterjee E1,2,3,11, Goozee K1,2,3,4,5,6,11, Lim CK1,11, James I8, Shen K8, Jacob KR1, Schrabi HR1,2,5,8, Shah T1,2,8, Aslin PR3,10, Dave P1,4, ManYan C4, Teddei K28, Lovejoy DB1, Guillenin GJ1, Martins RN1,2,3,5,6,7

1. Department of Biomedical Sciences, Macquarie University, North Ryde, NSW, Australia. 2. School of Medical Health and Sciences, Edith Cowan University, Joondalup, WA, Australia. 3. KaRa Institute of Neurological Disease, Sydney, Macquarie Park, Australia. 4. Anglicare, Sydney, Castle Hill, NSW, Australia. 5. School of Psychiatry and Clinical Neurosciences, University of Western Australia, Crawley, WA, Australia. 6. Australian Alzheimer’s Research Foundation, Nedlands, WA, Australia. 7. The Cooperative Research Centre for Mental Health, Carlton South, Australia. 8. Institute for Immunology & Infectious Diseases, Murdoch University, Murdoch, WA, Australia. 9. Australian eHealth Research Centre, CSIRO, Floreat, Australia. 10. School of Medical Sciences, University of New South Wales, Kensington, NSW, Australia. 11. Contributed equally to this work.

The kynurenine pathway (KP) has been reported to be dysregulated in several neurological diseases. Given that the current pre-mortem biomarkers of Alzheimer’s disease (AD), are either invasive or uneconomical, we investigated alterations in KP-metabolite serum concentrations in preclinical-AD prior to cognitive decline, to investigate potential preclinical-AD biomarkers. Based on the neocortical amyloid-β load (NAL), Kerr Anglican Retirement Village in Ageing Health (KARVIAH) cohort participants, having normal global cognition were stratified as, preclinical-AD (NAL+, n=35) and no apparent risk to AD (NAL-, n=65). KP-metabolites, tryptophan, kynurenine, 3-hydroxykynurenine, anthranilic acid (AA), 3-hydroxyAA measured employing ultra-high-performance liquid chromatography, while picolinic acid and quinolinic acid measured using gas-chromatography coupled with mass-spectrometry, were compared between NAL+ and NAL- participants. Serum kynurenine(p<.05) and AA(p<.01) were elevated in NAL+ versus NAL-, adjusting for covariates age, gender, APOE ε4 and education. Given that the KP is influenced by gender2-5, comparison of KP-metabolites between NAL+ versus NAL- participants after stratifying the KARVIAH cohort by gender, revealed significantly higher kynurenine(p=.0004) and anthranilic acid(p=.0001) concentrations in NAL+ versus NAL- female participants, while no significance was observed in male participants. To evaluate the potential of kynurenine or/and AA as potential biomarkers for preclinical AD in females, a receiver operating characteristic curve based on a logistic regression of the same covariates distinguished NAL+ from NAL-(at 91% sensitivity, specificity=43%), but was outperformed when AA was added to the same model(at 91% sensitivity, specificity=83%). The current findings may be attributed to inflammation, an early event in AD pathogenesis, and highlight the potential of KP-metabolite, AA, as a preclinical blood biomarker for AD.

Sonia Sanz Muñoz - University of Wollongong

EXTRACELLULAR APOLIPOPROTEIN E 25 KDA N-TERMINAL FRAGMENT FORMATION IS REGULATED BY HIGH-TEMPERATURE REQUIREMENT SERINE PEPTIDASE A1

Alzheimer’s disease (AD) is the most common form of dementia, characterized by cognitive impairment and memory loss. The ε4 allele of the APOE gene is the major genetic risk factor for AD. Proteolytic processing of apolipoprotein E (apoE) has been identified in human post mortem brain tissue. A 25 kDa fragment of apoE (apoE25) is more abundant in the ε3/3 background compared with ε4/4. A neuroprotective role of this fragment has been hypothesised, thus developing an in vitro system that generates apoE25 is essential for understanding the biological role of apoE25.

To address this, we utilised SK-N-SH neuroblastoma cells differentiated into neurons with all-trans retinoic acid (ATRA) treatment for up to 9 days. ApoE fragments were detected in cell culture supernatants but not in cell lysates. The most abundant apoE fragments were detected at ~25 kDa and ~28 kDa and both were recognised by an N-terminal specific antibody but not by a C-terminal antibody. The formation of apoE N-terminal fragments was reduced by >80% when a serine protease inhibitor (AEBSF) was added to the culture medium. A specific inhibitor of high-temperature requirement serine peptidase A1 (HtrA1) preferentially reduced the production of apoE25. Furthermore, HtrA1 levels were up-regulated by ATRA-
mediated neuronal differentiation. We also confirmed that knockdown of HtrA1 using siRNA inhibited apoE25 generation by 50%.

This study provides the first demonstration of endogenous production and cleavage of apoE in a cell culture system and confirms that HtrA1 contributes to the production of the N-terminal apoE 25 kDa fragment under physiological conditions.

Virginie Lam - Curtin Health Innovation Research Institute

**VITAMIN D, CEREBROCAPILLARY INTEGRITY AND COGNITION IN MURINE MODEL OF ACCELERATED AGEING**

Increased use of vitamin-D (vit-D) supplements has been attributed for improved cognitive performance, an important consideration given that vit-D deficiency becomes more common with older age. However, several lines of evidence suggest that chronically heightened plasma vit-D may paradoxically compromise cognitive function. The mechanism(s) for detrimental effects of exaggerated vit-D on central nervous system function have not been delineated. Senescence-accelerated-mouse-phenotype strains (SAMP) represent lines of AKR/J mice that simulate accelerated aging. Exaggerated capillary permeability in SAMP8 mice has been widely documented, however, it’s putative association with vit-D homeostasis has not yet been considered. Male SAMP8 mice and their age-matched controls, 6 and 20 weeks, were randomized to either control chow or a vit-D deficient diet. Capillary integrity, cognitive performance and peripheral vit-D status was assessed. An increase in serum vit-D, which progressively increased with age in SAMP8 mice, was found to occur concomitantly with poorer cognitive performance and increased capillary permeability. Latency time area-under-curve to rescue platform in Morris Water Maze increased by approximately 50% in SAMP8 mice at 20 weeks of age compared to baseline animals. Moreover, in the same mice, capillary permeability in the hippocampal formation and cerebral cortex was markedly increased. Strong evidence of causality between endogenous hypervitaminosis D and poorer maze performance is suggested by the finding that maintenance of SAMP mice on a vit-D deficient diet prevented the age-associated decline in maze performance, concomitant with maintenance of capillary impermeability. The findings may help explain paradoxical clinical data reporting associations of serum vit-D homeostasis and cognition.

Alexander Volkerling - University of New South Wales

**LKB1 AND TAU PHOSPHORYLATION IN ALZHEIMER’S DISEASE**

Alzheimer’s disease (AD) and frontotemporal lobar dementia with tau pathology (FTLD-tau) are the most prevalent forms of dementia with tauopathy. They are characterised by the presence of neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau, as well as neuronal dysfunction partially due to defective neuronal metabolism. Liver kinase B1 (LKB1) is a crucial regulator in neuronal energy homeostasis via activating AMP-activated protein kinase (AMPK). During brain development, LKB1 plays a vital role in regulating neuronal polarity via specific phosphorylation of tau. However, whether this pathway is involved in neuronal dysfunction in tau pathology is unknown. Our experiments show that LKB1 function is impaired when naturally occurring tau phosphorylation is dysregulated. This in turn has downstream effects on brain metabolism and cognition. Hence, we propose that site specific phosphorylated tau is able to inhibit LKB1-mediated AMPK activation in neurons. Furthermore, the LKB1-tau pathway may serve as a therapeutic target in tau-related neurodegeneration.

Wickliffe Abraham - University of Otago

**GLUTAMATE RECEPTOR TRAFFICKING AND PROTEIN SYNTHESIS MEDIATE THE FACILITATION OF LTP BY SECRETED AMYLOID PRECURSOR PROTEIN-ALPHA**

Mockett BG\(^1\), Guévremont D\(^2\), Parfitt K\(^3\), Elder M\(^2\), Peppercorn K\(^4\), Kochen L\(^5\), tom Dieck S\(^5\), Schuman E\(^5\), Tate WP\(^3\), Williams JM\(^2\), Abraham WC\(^1\).

\(^1\)Department of Psychology, \(^2\)Department of Anatomy, \(^3\)Department of Biochemistry, Brain Health Research Centre, Brain Research New Zealand, University of Otago, Dunedin, New Zealand. \(^4\)Department of
Secreted amyloid precursor protein-alpha (sAPPα) can facilitate long-term potentiation (LTP) and memory, yet the underlying mechanisms remain unclear. Here we demonstrate that a brief administration of sAPPα (1 nM) but not sAPPβ to rat hippocampal slices converted short-lasting LTP in area CA1 into the longer lasting late-LTP. We hypothesized that regulated glutamate receptor trafficking as well as de novo protein synthesis mediated this facilitation. We found using western blots that sAPPα facilitated the trafficking of GluA1-containing AMPA receptors, as well as NMDA receptors (GluN1) to the cell surface. Both the cell-surface receptor accumulation and LTP facilitation were maintained even after sAPPα wash-out, indicating a long-term effect of sAPPα treatment. Pharmacological inhibition of protein trafficking or protein synthesis prevented the receptor trafficking and the facilitation of LTP. Visualization of newly synthesized proteins using FUNCAT-PLA confirmed the ability of sAPPα to stimulate de novo protein synthesis, and revealed GluA1 as one of the up-regulated proteins. Thus sAPPα generates a coordinated synthesis and trafficking of glutamate receptors to the cell surface that facilitate LTP.

This research was supported by a grants from the Health Research Council of New Zealand, German Academic Exchange Service, a University of Otago postgraduate scholarship, the Max Planck Society, the European Research Council, and the Cluster of Excellence for Macromolecular Complexes.

Auditory and visual

Oral 11

Sam Merlin - Western Sydney University

OPTOGENETIC SILENCING OF FEEDBACK TO PRIMARY VISUAL CORTEX ALTERS RECEPTIVE FIELD SIZE AND RESPONSE GAIN

Merlin S* 1,2, Nurminen L* 2, Bijanzadeh M 2, Federer F 2, Angelucci A 2.
1. Western Sydney University, Campbelltown, NSW, Australia. 2. University of Utah, Salt Lake City, Utah, USA. * Equal contribution.

Visual information passes from primary visual cortex (V1), via feedforward connections, to various hierarchically arranged cortical areas, which in turn send feedback (FB) connections back to lower visual areas. The exact role of the reciprocal FB is not known, but is generally thought to be involved in higher cognitive roles such as figure-ground segregation, attention and expectation. We examined the role of early cortical FB on response properties in V1 of anaesthetized marmoset (Callithrix jacchus, 8 penetrations from 3 animals), using specific optogenetic inactivation of FB terminals. We inactivated FB terminals in V1, expressing virally-mediated Arch aerbodopsin, while simultaneously recording in V1 using a multichannel linear array. The surface of V1 was photostimulated with green laser (532nm), via fibre-coupled collimator, and intensity was tailored to each penetration. For a subset of V1 neurons recorded (76% of laser-modulated neurons), inactivation of FB resulted in size tuning curves shifting toward larger grating diameters, suggesting a fundamental role for FB in controlling receptive field (RF) size. Furthermore, weak FB inactivation resulted in increased maximum responses in almost half of these cells, and 75% of laser-modulated neurons, whether accompanied by increased RF size or not. Instead, high laser intensity lead to a general suppression of activity. This suggests FB from early extrastriate areas can modulate some fundamental neuronal response properties, such as RF size and response gain, and thus not only involved in higher cognitive processes.

Nicholas Price - Monash University

NONLINEAR TEMPORAL INTEGRATION OF VISUAL MOTION

Price NSC, Zavitz E, Oakley B, Rosa MGP
Department of Physiology; Biomedicine Discovery Institute – Neuroscience Program; ARC Centre of Excellence in Integrative Brain Function. Monash University, Clayton, VIC, Australia
It is well established that neuronal tuning curves are malleable. Even though a neuron’s average firing rate might reliably represent stimulus properties under a fixed testing protocol, the shape and gain of a tuning curve are affected by small changes in the testing protocol, including factors such as attention, adaptation and eye position. We have previously shown that direction- and speed-tuning curves of neurons in the middle temporal area (MT) are compressed or shifted laterally, if the neurons have been previously exposed to motion stimuli near the peak, or flank, of the tuning curve, respectively. Here, we examine how neurons in MT of sufentanil-anaesthetised marmosets (*Callithrix jacchus*) encode rapidly changing stimuli, in which the direction changes every 33 ms. In extracellular recordings from 142 neurons across 5 animals, we first characterised the dynamics of changes in tuning curves on these short time scales. Next, we demonstrated that temporal non-linearities in the way neurons integrate motion are necessary to account for these dynamic changes in tuning. Put simply, motion in one direction significantly affects the responses to subsequent motion in different directions. Finally, we demonstrate for the first time that, despite the rapidly changing visual stimulation, we can meaningfully characterise spike-count correlations between pairs of neurons, allowing us to quantify how populations of neurons collectively represent these rapidly changing stimuli. This work extends previous models of sensory integration in motion-sensitive neurons by explicitly incorporating stimulus history, and the associated rapid, non-linear adaptation.

Ulrike Grunert - University of Sydney

**RETNAL GANGLION CELL TYPES PROJECTING TO THE PULVINAR AND SUPERIOR COLLICULUS IN MARMOSET**

Grüner U1,2, Lee SCS1,2, Kwan WC3, Mundinano IC3, Martin PR1,2, Bourne JA3.

1. Save Sight Institute and Department of Clinical Ophthalmology, The University of Sydney, Sydney, NSW, Australia. 2. ARC centre of Excellence for Integrative Brain Function, The University of Sydney, NSW, Australia. 3. Australian Regenerative Medicine Institute, Monash University, Melbourne, VIC, Australia.

**Purpose:** At least 20 retinal ganglion cell types have been identified in humans and non-human primates. Targets of retinal ganglion cells include the dorsal lateral geniculate nucleus, superior colliculus (SC), pretectum and medial subdivision of the inferior pulvinar (PIm). Here we investigated the retinal ganglion cell types projecting to the inferior pulvinar and the superior colliculus in the marmoset.

**Methods:** Four retinas were obtained from two adult (>18 months) common marmosets (*Callithrix jacchus*) which had received neural tracer injections into the PIm (cholera toxin conjugated to Alexa 488) and into the SC (cholera toxin conjugated to Alexa 594) using an MRI-guided approach. Retinas were dissected, fixed in paraformaldehyde and wholemounts imaged. Retrogradely labelled retinal ganglion cells were subsequently intracellularly injected with the lipophilic dyes DiI or DiO, imaged with a Zeiss confocal microscope and classified according to dendritic field size, stratification and branch density.

**Results and conclusions:** The majority of the retrogradely labelled cells (~5000 per eye) were labelled from the SC injections; less than 10% were labelled from the PIm injections. Both the PIm and the SC received input from more than one retinal ganglion cell type but no cells were double-labelled, indicating independence of SC and PIm projections. Cells projecting to the SC included parasol cells and wide-field ganglion cell types, whereas cells projecting to the PIm were exclusively wide-field cells. We conclude that a sparse and exclusive cohort of wide-field ganglion cells projects to the marmoset pulvinar. The functional properties of these cells are not yet known.

Leo Lui - Monash University

**FEWER CELLS IN THE MIDDLE TEMPORAL AREA REPRESENT VISUAL SPACE INSIDE THE SCOTOMA AFTER CHRONIC LESIONS OF THE PRIMARY VISUAL CORTEX**

Hagan MA1,2, Chaplin TA1,2, Huxlin KR2, Rosa MG1,2, Lui LL1,2
Patients (and monkeys) who suffer damage to the primary visual cortex (V1) lose conscious vision in the associated parts of the visual field. However, they retain limited subconscious visual abilities within the scotoma. This phenomenon, known as blindsight, is thought to be mediated by the Middle Temporal Area (MT) via neural pathways that bypass V1. We recorded 188 MT neurons (single and multi-units) in four anaesthetised marmoset monkeys 7-11 months after V1 lesions, which represents the chronic phase when patients’ performance on visual tasks are stable, and improvements are no longer observed without specific intervention. While we have previously reported reduced direction selectivity in MT, distributions of receptive field locations have not been addressed. Substantial reorganisation occurred in MT as a result of the lesions. With respect to anatomical location, a disproportionate number of MT cells have receptive fields outside the scotoma ($X^2=13.62\ p<0.0001$) indicating that cells were less likely to represent space inside the scotoma after lesions. Furthermore, the majority of cells (63/80) with receptive fields inside the scotoma overlap the scotoma border; although cells with their receptive fields totally inside the scotoma were also clearly present. These findings imply that fewer MT cells represent visual space deep inside the scotoma, which, together with the reduced direction selectivity of individual neurons, will limit one’s ability to determine directions of motion after V1 lesions. However, the responses observed should be sufficient to account for the limited visual abilities of blindsight patients and monkeys.

Eugenia Zhi Wei Poh - The University of Western Australia

ONLINE REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION DURING A VISUAL LEARNING TASK: DIFFERENTIAL IMPACTS ON VISUAL CIRCUIT AND BEHAVIOURAL PLASTICITY IN ADULT EPHRIN-A2A5/- MICE

Poh EZ$^{1,2}$, Makowiecki K$^{1,3}$, Harvey AR$^{2,4}$, Rodger J$^{1,2,4}$.

1. School of Biological Sciences, The University of Western Australia, Perth, Australia. 2. School of Human Sciences, The University of Western Australia, Perth, Australia. 3. Present address: University of Goettingen, Department of Systems Neuroscience, JFB Institute of Zoology and Anthropology, Goettingen, Germany. 4. Perron Institute for Neurological and Translational Research, Perth, Australia.

Repetitive transcranial magnetic stimulation (rTMS) induces plastic changes in normal and abnormal neural circuits. Here we study the potential synergistic interactions between low-intensity rTMS (12 mT) and endogenous brain activity to promote beneficial long-term neural circuit reorganisation. We delivered rTMS to the visual cortex of awake, freely moving adult ephrin-A2A5$^{-/-}$ mice engaged in a visual learning task because their morphologically abnormal visual maps have been shown to be beneficially impacted by rTMS. Mice received chronic implantation of a detachable coil support and underwent 2 weeks of 10 minutes daily training in a two-choice visual discrimination task with concomitant rTMS or sham (no stimulation control). No-task controls were placed in the task arena without visual discrimination training. Visuomotor function, corticotectal and geniculocortical topography were assessed at the end of the intervention. The addition of a visual learning task prevented the beneficial anatomical reorganisation in the corticotectal projection induced by rTMS alone, but did not affect geniculocortical projections and visuomotor function. Intriguingly, rTMS delivery significantly increased the total number of trials completed by ephrin-A2A5$^{-/-}$ mice, but did not affect accuracy in the visual learning task. Our results suggest that interactions between intrinsic brain activity and rTMS may not always be synergistic, although rTMS may affect motor function and/or increase motivation and drive in ephrin-A2A5$^{-/-}$ mice. We have established a protocol to investigate the ‘online’ effects of rTMS in awake freely moving mice that can be used to better understand rTMS effects, an essential step in improving future clinical translation.
Srdjan Vlajkovic - The University of Auckland

PURINERGIC SIGNALING MODULATES AMINOGLYCOSIDE OTOTOXICITY

Lin SCY¹, Thorne PR², Housley GD², Vlajkovic SM¹.
1. Department of Physiology and The Eisdell Moore Centre, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand. 2. Department of Physiology and Translational Neuroscience Facility, School of Medical Sciences, UNSW, Sydney, Australia

Adenosine and adenosine-5′-triphosphate (ATP) are potent regulators of cellular and molecular processes in the inner ear acting on purinergic P1 and P2 receptors, particularly in response to cochlear stressors. The present study aimed to determine the role of adenosine and ATP and their synthetic analogues in the maintenance of the cochlear sensory hair cell population exposed to the ototoxic aminoglycoside neomycin. The study used an organotypic culture model of neomycin ototoxicity. Organ of Corti explants obtained from C57BL/6 mice at postnatal day 3 (P3) were incubated in normal culture medium or in culture medium containing ATP, UTP or their slowly hydrolysable analogues (ATP₆S, UTP₆S) for 19.5 hours prior to and after exposure to neomycin (1 mM, 3 hours). Cochlear explants were then fixed and labelled with Alexa Fluor 488-Phalloidin for hair cell counting. Neomycin caused substantial loss of the sensory hair cells, mostly in the middle turn of the cochlear explant. Addition of ATP and UTP did not affect hair cell survival, whilst the supplementation of ATP₆S and UTP₆S significantly enhanced neomycin-induced hair cell loss. In contrast, adenosine and particularly the A₁R-selective agonist adenosine amine congener (ADAC), conferred protection to the organ of Corti from neomycin toxicity. This study demonstrates that prolonged activation of P₂R aggravates neomycin-induced hair cell loss, whilst activation of A₁R has a protective effect. Based on these studies, we postulate that the balance of P₁ and P₂ receptor signalling is important for cochlear survival under ototoxic stress.

Jeremy Pinyon - University of New South Wales

BIONIC ARRAY DIRECTED GENE ELECTROTRANSFER OF A NEUROTROPHIN ENCODING PLASMID FREE OF ANTIBIOTIC RESISTANCE GENES ENABLES COCHLEAR NERVE REGENERATION

The utility of cochlear implant (CI) electrode arrays to enable gene electrotransfer of naked plasmid DNA has recently been demonstrated (Pinyon et al., Sci. Transl. Med. 2014). Plasmids that encode brain-derived neurotrophic factor (BDNF) stimulate outgrowth of the spiral ganglion neurites, decreasing the neural gap to the bionic interface, which enhances CI performance. Translation of this technology to the clinic could greatly improve hearing of many CI recipients once safety and efficacy are validated. The use of plasmids free of antibiotic resistance genes (pFARs) improves safety by eliminating the possibility of horizontal transfer of antibiotic resistance to potential pathogens. The utility of these plasmids also includes enhanced expression of the therapeutic protein due to their smaller size. Our objective was to test the amenability of pFARs to the CI application of bionic array-directed gene electrotransfer (BaDGE). Two weeks after deafening, guinea pigs underwent BaDGE to the left cochlea using a pFAR encoding neurotrophin-3 and BDNF. Neural innervation of the treated (left) and untreated (right) cochleae was compared two weeks after BaDGE (four weeks post deafening). Class III beta-tubulin immunohistochemistry of sectioned cochleae showed significant regeneration of the peripheral neurites of the spiral ganglion neurons, as determined by fluorescence intensity of the neurites within the osseous spiral lamina (p = 0.029; paired t-test, n = 4). These preclinical results have demonstrated the translational potential of pFARs encoding neurotrophins for gene electrotransfer-based CI augmentation.

Stephen Lomber - University of Western Ontario

VOCALIZATION PROCESSING REGIONS OF AUDITORY CORTEX MEDIATE ENHANCED FACE DISCRIMINATION ABILITIES OF THE CONGENITALLY DEAF
Lomber SG, Meredith MA, Wong C, Kral A
1. The Brain and Mind Institute, University of Western Ontario, London, ON, Canada. 2. Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA, USA. 3. Department of Experimental Otolgy, Medical University of Hannover, Hannover, Germany.

In the absence of acoustic input, it has been proposed that cross-modal reorganization of deaf auditory cortex may provide the neural substrate mediating compensatory visual function. Here, we will examine evidence in support of this hypothesis. By using a battery of visual psychophysical tasks we found that congenitally deaf, compared to hearing, cats have superior localization in the peripheral field and lower visual movement detection thresholds. Furthermore, reversible deactivation of posterior auditory cortex selectively eliminated superior visual localization abilities while deactivation of the dorsal auditory cortex eliminated superior visual motion detection. While these results demonstrate that dorsal pathway functions can be enhanced following hearing loss; only recently have similar results been obtained for ventral pathway functions. Here, we will show that deaf cats are significantly faster at learning to discriminate both human and conspecific faces compared to hearing cats. Moreover, bilateral deactivation of temporal auditory field (TAF) resulted in the elimination of the enhanced face (both conspecific and human) discrimination learning capabilities of the deaf cats. Unilateral deactivation of left TAF resulted in a partial, but significant, decrease in the enhanced face learning abilities of the deaf cats. These results provide evidence of a lateralization in the enhanced face learning abilities. Overall, these results show that enhanced visual cognition in deaf cats is caused by cross-modal reorganization within the ventral processing stream of “deaf” auditory cortex. Taken together, these results demonstrate a causal link between the cross-modal reorganization of auditory cortex and enhanced visual abilities of the deaf.

Oral 12
Neurodevelopment & neuroanatomy
Dhanisha Jhaveri - The University of Queensland

REGULATION AND CONTRIBUTION OF DISTINCT NEUROGENIC PRECURSORS IN THE ADULT MOUSE BRAIN

1 Mater Research Institute - The University of Queensland, QLD 4102, Australia
2 The University of Queensland, Queensland Brain Institute, Brisbane, QLD 4072, Australia

Neural stem/precursor cells drive the production of new, functional neurons (i.e. neurogenesis) in selected regions of the adult mammalian brain. These adult-born neurons are believed to play a pivotal role in the regulation of learning, memory and mood. However, their regulation and contribution in both health and disease remain to be fully elucidated. Our studies have revealed that two distinct populations of quiescent precursor cells exist in the adult hippocampus and that these are activated by different molecular mechanisms – one population being activated by KCl-mediated depolarisation and the other by the neurotransmitter norepinephrine. By developing a new cell-sorting protocol, we have purified these hippocampal precursors and have characterized their molecular identity. Also, we have found that these distinct precursors are differentially distributed along the septo-temporal axis of the adult hippocampus and are differentially regulated by well-known modulators of neurogenesis. Besides the hippocampus, our recent findings have demonstrated that the adult amygdala also harbours an endogenous population of neurogenic precursors that generate new neurons in vivo. Using retroviral birth-dating and lineage-tracing, we show that these newborn neurons mature and exhibit the electrophysiological properties of interneurons in the basolateral amygdala. Given that the hippocampus and amygdala are key brain regions implicated in the circuitry underlying neuropsychiatric disorders such as depression and anxiety, these findings provide the framework for understanding the functional
CIRC-SEQ ANALYSIS REVEALED COMPLEXITY OF CIRCULAR RNA EXPRESSION IN THE BRAIN WITH POTENTIAL AS MIRNA SPONGES

1. School of Biomedical Sciences, Faculty of Health and Medicine, University of Newcastle, Callaghan, NSW 2308, Australia. 2. Schizophrenia Research Institute, Sydney, Australia

Background: More recently, a novel type of non-coding RNA known as circular RNA (circRNA) was discovered in a variety of species, including humans, with a stable expression in the brain. CircRNAs are formed by the back-splicing of two RNA ends, generating a circle structure with a length ranging from one to many exons. CircRNAs act as transcription regulators, microRNA regulators, host gene expression modulators and template for translation. In this study, we analysed circRNA expression profiles in the post-mortem brain using circular RNA sequencing (Circ-Seq) to better understand their role in development and function of the brain.

Methods: Following enrichment for circRNA species by RNase R treatment, sequencing libraries were prepared from cerebral cortex (BA46) of 23 individuals using Illumina TruSeq (150 cycles) and sequenced by an Illumina NexSeq500. Sequencing data were analysed by CIRCexplorer2 pipeline to identify circRNA transcripts.

Results: The results revealed, surprisingly, a large number (52,000) of circRNAs, many of which were highly expressed across the brain samples. Interestingly, a large proportion of the identified circRNAs were rare or not previously reported. Furthermore, de novo assembly for circRNAs showed many of them are alternatively spliced, suggesting complexity of these molecules. We also discovered 2,440 novel circRNA that are spliced out of unannotated exons. Gene pathway analysis showed many of the circRNAs are transcribed from the genes implicated in important neurological activities, including synaptic function. Moreover, subsequent bioinformatics analysis indicated that many of the circRNAs potentially interact with miRNAs, supporting the miRNA sponging function for these circRNA. To validate the sequencing findings, real-time PCR was performed using outward primers sets designed to uniquely amplify circular transcripts, with the results confirming the observations in the Circ-Seq.

Conclusions: These findings indicated the abundance of circRNA as well as the expression complexity of these transcripts in the brain. Furthermore, our results support the hypothesis that circRNA are potentially functional, acting as a sponge, through binding to target miRNAs.

MATERNAL VITAMIN D TREATMENT REVERSES MATERNAL IMMUNE ACTIVATION INDUCED ALTERATIONS IN MESENCEPHALIC DOPAMINE NEUROGENESIS

Dopamine dysregulation is a feature present in the majority of patients with schizophrenia. We propose the aberrant ontogeny of mesencephalic dopamine (mesDA) systems as a common causal pathway for the pathogenesis of schizophrenia. We aim to test whether maternal administration of vitamin D could rescue the aberrant dopamine neurodevelopment induced by maternal immune activation (MIA) using double-strand viral mimic RNA (polyinosinic : polycytidylic acid, poly (I:C)). We administrated poly (I:C) or saline, and the active hormone form of vitamin D (1,25OHD) or its vehicle (corn oil) to pregnant C57BL/6 mouse dams at gestational day (GD) 9. Two days later, GD11, we assessed mesDA neurodevelopment using immunohistochemistry and automated image analysis of CellProfiler software. Four mesDA factors were employed for immunohistochemical analysis, including LIM homeobox transcription factor 1 alpha (Lmx1a), SRY-related HMG-box2 (Sox2), nuclear receptor related 1 protein (Nurr1), and tyrosine hydroxylase (TH). Four subgroups of early mesDA cells were therefore analyzed: mesDA progenitors, post-mitotic mesDA neurons, immature and mature mesDA neurons. The results revealed that MIA and 1,25OHD treatment reduced mesDA progenitors (Lmx1a+Sox2+), however, 1,25OHD treatment increased mature mesDA neurons (Nurr1+TH+). Single-cell quantification...
showed that 1,25OHD treatment increased the expression of Lmx1a, Nurr1 and TH in individual mesDA cells, but not of Sox2. MIA treatment instead had no obvious effects on the expression of these factors. In conclusion, our data demonstrates the neuroprotective effects of 1,25OHD on early mesDA neurogenesis possibly via its upregulation of mesDA factors, counteracting against the acute negative effects of MIA treatment.

Angela Laird - Macquarie University

EXPLORING THE ROLE OF MICRO RNA-218 IN MOTOR NEURON DEVELOPMENT

M Jayachandran¹, K Yuan², M Watchon², J Giacomotto² and A.S. Laird²

¹Brain and Mind Centre, University of Sydney, Sydney ²Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University ¹Sydney Medical School, University of Sydney ²Queensland Brain Institute, University of Queensland, Brisbane

MicroRNA-218 (miR-218) have been found to be specifically expressed within motor neurons in developing zebrafish, suggesting that mir-218 plays a role in motor neuron development. In this study, transgenic zebrafish with miR-218 knocked down (miR-218 sponge zebrafish lines) were compared to control zebrafish with the same genetic background. Motor axon outgrowth was measured in all groups and a significant decrease in motor axon length was found at 30 hours post fertilization (hpf) and 52 hpf compared to the controls (P<0.019, One-Way ANOVA followed by post hoc analysis). miR-218 sponge zebrafish also had aberrant motor function (decreased distance swum and increased periods of inactivity) throughout larval stages and development until 5 weeks old. The miR-218 sponge line also had decreased survival (P<0.0002), with a greater number of miR-218 sponge fish dying within 40 days post-fertilization. Together, these results suggest that miR-218 plays a role in normal motor axon development and that an imbalance in miR-218 levels can produce motor dysfunction and decreased survival reminiscent of a motor neuron disorder. Further investigation into levels of mir-218 in motor neuron disease patients and a possible role of miR-218 in motor neuron disease pathogenesis is warranted.

Zhe Zhang - The University of Queensland

LOSS OF THE SULFATE TRANSPORTER SLC13A4 ACTIVITY DURING NEONATAL DEVELOPMENT CAUSES AUTISM-LIKE BEHAVIOURS IN ADULT MICE

Zhang Z¹,², Piper M², Dawson PA², Simmons DG¹,²

¹School of Biomedical Sciences, The University of Queensland, Brisbane, Queensland, Australia. ²Mater Research Institute, The University of Queensland, Woolloongabba, Queensland, Australia

SLC13A4 is a sulfate transporter primarily expressed in the placenta and the choroid plexus of the brain. In the current study, we found that Slc13a4 haploinsufficient mice exhibit abnormal social interactions and social memory, and have alterations in exploratory behaviours in the open field and elevated plus maze paradigms. These phenotypes are consistent with those observed in several well-characterized mouse models of autism spectrum disorder (ASD). Furthermore, BrdU incorporation assay identified an increase in cell proliferation within the subventricle zone (SVZ), which is also evident in some mouse models of ASD and in human ASD postmodern brains. To investigate whether the ASD-like phenotypes we observed have a developmental origin, we crossed Slc13a4h/h mice with UBC-CreERT² mice and deleted Slc13a4 either in neonates or in adults by activating cre with tamoxifen administration. Deletion of Slc13a4 in adult mice did not result in behavioural or neurogenic impairments, however deletion of Slc13a4 in the neonate recapitulated our previous observations, demonstrating neonatal Slc13a4 activity is essential for establishing lifelong brain functions such as social behaviours and adult neurogenesis. Moreover, we found that N-acetyl-cysteine (NAC) administration during this developmental window ameliorated the phenotypes, while no improvement was evident in Slc13a4+/mice administered NAC in adulthood. In summary, our findings highlight the critical role of Slc13a4 activity during postnatal
development for normal adult social behaviours and neurogenesis, and identify NAC administration during the critical period as a potential treatment to prevent the acquisition of these adult phenotypes.

Andrew Shoubridge - SAHMRIA

TIME-COURSE STUDY OF DENDRITIC SPINE MORPHOLOGY AND DENSITY IN A PAEDIATRIC LYSOSOMAL STORAGE DISORDER

Mucopolysaccharidosis IIa (MPS IIa) is a paediatric-onset neurodegenerative lysosomal storage disorder (LSD) characterised by cognitive regression and motor abnormalities. Negligible cell loss occurs until late-stage disease, suggesting neurological dysfunction may result from discrete changes in neuronal structure and function. Indeed altered dendritic spine morphology and changes in dendritic spine number and maturity have been observed in other neurocognitive disorders, including autism spectrum disorders, Alzheimer’s disease, and other LSDs such as Niemann-Pick types A and C. This study sought to characterise spine morphology and number in the MPS IIa mouse brain over the disease time-course and determine age of onset and nature of spine abnormalities. Dendritic spines located on motor cortical pyramidal neurons were evaluated in perfusion-fixed brains taken from three- and six-week-old (pre-symptomatic), 12-week-old (early-symptomatic), and 20-week-old (late-symptomatic) MPS IIa-YFP and unaffected-YFP mice (n=3/genotype/age). Z-stack images of dendrites (36/mouse) were generated using a Leica TCS SP8X confocal microscope, and the density and morphology of individual dendritic spines was determined using Imaris (Bitplane) software, by an investigator blind to mouse genotype/age. Whilst no change in immature spine morphology was noted in affected animals, a statistically significant decrease in the number of mushroom (mature) dendritic spines was observed in 6-week-old (pre-symptomatic) MPS IIa mouse cerebral cortex (c.f. age-matched controls; p<0.001). Symptomatic-stage MPS IIa mouse cortical neurons exhibited similar spine deficits (p<0.01). Although electrophysiological studies are required for confirmation of our hypothesis, this data suggests that altered cell-cell communication may underpin symptom-expression in MPS IIa, providing a cogent target for therapeutic intervention.

Magdalena Lam - Macquarie University

THE ULTRASTRUCTURE OF SPINAL CORD PERIVASCULAR SPACES: IMPLICATIONS FOR THE CIRCULATION OF CEREBROSPINAL FLUID

Lam MA1, Hemley SJ1, Najafi E1, Vella NG2, Bilston LE1, Stoodley MA1

1Faculty of Medicine and Health Sciences, Macquarie University, 2Macquarie University Microscopy Unit, Faculty of Science and Engineering, Macquarie University, 3Neuroscience Research Australia, University of New South Wales, Sydney

Perivascular spaces play a pivotal role in the exchange between cerebrospinal and interstitial fluids, and in the clearance of waste in the CNS, yet their precise anatomical components are not well described. The aim of this study was to characterise the ultrastructure of perivascular spaces and their role in the transport of fluid, in the spinal cord of healthy rats, using transmission electron microscopy. The distribution of cerebrospinal fluid tracers injected into the subarachnoid space was studied using light, confocal and electron microscopy. Perivascular spaces were found around arterioles and venules but not capillaries, throughout the spinal cord white and grey matter. They contained fibroblasts and collagen fibres, and were continuous with the extracellular spaces of the surrounding tissue. At 5 min post injection, tracers were seen in the subarachnoid space, the peripheral white matter, the perivascular spaces of the penetrating blood vessels, the basement membranes, the extracellular spaces of the surrounding tissue, and in the lumen of blood vessels. The novel finding of transvascular clearance of tracers points out a novel CNS fluid outflow pathway, with implications for volume regulation in health and disease states, but also clinically for the detection of CNS-derived biomarkers in plasma, the immune response and drug pharmacokinetics.
Remarkable Hippocampal CA1 Expansion in Terrestrial Ungulates and Carnivores

We have found an unusual elaboration of CA1 of the septal hippocampus in terrestrial carnivores and ungulates. This phenomenon has not previously been reported. We examined sections of brains of 60 mammals on the brainmuseum.org website. In 7 artiodactyls (sheep, goat, deer, llama, pig, zebu, peccary) and one perissodactyl (zebra) CA1 is between 3 and 7 times as long as CA3 and CA2 combined, and the medial (distal) end of CA1 often forms a number of folds or pleats that look like gyri. In 14 carnivores (fox, dog, cat, etc) the CA1 extension is also prominent although not as extensive as in the ungulates. With four minor exceptions, this elaboration of CA1 is not seen in other groups of mammals (Euarchontoglires, Afrotheria, Xenarthra, Marsupialia, and Monotremata) where the length of CA1 is 1 to 2 times that of CA3. The CA1 expansion is also not seen in aquatic ungulates and carnivores or in members of the remaining orders of the Laurasiatheria (mole, hedgehog, shrew, bat). The CA1 area has been shown play a role in spatial memory, and its expansion may correlate with navigational ability over long migrations common to the ungulates, and across the wide territorial range of many carnivores.

Oral 13
Injury and repair

Peripheral Nerve Injury-Induced Heat Hyperalgesia and TRPV1 Responses Are Attenuated in Mice Lacking Seizure Protein 6 (SEZ6)

Teng K-S1, Lovric MM1, Thek KR2, Daykin HJ3, Eroglu Ç4, Dolphin AC5, Graham BA6, McDougall SJ2, Gunnersen JM1

1. Department of Anatomy and Neuroscience, University of Melbourne, Parkville VIC 3010. 2. Florey Institute of Neuroscience and Mental Health, Parkville VIC 3010. 3. Department of Pharmacology, University of Melbourne, Parkville, VIC, 3010. 4. Department of Cell Biology, Duke University Medical Center, Durham, NC 27710, USA. 5. University College London, UK. 6. University of Newcastle, Callaghan NSW 2308. *Equal author contributions.

Chronic pain arising from nervous system injury or disease, termed neuropathic pain, is debilitating, difficult to treat and an enormous societal burden. Sensitization and exaggerated pain responses, including allodynia and hyperalgesia, are consequences of maladaptive changes at sensory afferent neuron synapses in the spinal cord dorsal horn. The drugs gabapentin and pregabalin are used to treat neuropathic pain and they exert their effects via the α2δ receptor, an accessory subunit of voltage-sensitive calcium channels. Growing evidence implicates α2δ in excitatory synaptogenesis and our data reveal that Seizure-related gene 6 (Sez6) protein binding to α2δ can promote this effect. Thus, we hypothesized that Sez6 contributes to the synaptic gain-of-function in spinal cord dorsal horn neurons in neuropathic pain. We tested Sez6 knockout (KO) mice and wild-type (WT) controls for mechanical (von Frey) and heat/cold hyperalgesia before and after either partial sciatic nerve chronic constriction (CCI) or sham surgery. Spinal cord and brain tissue sections were processed for Golgi-Cox analysis and patch clamp recordings were made from LII outer dorsal horn neurons in spinal cord slices. Sez6 KO mice showed diminished and faster-resolving heat hyperalgesia, attenuated TrpV1-dependent activity in spinal cord dorsal horn and lack of an injury-induced increase in mature dendritic spines in the prefrontal cortex compared to WT controls. These results indicate that blocking Sez6 function after peripheral nerve injury could prevent the development of heat hyperalgesia, elevated TrpV1 responses and pathological plasticity in ascending pain pathways.

Rohan Walker - University of Newcastle
WHY HASN’T THE GARBAGE BEEN COLLECTED? UNDERSTANDING THE RELATIVE CONTRIBUTIONS OF GLYMPHATIC FLOW AND METABOLIC PROTEOSTASIS IN THE ACCUMULATION OF NEUROTOXIC PROTEINS WITHIN THE BRAIN POST-STROKE

Rohan Walker, Zidan Zhao, Murielle Klugé, Lin Kooi Ong, Michael Nilsson
University of Newcastle and the Hunter Medical Research Institute.

Cognitive function progressively deteriorates post-stroke. In investigating why this occurs we and others have identified that certain nuclei within the brain post-stroke exhibit a number of neurodegenerative features. Most strikingly, areas with the highest levels of degeneration are characterised by the accumulation of soluble amyloid-beta oligomers. In an attempt to provide some new insights about why this accumulation occurs, our research team has recently begun examining two potential explanations. The first is a failure of glymphatic clearance and the second is a failure in proteostasis. To consider glymphatic mechanisms, we have turned to in-vivo multiphoton imaging. Here we have identified that stroke is associated with a dramatic slowing of glymphatic flow, a process known to be critical to facilitating the removal of waste proteins from the brain. Consistent, with this data we have further identified that stroke is associated with robust impairments in aquaporin-4, the water channel that facilitates the convective flow that ‘rinses’ the CNS of waste proteins. We have also now obtained evidence that microglial function in areas characterised by high amyloid loads is also grossly impaired. Specifically, using slice based real time imaging we have identified that microglial ability to respond to damage cues and engage in phagocytosis is significantly compromised. Together, these novel findings suggest that stroke results in impairment of two of the most significant pathways involved in waste clearance within the brain. Strategies to remediate these deficits are now being considered, with a specific emphasis of promoting the establishment on new vasculature.

Jennifer Keller - University of Melbourne

MODELLING SENSORY NEURON PLASTICITY IN PULMONARY DISEASE USING PRIMARY AND STEM CELL DERIVED SENSORY NEURONS

Keller JA¹, Hudson JE², Mackay GA³, Dottori M⁴, McGovern AE¹, Mazzone SB¹
1. Department of Anatomy and Neuroscience, University of Melbourne, Victoria, Australia. 2. School of Biomedical Sciences, University of Queensland, Brisbane, Australia. 3. Department of Pharmacology and Therapeutics, University of Melbourne, Victoria, Australia. 4. Centre for Neural Engineering, University of Melbourne, Victoria, Australia.

Hypersensitivity of the sensory circuitry innervating the respiratory tract accompanies mucosal dysfunction in a variety of pulmonary diseases, and contributes significantly to patient morbidity. The interplay between respiratory epithelial cells and sensory neurons is poorly defined and difficult to study in vivo. We set out to develop novel in vitro preparations consisting of primary vagal or stem cell derived sensory neurons and human airway epithelial cells. Murine primary vagal sensory neurons were enzymatically dissociated and cultured. H9 human embryonic stem cells (hESC) were differentiated into sensory neurons using small inhibitors and growth factors. hESC-derived neurons stain positive for β-tubulin III, Neurofilament, Peripherin, TRPV1, Piezo2, vGluts1/2, and Calbindin neuronal markers and express key sensory genes (TAC1, SCN9A, P2RX3, TRPV1, ASIC2). Media conditions were optimised to allow human epithelial cells grown at air-liquid interface (ALI) to be co-cultured with primary vagal or hESC-derived sensory neurons. Neurons co-cultured with differentiated epithelial cells at ALI possess significantly longer neurites than those grown alone (neurons only = 640±212cm; co-culture = 916±328cm; P<0.05, paired T-test). In addition, co-cultured neurons displayed molecular expression profiles more similar to acutely isolated cells compared to neurons cultured for the same time alone. Altered growth and gene expression profiles of neurons in co-culture conditions suggests the existence of epithelial paracrine mediators in the co-culture system that support the maintenance of neurons. Future experiments will utilise hESC-derived neurons and epithelial cells derived from patients with active respiratory diseases to better
Australasian Neuroscience Society Annual Scientific Meeting 2017  
International Convention Centre, Sydney, December 3rd – 6th 2017

<table>
<thead>
<tr>
<th>Define mechanisms of sensory nerve plasticity.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leon Teo - Australian Regenerative Medicine Institute</td>
</tr>
</tbody>
</table>

**REACTIVATING INFANT SCARRING PATHWAYS TO ATTENUATE GLIAL SCARRING AND IMPROVE FUNCTIONAL SPARING AFTER STROKE IN ADULTS**

**Teo L, Homman-Ludiye J, deSouza M, Kwan WC, Carril-Mundinano I, Bourne JA**  
**Australian Regenerative Medicine Institute, Monash University, Melbourne VIC**

**Background:** The glial scar, formed by reactive astrocytes after CNS injury, severely impedes neuroregeneration. However, infants possess greater potential for functional recovery compared to adults, correlated to glial scar severity. **Hypothesis:** The infant and adult brain differentially regulate astrogliosis post-stroke, resulting in diverging chronic scar outcomes. Additionally, reactivation of infant scarring pathways in the injured adult brain improves functional sparing.  

**Methods:** A clinically translatable infant and adult nonhuman primate (NHP) model of stroke (n=44), possessing identical astrogliotic profiles with humans, was utilized.  

**Results:** The chronic infant scar is significantly smaller than adults (p<0.05). The earlier onset of astrocyte proliferation and absence of crucial regulators of astrogliosis (STAT3/Lcn2) in infants suggests that astrogliosis is differentially regulated compared to adults. Activators of EphA4, a modulator of astrogliosis, were differentially upregulated: In infants, ephrin-A1 upregulation induced astrocyte repulsion, reduced proliferation and overall reactivity, correlating to discrete scarring. In adults, ephrin-A5 upregulation induced opposing cellular responses, correlating to dense, widespread scarring. More importantly, the reintroduction of ephrin-A1 signalling in post-stroke adults successfully attenuated astrogliosis, resulting in a ~50% (p<0.05) reduction in chronic scar volume as well as reduced density and severity. Ephrin-A1 treatment in vivo improved neuronal survival and functional sparing of neural circuitry after adulthood stroke.  

**Conclusion:** Astrogliosis and glial scarring are differentially regulated in the infant vs. adult NHP brain post-stroke, correlating to smaller, discrete scars in infants. Reactivation of infant ephrin-A1 signalling in adults successfully attenuated astrogliosis resulting in more discrete scarring, which was permissible to functional sparing after stroke.

| Mian Bi - University of New South Wales |

**TAU DEPLETION AMELIORATES NMDA-RECEPTOR MEDIATED EXCITOTOXICITY AND INJURY IN STROKE**

**Bi M1, Gladbach A1, van Eersel J1, Ittner AA1, Przybyla M1, Ke YD1 & Ittner LM1,2**  
1. Dementia Research Unit, School of Medical Sciences, University of New South Wales, Sydney, Australia  
2. Neuroscience Research Australia, Sydney, Australia

Stroke is a leading cause of death and disability in Australia. The majority of strokes are ischaemic, resulting in focal anoxic injury followed by secondary excitotoxic injury. Neuronal excitotoxicity due to hyper-excitation of glutamatergic receptors contributes to brain injury in stroke. We have previously shown that tau-deficient mice are protected from Aβ-induced toxicity in an APP23 model of Alzheimer’s disease. Here, we utilised a filamentous middle cerebral artery occlusion (MCAO) with reperfusion model to generate stroke in tau-deficient (tau−/−) and wildtype (tau+/+) mice. We showed that tau−/− mice are profoundly protected from ischemic injury and functional deficits following MCAO stroke. We also showed that tau depletion ameliorates NMDA-mediated excitotoxicity in vitro as well as in an intra-cerebral injection model of excitotoxicity. Mechanistically, we also show that this protection is due to site-specific inhibition of excitotoxicity by glutamatergic and Ras/ERK-mediated pathways. Consequently, perturbation of this pathway by an AAV-shRNA vector restored the susceptibility of tau−/− animals to ischaemic injury. Our findings introduce a new role for tau in stroke, making tau-dependent processes relevant beyond progressive age-related neurodegenerative disorders such as Alzheimer’s disease.
NEUROPHYSIOLOGICAL DYSFUNCTION ASSOCIATED WITH CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY IN CISPLATIN TREATED PATIENTS

Timmins HC1, Li T1, Grimson P2,3, Murray JE4, Cox KM2,5, Horvath LG2,3,6, Lewis CR4,7, Goldstein D4,7, Kiernan MC1, Park SB.

1. Brain and Mind Centre, University of Sydney; 2. Chris O’Brien Lifehouse, Sydney, Australia; 3. Sydney Medical School, University of Sydney, Australia; 4. Prince of Wales Clinical School, UNSW, Australia; 5. Sydney Nursing School, University of Sydney; 6. Department of Oncology, Royal Prince Alfred Hospital, NSW, Australia; 7. Department of Medical Oncology, Prince of Wales Hospital, NSW, Australia.

Chemotherapy-induced peripheral neuropathy (CIPN) is a dose-limiting side effect of cancer treatment, producing paraesthesia, numbness and functional impairment. The present study examined the effects of the chemotherapy cisplatin on clinical, functional and neurophysiological measures of nerve function. Neuropathy was assessed via the validated patient questionnaire EORTC-CIPN20, clinician-based grading scale NCI-CTCAE and Total Neuropathy Score clinical version (TNSc), comprising pinprick, vibration-sensibility, deep tendon reflexes, strength and symptom report. Nerve conduction and axonal excitability studies were undertaken, recording compound sensory action potentials (CSAPs) from sural and median nerves. Twenty patients (M=18, Age:49.5±3.8 years) with a median post-cisplatin completion of 5 months (IQR:3.26.3 mean cumulative dose:304±18.4 mg/m²) were assessed. 60% of patients reported numbness and tingling in the hands or feet, with 10% reporting ‘quite a bit’ or ‘very much’. 65% had ≥2 abnormalities on clinical examination, with 28% having sural amplitudes below normative range. TNSc was inversely correlated with time since completion of treatment (r=−.498, p<.05), as was patient report of lower limb numbness (r=−.469, p<.05). Sensory axonal excitability studies revealed significantly smaller median CSAPs (23.2±1.1µV) compared to controls (N=30, Age:47.3±3.5years, 44.1±1.1µV, p<.01), with higher stimulus thresholds required to reach 50% maximal response (Cisplatin:5.0±1.1mA, control:3.1±1.1mA, p<.01) and significantly increased threshold change in excitability parameter TEh90-100ms (Cisplatin:−128.7±4.7%; control:−116.6±3.1%, p<.05). TEh90-100ms was correlated with median (r=.524, p<.05) and sural amplitudes (r=.684, p<.01), suggesting a link between abnormal excitability and neurophysiological dysfunction. Abnormalities in objective neurophysiological parameters may serve as a marker of axonal dysfunction and help identify patients at risk of severe neuropathy.

SOMATOTROPIN (GROWTH HORMONE) AS NEURO-RESTORATIVE THERAPY AFTER STROKE

Ong LK1,2,3, Chow WZ1,2,3, Tebay T1, Kluge M1,2,3, Pietrogrande P1,2,3, Zalewska K1,2,3, Johnson SJ2,4, Isgaard J1, Nilsson M1,2,3, Walker FR1,2,3.

1. School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, NSW, Australia. 2. The Priority Research Centre for Stroke and Brain Injury, University of Newcastle and Hunter Medical Research Institute, NSW, Australia. 3. NHMRC Centre of Research Excellence Stroke Rehabilitation and Brain Recovery, Australia. 4. School of Electrical Engineering and Computing, University of Newcastle, Callaghan, NSW, Australia. 5. University of Gothenburg, Gothenburg, Sweden.

The 470,000 Australians living with stroke are at high risk of developing cognitive deficits and dementia. There are currently no generally accepted therapeutic interventions for improving cognition post-stroke. A potential candidate which has had an exemplary track record of safety and efficacy in clinical setting is growth hormone. Far beyond of its classical actions on longitudinal body growth and intermediate metabolism, growth hormone plays an important role in the...
regulation of cell proliferation and survival of the brain and its effect on cognitive functions. In this study we treated experimentally stroked mice with growth hormone subcutaneously, via mini-osmotic pumps for 28 days starting 3 days post-stroke. Using mouse touchscreen platform of paired-associate learning task, we found that growth hormone treatment significantly improved cognitive performance in stroked mice. Histological and biochemical analysis suggested that growth hormone treatment significantly increased pro-regenerative factors (IGF-1 and VEGF), promoting markers of neuroplasticity (synapsin 1 and myelin basic protein) and vasculogenesis (CD31 and Collagen IV), and reduced the area of tissue loss within the peri-infarct area. Our results are striking, supporting the effectiveness of growth hormone in facilitating the formation of brain's neuronal and vascular networks and neuroprotection, leading cognitive recovery after stroke.

Rosina Giordano-Santini - The University of Queensland

BEHAVIOURAL CONSEQUENCES OF NEURONAL CELL-CELL FUSION

Giordano-Santini R, Kaulich E, Hilliard MA.

Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane QLD 4072, Australia

Recent studies have shown that neuronal cell-cell fusion can occur following viral infection, axonal injury, or after bone marrow transplantation. Although the circumstances leading to these fusion events have been characterized, little is known about their consequences at the physiological and behavioural level. Here, we have developed and validated a model to study the functional consequences of neuronal cell-cell fusion in vivo in the nematode Caenorhabditis elegans. The Amphid Wing “C” neurons Left and Right (AWCL/R) are a pair of chemosensory neurons located in the head of the animal, each responsible for attraction to specific odours. By misexpressing nematode fusogens in AWCL/R, we were able to fuse these neurons. By analysing the chemosensory response of animals with neuronal cell-cell fusion, we found that fused neurons are viable and still able to mediate attraction to odours, which confirms the robustness of their response. However, preliminary data shows that fusion with the fasciculating Amphid Wing “B” Left and Right neurons, another pair of neurons which mediates avoidance to specific odours, suppresses the attraction response mediated by AWCL/R. These data suggest that cell-cell fusion between neurons of the same class does not alter neuronal response, but fusion between different neuronal classes can impair the animal behavioural output. To our knowledge, this is the first study that shows how neuronal fusion affects the function of the nervous system in vivo. We propose neuronal cell-cell fusion as a synthetic biology approach to study neuronal circuits and how changes in neuronal connectivity modify behaviour.

Oral 14
Synaptic function and plasticity

Brian Billups - Australian National University

MAINTAINING GLUTAMATERGIC TRANSMISSION: THE ROLE OF PRESYNAPTIC GLUTAMINE TRANSPORT IN THE BRAINSTEM AND HIPPOCAMPUS

Hulme SR, Billups B
The Eccles Institute of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, ACT, Australia.

To maintain synaptic transmission it is essential that glutamate released from neurons is rapidly replenished. Recycling glutamate via astrocytic uptake of released glutamate, conversion of glutamate to glutamine and subsequent return of glutamine to neurons for ongoing glutamate synthesis (the “glutamate-glutamine cycle”) is commonly believed to be critical for maintaining presynaptic glutamate supply. However, the amino acid transporter that sequesters glutamine from
the extracellular space into presynaptic terminals has not been identified, and its role in replenishing synaptic glutamate under physiological levels of neurotransmission has not been determined.

To investigate presynaptic glutamine transport we have performed whole-cell patch-clamp recordings from presynaptic terminals in the brainstem (the calyx of Held) and in the hippocampus (mossy fibre boutons) in acutely isolated brain slices. In both preparations, we show that glutamine is sequestered into presynaptic terminals by an electrogenic neutral amino acid transporter. Furthermore, recording postsynaptic responses during physiologically relevant patterns of neuronal activity reveals that inhibiting this presynaptic glutamine transport impedes the replenishment of synaptic glutamate over time.

These results are the first direct recordings of presynaptic glutamine transporter activity in individual forebrain neurons, and clearly demonstrate that they play a role in recycling glutamate at excitatory synapses. It is therefore clear that developing pharmacological inhibitors of presynaptic glutamine transport represents a novel and promising way of modulating glutamatergic synapses.

James Daniel - Max Planck Institute of Experimental Medicine

SUMO1-MODIFICATION OF PROTEINS IS NOT OBSERVED AT SYNAPSES

Daniel J. A.¹, Cooper B.², Palvimo J.², Zhang F.², Brose N.¹, Tirard M.¹.
1 Max Planck Institute of Experimental Medicine, Molecular Neurobiology, Göttingen, Germany.
2 Institute of Biomedicine, University of Eastern Finland, Kuopio, Finland.
3 Institute of Biomedicine and Turku Center for Disease Modeling, University of Turku, Turku, Finland.

Post-translational modification of proteins by covalent conjugation of small ubiquitin-like modifiers (SUMOs) is a key mechanism by which protein interactions, localisation, solubility, and degradation can be regulated. Conjugation of the SUMO isoform SUMO1 to proteins at synapses has been proposed as critical to synaptic transmission, regulating synaptic vesicle endocytosis, plasticity and other functions. However, the published evidence for SUMO1-conjugation at synapses is contradictory. To examine the presence of SUMO1-conjugated proteins at synapses, we used genetically-modified mouse models, stringent biochemistry and confocal microscopy. Using a knock-in reporter mouse line expressing SUMO1 tagged with both His6 and HA, we examined seven synaptic proteins previously proposed as SUMO1-targets in mouse brain. In all cases we report no biochemical evidence of SUMO1 conjugation. We next used antibodies to examine the presence of SUMO1 at synapses using quantitative immunocytochemistry in cultured hippocampal neurons. Neurons from mice lacking the SUMO1 protein (SUMO1KO) was included as an essential negative control to ensure antibody specificity. Quantification of anti-SUMO1 immunolabelling shows no specific anti-SUMO1 immunolabelling is present at synapses using two independent methods of quantification. By contrast, nuclear anti-SUMO1 immunolabelling is significantly decreased in the SUMO1KO. Our findings indicate that SUMO1-conjugation of synaptic proteins does not occur or is very rare and hence not detectable using current methodology. These data bring into question the notion of wide-spread regulation of synaptic proteins by SUMO1 modification, and highlight the importance of using robust tools for identifying SUMO1 substrates to faithfully elucidate the role of SUMO conjugation at synapses.

Angelo Keramidas - University of Queensland

INHIBITORY SYNAPSE DEFICITS CAUSED BY FAMILIAL ALPHA1 GABAA RECEPTOR MUTATIONS IN EPILEPSY

Chen X¹, Durisic N¹, Lynch JW² and Keramidas A¹
1. Queensland Brain Institute, The University of Queensland, Brisbane, Queensland 4072, 2. School of Biomedical Sciences, The University of Queensland, Brisbane, Queensland 4072
Epilepsy is a spectrum of neurological disorders with many causal factors. The GABA type-A receptor (GABA\(_A\)R) is a major genetic target for heritable human epilepsies. We examine the functional effects of three epilepsy-causing mutations to the \(\alpha_1\) subunit (\(\alpha_1^{T107}, \alpha_1^{D192N}\) and \(\alpha_1^{A295D}\)) on inhibitory postsynaptic currents (IPSCs) mediated by the major synaptic GABA\(_A\)R isoform, \(\alpha_2\beta_2\gamma_2\)L. We employed a neuron - HEK293 cell heterosynapse preparation to record IPSCs mediated by mutant-containing GABA\(_A\)Rs in isolation from other GABA\(_A\)R isoforms. IPSCs were recorded in the presence of the anticonvulsants drugs, carbamazepine and midazolam, and at elevated temperatures (22, 37 and 40 °C) to gain insight into mechanisms of febrile seizures. We found that IPSCs mediated by \(\alpha_1^{T107}\), \(\alpha_1^{A295D}\), \(\alpha_1^{D192N}\)GABA\(_A\)Rs decayed faster than those mediated by \(\alpha_1\)GABA\(_A\)Rs. IPSCs mediated by \(\alpha_1^{D192N}\) and \(\alpha_1^{A295D}\)GABA\(_A\)Rs also exhibited a heightened temperature sensitivity. In addition, the \(\alpha_1^{T107}\)GABA\(_A\)Rs were refractory to modulation by carbamazepine or midazolam. In agreement with previous studies, we found that \(\alpha_1^{A295D}\)GABA\(_A\)Rs were retained intracellularly in HEK293 cells. However, pre-incubation with 100 nM suberanilohydroxamic acid (SAHA) induced A295D GABA\(_A\)Rs to mediate IPSCs that were indistinguishable in magnitude and waveform from those mediated by \(\alpha_1\)GABA\(_A\)Rs. These results provide new insights into the mechanisms of epileptogenesis of \(\alpha_1\) epilepsy mutations and suggest possible leads for improving treatments for patients harbouring these mutations.

Dennis Cheung - University of New South Wales

UPREGULATING KCC2 AS A TARGET FOR SEIZURE THERAPIES

Cheung DL\(^1\), Goulton CS\(^1\), Watanabe M\(^2\), Nabekura J\(^3\), Moorhouse AJ\(^1\)
1. School of Medical Sciences, UNSW Sydney, Sydney, New South Wales, Australia. 2. Department of Neurophysiology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan. 3. Division of Homeostatic Development, National Institute for Physiological Sciences, Okazaki, Aichi, Japan.

During a seizure, the balance between excitatory and inhibitory activity is disrupted. Given its central role in Cl\(^-\) homeostasis, we hypothesized that increased KCC2 activity would enhance GABAergic inhibition thus improving resistance to seizures. We tested this using a systemic kainic acid (KA) seizure model, in control and KCC2 upregulated mice. We used a tetracycline conditional expression mouse to overexpress KCC2 via withdrawal of doxycycline dietary supplementation. In this seizure model, mice underwent an escalating dose regime receiving two KA injections (5 mg/kg, IP) administered one hour apart. Behavioural seizures were terminated after the second hour by a single diazepam injection (5 mg/kg, IP). EEG was recorded throughout the entire procedure.

We assessed seizure severity by counting the number of seizure spikes per hour in the EEG traces and the percentage time spent in seizure. In both measures, there was no significant difference between control and KCC2-upregulated mice post KA. However, post diazepam there was a significant reduction in both seizure measures in KCC2-upregulated mice (\(n = 11\)) as compared to control (\(n = 10\)) \((p < 0.0001\) both, Kruskal-Wallis test). Additionally, the power spectrum density (PSD) post diazepam was significantly reduced as compared to prior diazepam PSD in KCC2-upregulated mice only \((p < 0.001,\) Wilcoxon matched-pairs sign rank test).

Our results suggest that increased KCC2 activity by itself has only a limited effect on seizure thresholds. However, its ability to potentiate the effectiveness of diazepam in ameliorating seizures represents a novel approach for improving current seizure pharmacotherapies.

Sumasri Guntupalli - Queensland Brain Institute

GLUA1 SUBUNIT UBQUITINATION MEDIATES AMYLOID-B-INDUCED LOSS OF SURFACE AMPA RECEPTORS
The accumulation of soluble amyloid-β (Aβ) peptides produces profound neuronal changes in the brain during the pathogenesis of Alzheimer’s disease. Excessive levels of Aβ disrupt excitatory synaptic transmission by promoting the removal of synaptic AMPA receptors (AMPARs), dendritic spine loss, and synaptic depression. Recently, activity-dependent ubiquitination of the GluA1 subunit has been shown to regulate the intracellular sorting of AMPARs toward late endosomes for degradation. However, whether this ubiquitin signaling pathway mediates Aβ-induced loss of surface AMPARs is unknown. In this study, we demonstrate that acute exposure of cultured neurons to soluble Aβ oligomers induces AMPAR ubiquitination concomitant with the removal of AMPARs from the plasma membrane. Importantly, expression of the GluA1 ubiquitin-deficient mutants inhibited the adverse effects of Aβ on the surface expression of AMPARs in neurons. Furthermore, we revealed the cross-talk between GluA1 ubiquitination and phosphorylation, in particular phosphorylation at Ser-845, which is crucial for AMPAR recycling and is known to be dephosphorylated in the presence of Aβ. Our data showed that the GluA1 ubiquitin-deficient mutant enhances GluA1 phosphorylation on Ser-845. Conversely, the GluA1 S845D phosphomimetic mutant reduced binding with Nedd4-1 and hence the ubiquitination of AMPARs. Importantly, the GluA1 S845D mutant also prevented Aβ-induced removal of surface AMPARs. Taken together, these findings provide the first demonstration of the dynamic cross-modulation of GluA1 ubiquitination and phosphorylation, a process that is perturbed by Aβ, in regulating the membrane sorting decision that ultimately determines the number of AMPARs on the cell surface.
GluA1 ubiquitination and phosphorylation, a process that is perturbed by Aβ, in regulating the membrane sorting decision that ultimately determines the number of AMPARs on the cell surface.

Jocelyn Widagdo - The University of Queensland

THE ACTIVITY-INDUCED LONG NON-CODING RNA MEG3 MODULATES AMPA RECEPTOR SURFACE EXPRESSION IN PRIMARY CORTICAL NEURONS

Widagdo J1,2, Tan MC1,2, Chau YQ1,2, Zhu T1,2, Wong JJ-L3, Cheung A2, and Anggono V1,2
1. Clem Jones Centre for Ageing Dementia Research, The University of Queensland, Brisbane, Queensland, Australia. 2. Queensland Brain Institute, The University of Queensland, Brisbane, Queensland, Australia. 3. Gene and Stem Cell Therapy Program, Centenary Institute, Sydney, New South Wales, Australia.

Transcription of new RNA is crucial for maintaining synaptic plasticity, learning and memory. Although the importance of synaptic plasticity-related messenger RNAs (mRNAs) is well established, the role of a large group of long non-coding RNAs (lncRNAs) in long-term potentiation (LTP) is not known. In this study, we demonstrated the expression of a lncRNA cluster, namely maternally expressed gene 3 (Meg3), retrotransposon-like gene 1-anti-sense (Rtl1-AS), Meg8 and Meg9, which is located in the maternally imprinted Dlk1-Dio3 region on mouse chromosome 12qF1, in primary cortical neurons following glycine stimulation in an N-Methyl-D-aspartate receptor (NMDAR)-dependent manner. Importantly, we also validated the expression of Meg3, Meg8 and Meg9 in the hippocampus of mice following cued fear conditioning in vivo. Interestingly, Meg3 is the only lncRNA that is expressed in the nucleus and cytoplasm. Further analysis revealed that Meg3 loss of function blocked the glycine-induced increase of the GluA1 subunit of AMPA receptors on the plasma membrane, a major hallmark of LTP. This aberrant trafficking of AMPA receptors correlated with the dysregulation of the phosphatidylinoside-3-kinase (PI3K)/AKT signaling pathway and the downregulation of the lipid phosphatase and tensin homolog (PTEN). These findings provide the first evidence for a functional role of the lncRNA Meg3 in the intricate regulation of the PTEN/PI3K/AKT signaling cascade during synaptic plasticity in neurons.

Andrea Kwakowsky - University of Auckland

AGE- AND GENDER- SPECIFIC EXPRESSION CHANGES OF GABAA RECEPTOR SUBUNITS IN THE HUMAN CORTEX

Andrea Kwakowsky, Madhavi Pandya, Henry J Waldvogel, Richard L Faull

Centre for Brain Research, Department of Anatomy and Medical Imaging, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the nervous system. GABA A receptors (GABAAR) are pentameric ionotropic channels. Subunit composition of the receptors is associated with the affinity of GABA binding and its downstream inhibitory actions. Fluctuations in subunit expression levels with increasing age has been demonstrated in animal and human studies. Also, a few studies found that hormonal changes have implications on the GABAAR expression suggesting gender-related changes in GABAAR subunit composition. However, our knowledge is highly based on animal models that produce inconsistent findings. This study is the first detailed analysis of the age- and gender-specific changes of the GABAAR subunit expression in the human superior- (STG), middle- (MTG), inferior temporal gyrus (ITG) and cerebellum (CER) using Western blotting and immunohistochemistry. We observed a significant gender-dependent difference in alpha1 subunit expression; males presenting significantly higher levels compared to women across all stages of life in STG. No significant age- or gender-related differences were found in alpha2, beta3 and gamma2 subunit expression in the STG. However, we found significantly lower GABAAR alpha3 subunit expression in the STG in young females compared to young males (P<0.05) and old males showed higher expression compared to young males (P<0.001). Older females
showed significantly lower alpha5 subunit expression compared to old males (P<0.05) in the STG. Furthermore, GABAARs were well preserved during normal aging and between genders in the human MTG, ITG and CER. In summary, age- and gender-related GABAAR subunit composition changes might influence GABAAR function and affect GABAergic neurotransmission.

Kathryn Munro - The University of Melbourne

KNOCKOUT OF SEIZURE-RELATED GENE 6 (SEZ6) FAMILY PROTEINS ALTERS EXCITATORY SYNAPSE FORMATION, COGNITION AND MOTOR FUNCTION

K. Munro*1, A. Nash*1, E. Ong-Palsson1, K. Teng1, H. Takeshima2, J. Power3, M. Pignoni4, S. Lichtenthaler4, J. Gunnersen1
1. Dept. of Anatomy and Neuroscience, University of Melbourne, Australia. 2. Graduate School of Pharmaceutical Sciences, Kyoto University, Japan. 3. School of Medical Sciences, UNSW Sydney, Australia. 4. German Centre for Neurodegenerative Diseases, Germany

Mice lacking Sez6 throughout development display dendritic branching abnormalities and fewer spines on pyramidal neurons1. The persistence of Sez6 expression, and that of family members Sez6L and Sez6L2, suggests an ongoing role for these proteins in the mature brain including the cortex and hippocampus. Understanding the function of Sez6 family proteins is important as all are substrates of the Alzheimer’s disease protease BACE1, and their processing may be affected by BACE inhibitors being developed for the treatment of Alzheimer’s disease. Using a tamoxifen-inducible conditional knockout (KO) model (Sez6 flox/KO x CaMKIIαCreERT2) in which Sez6 is deleted in pyramidal neurons, we found that Sez6 plays an ongoing role in excitatory synapse function in the mature brain. In adult-deleted Sez6 conditional KO mouse hippocampus, evoked excitatory post-synaptic currents and dendritic spines of CA1 pyramidal neurons were smaller compared to controls. In behavioural tests, Sez6 conditional KO mice show abnormally strong and persistent contextual fear memory. Mice lacking all Sez6 family members (TKO mice) do not perform as well as wild-type (WT) mice in context fear conditioning and the Morris Water Maze, have significant deficits in motor function, and have fewer mature spine types in the cortex. Sez6L, recently validated as a BACE1 substrate in vivo, is localised widely within the cortex and hippocampus2. Preliminary analyses of Sez6L KO mice indicate deficits in motor function. Our results identify important roles of Sez6 family proteins in synapse formation, maintenance and behavior.

Oral 15
Autonomic nervous system and metabolism

Michal Toborek - University of Miami, School of Medicine

BIOENERGETIC REGULATION AT THE BLOOD-BRAIN BARRIER: ROLE OF OCCLUDING

Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL 33136, USA

Energetic regulation at the blood-brain barrier (BBB) is critical for maintaining its integrity, transport capabilities, and brain demands for glucose. However, the underlying mechanisms that regulate these processes are still poorly explored. We recently characterized the protein occludin as a NADH oxidase and demonstrated its influence on the expression and activation of the histone deacetylase SIRT-1. Because SIRT-1 works in concert with AMP-dependent protein kinase (AMPK), we investigated the impact of occludin on this metabolic switch. Here we show that in BBB pericytes, occludin promotes AMPK expression and activation, influencing the expression of glucose transporters GLUT-1 and GLUT-4, glucose uptake, and ATP content. Furthermore, occludin expression, AMPK activity, and glucose uptake were altered under inflammatory (TNFα) and infectious (HIV) conditions. We also show that pericytes share glucose and mitochondria with astrocytes, and that occludin levels modify the ability of pericytes to share those energetic resources.
resources. In addition, we demonstrate that murine mitochondria can be transferred from live brain microvessels to energetically-impaired human astrocytes, promoting their survival. Our findings demonstrate that occludin plays an important role in BBB pericyte metabolism by influencing AMPK activity, glucose uptake, ATP production, and by regulating the ability of pericytes to metabolically interact with astrocytes. Supported by HL126559, MH072567, MH098891, and DA044579.

Andrea Harrington - University of Adelaide

IDENTIFYING SPINAL CORD DORSAL HORN NEURONS ACTIVATED BY COLORECTAL MECHANOSENSORY INPUT

Harrington AM1,2,3 and Brierley SM1,2,3.
1. Visceral Pain Research Group, Flinders University. 2. Centre for Nutrition and Gastrointestinal Disease, University of Adelaide. 3. Infection and Immunity, South Australia Health and Medical Research Institute, Adelaide, SA, 5000, Australia.

Mechanosensory events from the colon and rectum are signaled into the thoracolumbar (T10-L1) and lumbosacral (L6-S1) spinal cord dorsal horn via the splanchnic and pelvic afferent pathways respectively. The spinal cord neurons activated by such colorectal afferent input remain uncharacterized. We used immunolabelling for the neuronal activation marker phosphorylated MAP Kinase ERK1/2 (pERK) to identify the spinal cord dorsal horn neurons activated by in vivo colorectal distension. The number of pERK-immunoreactive neurons (mean±SEM/tissue section) was compared following non-noxious (20mmHg) and noxious (80mmHg) distension pressures and no distension controls (N=3-5 mice). In the thoracolumbar T13-L1 spinal regions, there was no significant difference between controls (3±0.15) and non-noxious (4±0.15) pressures of distension. Whereas, noxious colorectal distension significantly increased the number of neurons activated (9±0.15; p=0.0018), with pERK-immunoreactive neurons primarily localised to the superficial dorsal horn. In the lumbosacral spinal regions L6-S1, non-noxious colorectal distension evoked a significant (p=0.002) increase in pERK-immunoreactive neurons (9±0.75) relative to no distension controls (3±0.92). This was significantly (p=0.006) increased following noxious colorectal distension (13±0.83). pERK-immunoreactive neurons in L6-S1 were present within mid-dorsal horn laminae and the dorsal column in addition to the superficial dorsal horn. This study identifies the spinal cord dorsal horn neurons activated by colorectal mechanosensory events. These results indicate the different role splanchnic and pelvic afferent pathways have in relaying noxious and non-noxious colorectal mechanosensory events into the spinal cord.

Paul Mirabella - Monash University

GLUCOSE-SENSING NEURONS OF THE MEDIOBASAL HYPOTHALAMUS PROJECT TO BROWN ADIPOSE TISSUE (BAT)

Mirabella PN, Stefanidis A, Spanswick DC, Oldfield BJ.
Department of Physiology, Monash University, Melbourne, Australia

Neural input to BAT remains a critical feature of its functional recruitment. In the case of postprandial thermogenesis, evidence suggests a central nutrient-sensing mechanism regulating sympathetic nerve activity to BAT. It is hypothesised that BAT-directed neurons in the mediobasal hypothalamus respond to changes in extracellular glucose, and that the nature of their monosynaptic projection is the basis of the observed heterogeneity in their responsiveness to glucose. Injection of the GFP-tagged, transsynaptic retrograde virus, pseudorabies virus (PRV), into the interscapular BAT of rats allowed the identification of neurons projecting polysynaptically to BAT. Whole-cell patch clamp recordings were performed on GFP+ hypothalamic neurons. Increasing the extracellular glucose concentration revealed glucose-excited neurons in the arcuate nucleus (ARC) (Δ5.55±1.54mV, p<0.0001; n=13) and in the retrochiasmatic area (RCA) (Δ7.48±2.64mV, p<0.001; n=8). Retrospective immunohistochemical analyses of recorded cells showed that glucose-sensitive
neurons in both the ARC and RCA express the anorectic peptide, POMC (n=6 and 8, respectively). In attempt to delineate the heterogeneity of glucose-sensitive neurons based on their monosynaptic projections, retrobeads were injected into brain regions downstream of ARC/RCA, and the glucose responsiveness of double-labelled (bead+/PRV+) neurons was tested. Of those recorded, glucose-sensitive neurons of the ARC projected largely via the paraventricular nucleus (46%), whereas those in the RCA terminated primarily in the sympathetic pre-ganglionic neurons of the IML (64%). These data provide a basis for the postprandial regulation of BAT thermogenesis through central glucose-sensing mechanisms and suggest an importance of their axonal trajectories. These will inform future studies investigating the hypothalamic control of BAT.

Mathusi Swaminathan - University of Melbourne

MICE LACKING ALPHA- SYNUCLEIN HAVE ALTERED CHOLINERGIC FUNCTION IN THE COLON

Swaminathan M1, Fung C1, Finkelstein DI2, Bornstein JC1, Foong JPP1.

1. Enteric Neuroscience Laboratory, Department of physiology, University of Melbourne, Melbourne, Australia. 2. Parkinson’s Disease Laboratory, Florey Institute of Neuroscience and Mental Health, Melbourne, Australia.

α-Synuclein (α-Syn), is a pre-synaptic protein in the brain that is strongly linked with Parkinson’s disease (PD). PD patients commonly suffer from gastrointestinal disorders and α-Syn is expressed in the nervous system of the gut (enteric nervous system, ENS), but its role in the gut remains largely unknown. We examined the effects of α-Syn depletion on the structure and function of the ENS by comparing isolated colons of adult α-Syn knockout (KO) and wild-type (WT) mice. α-Syn immunoreactivity was found in myenteric neurons and in varicosities within myenteric ganglia and smooth muscle layers of WT, but not KO mice. The incidence of spontaneous isotonic contractions of circular muscle preparations was significantly lower in α-Syn KOs (6/6 WT and 4/9 KO mice had contractions; p < 0.05). These contractions are typically mediated by muscarinic receptors. Nearly all varicosities within the myenteric ganglia that contain the excitatory neurotransmitter acetylcholine (vAChT-immunoreactive) contain α-Syn. α-Syn KOs have a higher myenteric neuron density and a greater proportion of a subset of cholinergic neurons in the myenteric plexus (n=3, p<0.05). Synaptic transmission was examined between myenteric neurons loaded with the calcium indicator, Fluo4AM. α-Syn KOs have significantly larger 1 pulse-evoked calcium transients (WT, 40 neurons; KO, 75 neurons, p<0.05), a response that is typically mediated by nicotinic receptors. Overall, our findings suggest that α-Syn KO mice have altered synaptic transmission between myenteric neurons and at neuromuscular junctions. Some of these effects are likely to involve changes in the function of cholinergic nerve terminals.

Lin Yung Hung - University of Melbourne

PERTURBATION OF GUT BACTERIA BY ANTIBIOTIC VANCOMYCIN REDUCES SEROTONIN PRODUCTION AND DISRUPTS ENTERIC NERVOUS SYSTEM DEVELOPMENT IN EARLY POSTNATAL MICE

Hung LY1, Unterweger P1, Parathan P1, Savidge TC2, Bornstein JC1, Foong JPP1.

1. University of Melbourne, Parkville, Victoria, Australia. 2. Baylor College of Medicine, Houston Texas, USA.

Early postnatal life is the critical stage where enteric nervous system (ENS) development coincides with the colonisation of early microbes within the gut. While it is now known in the adult that the microorganisms living in the bowel (microbiota) can influence firing of enteric neurons and gut functions, how microbiota influence the ENS during development remains unclear. In this study, we investigated whether antibiotic exposure early in life alters gut microbiota and ENS development. Mouse pups from each litter were given a single oral dose of antibiotic (vancomycin, 83.3g/kg/day) or water each day from birth (postnatal day, P0) until P10/11 when their duodenums and colons...
were examined. Intestinal contents and mucosa were sampled for microbiota and serotonin analysis via 16S rRNA sequencing and mass spectrometry respectively. We investigated structural and functional changes of the ENS using immunohistochemistry and video imaging of gut motility. Vancomycin significantly shifted microbial composition in colonic mucosa. Vancomycin pups had more frequent and faster colonic motor patterns \( (n=8-10; \text{colon} \ P<0.05) \), lower myenteric neuron density and altered proportions of nitriergic and calbindin-immunoreactive neurons \( (n=10; \ P<0.05) \) in their colons compared to water-fed littermates. Conversely the duodenum was not affected. Vancomycin-fed pups also had significantly lower levels of mucosal serotonin. Co-administration of 5-Hydroxytryptophan with vancomycin from P0 prevented vancomycin-induced effects on neuron density and colonic motility at P10-11. Overall, we show that exposure to vancomycin alters ENS development and motility patterns in the colon in response to aberrant microbial colonization after birth via a mechanism that involves mucosal serotonin.

Khalid Elsaafien - The Florey Institute of Neuroscience and Mental Health

THE ROLE OF THE CHEMOATTRACTANT CCL2 AND LEUKOCYTES IN NEUROGENIC HYPERTENSION

Elsaafien K, Korim WS, May CN, Yao ST
The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Victoria 3052, Australia.

The chemokine C-C motif ligand 2 (CCL2) has been shown to mediate the development of increased blood pressure in hypertension. Studies in primary and secondary hypertensive animal models reported 3-fold increases in CCL2 levels in the paraventricular nucleus of the hypothalamus (PVN). However, the underlying neural mechanisms of CCL2 signalling in hypertension remain unclear. We have investigated whether CCL2 in the PVN contributes to sympathetically-mediated increases in blood pressure. We show that CCR2 receptors are expressed predominantly by astrocytes that make close appositions with RVLM-projecting PVN neurons. Activation of CCR2 receptors in the PVN elicited biphasic responses in mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) that are abolished by prior blockade of CCR2 receptors with the competitive CCR2 antagonist (RS-102895). The biphasic response was characterised by an early sympathoinhibition followed by long-lasting increases in RSNA and MAP. The first phase is dependent on purinergic transmission, putatively between astrocytes and neurons, whereas monocyte and lymphocyte extravasation into the PVN appears to be responsible for the sympathoexcitation. Finally, we showed that in renovascular hypertensive rats, lymphocyte infiltration into the PVN is upregulated compared with normotensive rats. These findings suggest that neuro-inflammation within the PVN leads to increases in sympathetic nerve activity and may contribute to the development of hypertension.

Alice McGovern - University of Melbourne

A NOVEL ROLE OF THE DESCENDING ANALGESIA SYSTEM IN THE REGULATION OF VAGAL REFLEXES

McGovern AE, Kerr NF, Farrell M, Mazzone SB
1. Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Victoria, AUSTRALIA. 2. Monash Biomedical Imaging, Department of Radiology, Monash University, Clayton, Victoria, AUSTRALIA.

The submedius nucleus of the thalamus (SubM), ventrolateral orbital cortex (VLO) and periaqueductal grey (PAG) constitute a well-described pain modulatory pathway. Activation leads to depression of nociceptive inputs in the spinal cord via the rostromedial medulla and brainstem descending inhibitory systems. Using transynaptic anterograde viral tracing we noted a neural circuit derived from jugular ganglia vagal afferents innervating the respiratory tree projecting to the SubM, suggestive that vagal afferent pathways may be regulated by SubM-VLO-PAG descending modulatory system. In functional studies using urethane anesthetized Sprague Dawley rats, electrical stimulation of the larynx to activate vagal afferent fibres evoked respiratory slowing in a
stimulus frequency dependent manner. Concomitant SubM activation via microinjections of 5ug serotonin significantly inhibited reflex reductions in respiration when compared to vehicle controls (e.g., baseline respiratory rate of 117±4.7 breaths per minute (bpm), to 12±2.2bpm and 69±5.9bpm in vehicle and serotonin animals, respectively; p=0.0001, n=9/group). Chemogenetic activation of the SubM with the Gq-DREADD demonstrated a similar inhibitory effect on respiratory slowing (86±7.6bpm and 15±62.4bpm in +m3D+1mg/kg CNO and –m3D+1mg/kg CNO animals, respectively; p=0.0001, n=3/group). This inhibitory effect was absent in animals receiving prior electrolytic lesion of the VLO. Moreover, lesion of the VLO facilitated respiratory slowing caused by laryngeal stimulation (6±6.6bpm and 73.5±6.7bpm in lesion and sham animals, respectively; p=0.0001, n=5/group), consistent with a tonic descending inhibitory influence over respiratory vagal afferent processing. Taken together, these data support the notion that the SubM-VLO-PAG descending modulatory system plays an import role in the regulation of bulbar visceral afferent processing.

Ian Johnston - The University of Sydney

HIGH FAT HIGH SUGAR DIET INCREASES IMPULSIVITY

Ian N. Johnston, Mona Abdelraheem, Linda Yan, Joel Raymond

School of Psychology, The University of Sydney

There are strong correlations between obesity, a high fat, high sugar (HFHS) diet, and low self-control. It is commonly assumed that obesity is due to low self-control: Obese individuals tend to choose HFHS foods and therefore become obese. We tested an alternative hypothesis: That a HFHS diet causes individuals to become highly impulsive.

Methods: Baseline impulsivity was assessed in laboratory rats with a delay discounting task, and were then randomised to either a control diet or a 6 week diet of sweetened condensed milk (SCM; Experiment 1) or a Western cafeteria diet (WCD; Experiment 2). They were then reassessed for impulsivity on the delay discounting task. Memory was also assessed in the novel location recognition task. Neural tissue was collected at the end of the Experiment 2 and assessed histologically.

Results: Initial impulsivity did not predict SCM or WCD consumption. However, SCM or the WCD consumption caused the rats to become more impulsive compared to control rats. The HFHS diets also caused the rats to display poorer spatial memory. The HFHS diets also upregulated the expression of microglial Iba-1 antigens in the prefrontal cortices.

Conclusions: These experiments show that daily consumption of a HFHS diet cause laboratory rats to become more impulsive, and that this diet also causes neuroinflammatory changes within the prefrontal regions of the brain associated with impulsive choice. We argue that there is a dynamic relationship between diet and self-control: HFHS diets reduce the capacity for individuals to control impulsive choices, and this may exacerbate the effect of HFHS diets on obesity.
Subsection 8: ANS Student Body Symposia (Symposium 1-2)

Symposium 1: Grant and fellowship writing tips
Lezanne Ooi (University of Wollongong)

Abstract still to come.

Symposium 2: Post PhD pathways
Stu Fillman (Creso Pharma) – Industry
Abstract still to come.

Dion Petorious (Science and Technology Australia) - Science communication
Abstract still to come.

Christelle Damiens (Exportia)
Abstract still to come.
**Posters**

History of neuroscience and neuroscience teaching

Femke Buisman-Pijlman - University of Adelaide

**HOW CAN I USE ONLINE TOOLS IN MY TEACHING OF NEUROSCIENCE COURSES?**

Online teaching activities have become more and more integrated in formal degree programs. Students expect them and universities start to demand them. But how can you optimally use online tools to support your teaching? This paper reviews the place for specific tools and matches it to teaching aims. How can you make it work for you? First identify the problem that you are trying to address (i.e. low grades, low retention, impossible to get clinical lecturers in). Secondly, identify the aim of using the activity (i.e. providing bridging material, extent students, offer revision tools, add a clinical or multi-disciplinary perspective). The next step is to make, or ideally find, the right resource to support teaching. The last and most important step is to embed the content or activity in the course in a way that supports student learning. Just putting videos online is not the answer. How can you link it to their course objectives or learning goals? How do you refer to it in class activities? At the University of Adelaide we have adopted a university-wide approach to improve the uptake of online tools. Years of experience has helped us to fine-tune the development and use of online resources and activities in neuroscience teaching. Data will be presented of student’s engagement with a range of tools in different learning environments including face-to-face courses, MOOC’s and fully online degree programs.

Dominic Hare - The Florey Institute Of Neuroscience And Mental Health

**A HISTORY OF IRON AND PARKINSON’S DISEASE: (NEARLY) ONE HUNDRED YEARS IN TEN-TO-FIFTEEN MINUTES**

Hare DJ, Double KL.

1. The Florey Institute of Neuroscience and Mental Health, Parkville, Victoria, Australia. 2. Department of Medicine (Royal Melbourne Hospital) at the Doherty Institute, The University of Melbourne, Parkville, Victoria, Australia. 3. Sydney Medical School, University of Sydney, Darlington, New South Wales, Australia. 4. Brain and Mind Centre, University of Sydney, Camperdown, New South Wales, Australia.

The first report of abnormal deposits of iron in the post mortem Parkinson’s disease brain was published by Lhermitte and colleagues in 1924. Although often misrepresented as the origin of the theory that elevated brain iron contributes to Parkinson’s disease pathology, and somewhat at odds with our contemporary understanding of the neuropathology of iron, this study marked the beginning of nearly a century’s worth of research into how a metal essential to nearly every form of life on Earth could be a contributing factor to second most common neurodegenerative disease. Even with this effort, whether iron is a cause or effect of the disease remains a hotly debated topic. In this talk, advances in knowledge are discussed in line with the advances in technology that brought them about, drawing a historical line from early observational studies to clinical trials directly targeting iron in the brain currently being conducted. The future of this often-controversial field will also be considered, and we speculate whether elucidating the role of iron in Parkinson’s disease will remain as enigmatic as the metal itself in the years to come.
Sharna Jamadar – Monash University

NEW ADVANCES IN SIMULTANEOUS BOLD-fMRI AND DYNAMIC [18F]FDG-PET IMAGING OF HUMAN BRAIN FUNCTION

Jamadar SD1,2,4, Sforazzini, F1, Ward, PD1,2,4, Li, S1, Baran, J1, Chen, Z1,3, Egan, GF1,2,4.


BOLD-fMRI provides a regionally-specific, but non-quantitative and indirect measure of neuronal activity. [18F]-fludeoxyglucose positron emission tomography (FDG-PET) provides an index of neuronal glucose metabolism, which is quantitative and tightly coupled to synaptic activity. However, while the temporal resolution of fMRI is in the order of seconds, standard FDG-PET represents a static snapshot of glucose metabolism over the course of a ~45-min scan. Here, we develop a novel protocol that increases the temporal resolution of FDG-PET to 1-min, while simultaneously providing BOLD-fMRI contrast. In 12 participants, 100MBq of [18F]-FDG was infused over the course of a 90-min scan. Infusion start-time was locked to the onset of the PET scan (no uptake period). To allow the FDG-PET signal to rise to detectable levels, non-functional MRI was acquired during the first 20mins after infusion onset. For the remaining 70-mins, a flashing checkerboard stimulus was presented in an embedded on/off design. A slow on/off design (e.g., 10-mins blocks) provided FDG-PET contrast, and a fast (e.g. 32/16-sec) design provided BOLD-fMRI contrast. Results confirmed that this embedded design provides both BOLD-fMRI and FDG-PET contrast within the visual cortex. Joint independent component analysis (ICA) showed that BOLD-fMRI visual cortex activity was positively associated with FDG-PET metabolism in that region, and negatively associated with FDG-PET signal in the vasculature. This novel design demonstrates (a) that a temporal component can be introduced to FDG-PET imaging using slow infusion; (b) an embedded on/off design provides both FDG-PET and BOLD-fMRI contrast; and (c) a previously-unknown metabolic-oxygenation relationship during neural activity.

Adam Martin – University Of New South Wales

Establishing neural networks in peptide hydrogels

Martin AD1,2, Ke, YD1, Chua SW1, Thordarson P2, Ittner LM1,3

1. School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia
2. School of Chemistry, University of New South Wales, Sydney, NSW, Australia
3. Neuroscience Research Australia, Sydney, Australia

Alzheimer’s Disease (AD) is the most common form of dementia and is projected to affect over half a million Australians by the year 2020. Currently, there is no known cure and limited therapies available. A major factor in the ineffectuality of current treatments is centred on the difficulty in diagnosing AD. By the time clinical and behavioural symptoms are established, the disease is at an advanced state, limiting treatment options. Therefore, a strategy is needed to identify early diagnostic markers of Alzheimer’s Disease, either biomarkers or physical changes in the brain. One way to achieve this aim is to design materials which mimic the environment of the brains extracellular matrix (ECM).

The ECM is a fibrous mesh which provides physical and chemical cues for various cellular processes. Hydrogels are composed of cross-linked fibres, and represent an opportunity to mimic the structure of the native ECM. Using short peptides to form hydrogels allows physical and chemical properties of the gel matrix to be tuned, such as stiffness, chemical environment and mesh size. Here we report peptide hydrogels that support the growth of primary neurons in 2D and 3D systems. Neurons can be cultured for over 40 days on these hydrogels whilst maintaining viability, and show synaptic development and electrical activity. The hydrogel can be controllably disassembled, unlike current 3D gel materials which require mechanical shearing. Such a well-defined, tuneable 3D hydrogel matrix holds significant promise for future applications in early diagnosis for various neurodegenerative diseases.
DEVELOPING A MICROFLUIDIC MODEL OF THE HUMAN BLOOD BRAIN BARRIER

Oikari LE, Quek H, White AR

Mental Health Program, QIMR Berghofer Medical Research Institute, Herston, QLD, Australia.

The blood-brain barrier (BBB) is a structure that lines the walls of brain microvessels, inhibiting the entry of toxic molecules into the brain while enhancing the uptake of key nutrients. Brain endothelial cells (BECs), a key cell type of the BBB, express high levels of tight junction proteins that inhibit the passive diffusion of molecules across the cell barrier. The BBB has a critical function in protecting brain health, but its tight structure challenges the delivery of drugs into the central nervous system. For continued development of BBB permeable drugs, establishing a reliable in vitro model of the human BBB is critical. A central limitation of the current BBB models is the lack of in vivo relevance, resultant by the use of artificial membranes and a static culture environment. To overcome this, we have utilised the commercially available OrganoPlate® (Mimetas) as a platform for the development of a human BBB model that allows cells to be grown in a microfluidic environment without physical separation. Human immortalised BEC line, hCMEC/D3, was used as a cell model to induce barrier formation in the OrganoPlate®. We confirmed that hCMEC/D3 cells express BBB tight junction proteins occludin, claudin-3 and ZO-1, with cells maintaining viability when seeded in the OrganoPlate® against a supporting matrix. Following culture in the OrganoPlate® hCMEC/D3 cells formed a tube-like structure, mimicking a blood-vessel, indicating BBB formation. Our results support the use of the OrganoPlate® as a reproducible platform for BBB model, with potential to be used in the screening of BBB permeable compounds.

Developing a 3D model system using microglia-like cells derived from human monocytes


Quek H, Oikari LE, White AR

Mental Health Program, QIMR Berghofer Medical Research Institute, Herston, QLD, Australia.

Neuroinflammation is a major contributor to various neurodegenerative diseases, and is largely mediated by microglia, an innate immune cell of the brain. To date, generating microglia from human stem cells or post-mortem human brain tissue lacks reproducibility. In addition, the 2D culture environment commonly used to study microglia can induce abnormal inflammatory responses. Further, the use of animal derived microglia does not fully recapitulate fully diverse responses of human microglia. To overcome these issues, we propose a unique cell model system utilising human induced microglia-like cells (hiMG) generated directly from human peripheral blood monocytes. This is a non-invasive, rapid and reproducible process. Human monocytes are isolated by using Ficoll-Paque density gradient centrifugation and differentiated to microglia-like cells by culturing with GM-CSF and IL-34, as described in Ohgidani et al, 2014. Microglia-like cells are cultured in commercially available OrganoPlate® (Mimetas) as a 3D platform that mimics the microenvironment of the brain. We have successfully grown hiMG in 3D OrganoPlates for up to 70DIV, where cells revealed high expression of microglial markers such as P2ry12 and Cx3cr1, and low expression of macrophage marker, Ccr2. We further co-cultured hiMG with neural stem cells to examine the interaction of microglia-like cells with astrocytes and neurons, showing that our 3D model system has the potential to create a microenvironment similar to the human brain and provide as a platform for compound screening.
A multicellular organism is composed of various types of cells inside, where they connect with each other to work in a coordinated manner. Organism-level biological functions, such as sleep-wake behavioral rhythm, are mainly regulated by these cellular circuits. To investigate such multicellular systems, we developed an omics-type comprehensive cell and cell circuit analysis pipeline termed CUBIC (Clear, Unobstructed Brain/Body Imaging Cocktails and Computational analysis), which includes an efficient and reproducible whole organ and body clearing method, a rapid imaging with light-sheet fluorescence microscopy and computational image informatics (Susaki et al. *Cell* 2014; Tainaka et al. *Cell* 2014; Susaki et al. *Nature Protocols* 2015). CUBIC aims to realize an omics-type approach in the cell and cell circuit layer (Cell-omics) and enables multi-modal, multi-sample comparing analysis. The technology for organism-level systems biology thus can be applicable to the wide range of life science and medical researches, and will facilitate our understanding of the complicated multicellular living systems.

Georg Von Jonquieres – UNSW Sydney

**AAV MEDIATED EXPRESSION OF CALCIUM INDICATORS IN COCHLEAR INNER HAIR CELLS. A NEW TOOL TO EXAMINE CALCIUM DYNAMICS.**

von Jonquieres G1, Parmar J1, Pinyon JL1, Klugmann M1, Housley GD1

1. Translational Neuroscience Facility, Department of Physiology, UNSW Sydney, NSW, Australia

Tight regulation of Ca\(^{2+}\) homeostasis is essential for development and function of cochlear inner hair cells (IHCs), enabling proper mechanotransduction and signal propagation. Our understanding of the role and regulation of Ca\(^{2+}\) dynamics in IHCs is emerging, but has been hampered by constraints inherent to conventional Ca\(^{2+}\) indicators. Genetically encoded Ca\(^{2+}\) indicators are novel protein-based fluorophores that exhibit minimal baseline fluorescence in the absence of Ca\(^{2+}\), but greatly increase fluorescence intensity upon Ca\(^{2+}\) binding. Recombinant adeno-associated virus (rAAV) vectors are an emerging safe and efficient tool for somatic gene transfer to the inner ear. We employed a novel AAV vector for delivery of GCaMP5g to the inner ear of neonatal mice. This approach enabled fast and efficient IHC Ca\(^{2+}\) imaging from around the onset of hearing. It circumvents challenges of conventional Ca\(^{2+}\) imaging, including variable dye loading and allows for reliable and repeatable signal acquisition and improved hair cell survival. The dynamic range (ΔF/F\(_0\)) measured in two separate experiments with cycling of extracellular Ca\(^{2+}\) of isolated organ of Corti from 2 week old mice was 0.85 ± 0.17 (mean ± sem), which was ~ double the dynamic range of comparable studies using Fluo4-based Ca\(^{2+}\) imaging (Wong et al. Eur. J. Neurosci. 2013). Our results indicate that rAAV - mediated GCaMP5g somatic cell gene transfer to the inner ear will advance understanding of hair cell Ca\(^{2+}\) homeostasis and facilitate drug screens targeting hearing loss.

Debbie Young – The University of Auckland

**CHARACTERISATION OF A NOVEL MOLECULAR SWITCH FOR USE IN GENE THERAPY**

Fong DM1,2, Musa H1,2, Mouravlev A1,2, Wu A1,2, Young D1,2

1. Department of Pharmacology & Clinical Pharmacology, The University of Auckland, Auckland, New Zealand. 2. Centre for Brain Research, The University of Auckland, Auckland, New Zealand.

Gene therapy strategies for neurodegenerative diseases involve using gene delivery vehicles such as adeno-associated viral (AAV) vectors to mediate transfer of a therapeutic transgene under the control of a constitutively active promoter to the region of interest. Unregulated, long-term production of therapeutic protein that non-discriminately affects both sick as well as healthy cells is potentially problematic. We have developed a novel gene regulation system that could restrict transgene expression to at-risk cells only for use in gene therapy. Our molecular switch relies on elevated calpain or caspase activity to enable the translocation of an ARF5 transcription factor from the cytosol to the nucleus, thus allowing ARF5 to bind to a target DNA binding response element to drive therapeutic gene expression. The aim of the study is to optimise and characterise the functionality of this system in regulating a dual artificial microRNA targeting huntingtin (miRhtt) and brain-derived neurotrophic factor gene therapy cocktail for Huntington’s disease. We found that truncation of ARF5 to a 0.3 kb DNA binding domain sequence did not lead to any significant attenuation in its ability to regulate GFP reporter gene expression levels. *In vitro* cell models expressing pathogenic N-terminal huntingtin fragments regulated expression of miRhtt leading to silencing of mutant huntingtin protein expression, whereas non-
pathogenic huntingtin fragments did not. Our results to date suggest we have developed a gene regulation system with flexibility in regulating transgenes as well as artificial miR-based gene silencing sequences within the constraints of the 4.8kb DNA insert capacity of AAV vectors.

Rakesh Balachandar - Simon Fraser University

IS ARTERIAL SPIN LABELLING A TOOL OF CHOICE TO INVESTIGATE ALZHEIMER’S DISEASE: A SYSTEMATIC REVIEW

Balachandar R 1, Soundararajan S 2.
Simon Fraser University, Burnaby Canada
National Institute of Mental Health and Neurosciences, Bangalore, India

Recent studies have emphasized the contribution of vascular insufficiency and perfusion aberrations in the onset and progression of Alzheimer’s disease (AD). Cerebral blood flow and perfusion can be non-invasively quantified using Arterial spin labelling (ASL). However, ASL is yet to be recognized as a tool for early diagnosis of AD and mild cognitive impairment (MCI). Systematic review of ASL studies, investigating AD would offer clues towards its application in early diagnosis of AD.

Systematic review according to the guidelines of “Preferred Reporting Items for Systematic Reviews and Meta-Analysis” was followed. Keywords such as “ASL”, “mild cognitive decline”, Alzheimer’s disease” and “vascular perfusion” with additional filters “performed on humans” and “English language” were used to search the studies in “Pubmed”, “google scholar” and “sciencedirect”.

Of the list of studies resulted, 39 manuscripts were identified as relevant. Thirty of them were original articles while 9 were review articles. Hypoperfusion of precuneus and posterior cingulate gyrus is consistently reported group changes during prodromal AD (MCI). While absence of hypoperfusion in frontal regions and subcortical regions differentiates from future development of frontotemporal dementia and vascular dementia respectively. However, support vector analysis yielded moderate receiver operating characteristic values in reliably discriminating early stages of AD.

ASL changes are attributed to local neuronal number, size, synaptic density and activity. However, the low resolution of ASL images, susceptibility to motion limits its standalone tool in early identification of AD. Hence, ASL along with other pathological biomarkers would be a useful tool for early detection of AD.

Harrison Evans - Queensland Brain Institute

INVESTIGATING THE DE NOVO PROTEOME IN NEURODEGENERATIVE DISEASES- A CLICK CHEMISTRY APPROACH

Evans HT1, Bodea LG2, Götz J1
1. Clem Jones Centre of Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, St Lucia Campus, Brisbane, QLD, 4072, Australia

Dynamic changes in the de novo proteome have been previously demonstrated to underlie many complex neurological phenomena, such as memory formation, synaptic remodelling and microglial activation. While candidate-based approaches have identified some proteins that are required to be synthesised in order to facilitate such processes in vivo, technical limitations have prevented analysis of the whole de novo proteome. To overcome this, recent studies have examined the whole de novo proteome by using bio-orthogonal non-canonical amino acid tagging (BONCAT) to label de novo synthesised proteins with the methionine surrogate azidohomoalanine (AHA). Here we detail the first robust use of this method to examine the de novo proteome in the brains of adult mice. In this study we establish the optimal dosage AHA and demonstrate that AHA can label proteins de novo synthesised in the brain as little as 4 hours after being delivered via intraperitoneal injection. We also examined the optimal AHA treatment time and determined that maximal AHA labelling is observed 16 hours post injection. Finally we used this method to investigate de novo proteomic changes in the K3 mouse model of frontal-temporal dementia (FTD). Interestingly we found that the presence of pathological tau significantly alters protein synthesis. Together these results demonstrate that AHA labelling is a novel and exciting method for investigate how the de novo proteome is altered in vivo during complex neurological phenomena.

Qiang Ma - Peking Union Medical College

Dissecting dorsal raphe circuits with genetically-targeted technology
Ma Q¹, Xiu J¹, Xu Q¹

¹ State Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences Chinese Academy of Medical Sciences, School of Basic Medicine Peking Union Medical College, Beijing, China

The dorsal raphe nucleus (DRN) projects serotonergic axons throughout the brain. However, it also includes a large population of cells that contain other neurotransmitters. Although, DRN play an important role in regulation of many physiological functions, the circuit mechanisms are poorly understood. Here, we used the modified adeno-associated virus to map the output of serotonergic and nonserotonergic DRN neurons. To explore DRN heterogeneity, we used a simultaneous two-vector knockout strategy, as well as cre-induced and cre-silenced vectors in a cre-expressing transgenic mouse line. We found that topographic patterns of the axonal projection were different among different DRN neurons, that is, the DRN nonserotonergic neurons, for which the main projection target is the ventral tegmental area (VTA) and the DRN serotonergic neurons, for which the main projection target is the intermediate portion of the lateral septum (LSi). Furthermore, individual brain areas receive inputs from both serotonergic and nonserotonergic DRN neurons. These findings indicate that a subpopulation of serotonergic and nonserotonergic dorsal raphe nucleus cells may act to co-modulate processing in these nuclei.

Rucha Pandit – Queensland Brain Institute

Understanding the effects of low-intensity ultrasound on a tau mouse model of Alzheimer’s Disease

¹Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, Brisbane, QLD, Australia.

Scanning ultrasound (SUS) is a new treatment modality that has been shown to reduce amyloid-β pathology in Alzheimer’s disease¹. However, its effect on the second pathological feature of AD, tau deposition, while being studied for antibody delivery across the blood-brain barrier (BBB)², has not been mechanistically addressed. Here we show the effect of SUS on a tau-transgenic mouse model with motor and memory impairment. Histochemical and biochemical tests were used to quantify the changes associated with repeated ultrasound-mediated BBB opening, while a paradigm of behaviour tests was used to correlate these changes to memory and motor functions in the mouse model. Following 15 well-tolerated ultrasound treatments, which is the highest number of treatments used until date, we saw a significant reduction in disease-specific hyperphosphorylation of tau, with an improvement in memory and motor functions in the transgenic mouse model.

Our results, for the first time, show a positive effect on reducing hyperphosphorylated tau in a transgenic mouse model following repeated SUS treatments, which is accompanied by an amelioration of memory and motor function deficits. These results further advocate the use of ultrasound as a potential non-invasive therapy for neurodegenerative disorders.

Bhedita Seewoo - The University of Western Australia

RESTING-STATE FMRI STUDY OF BRAIN ACTIVATION USING LOW-INTENSITY REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION IN RATS

Seewoo BJ¹, ², ³, Feindel KW², Etherington SJ³, Rodger J¹, ².

¹. Experimental and Regenerative Neurosciences, School of Biological Sciences, The University of Western Australia, Perth, Australia. 2. Centre for Microscopy, Characterisation and Analysis, Research Infrastructure Centres, The University of Western Australia, Perth, Australia. 3. School of Veterinary and Life Sciences, Murdoch University, Perth, Australia. 4. Perron Institute for Neurological and Translational Research, Perth, Australia.

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive neuromodulation technique used to treat many neuropsychiatric conditions. However, the mechanisms underlying its mode of action are still unclear. This is the first rodent study using resting-state functional MRI (rs-fMRI) to examine low-intensity (LI) rTMS effects, in an effort to provide a direct means of comparison between rodent and human studies. Using anaesthetised Sprague-Dawley rats, rs-fMRI data were acquired before and after control or LI-rTMS at 1 Hz, 10 Hz, continuous theta burst stimulation (cTBS) or biomimetic high-frequency stimulation (BHFS). Independent component analysis revealed LI-rTMS-induced changes in the resting-state networks (RSN): (i) in the somatosensory cortex, the synchrony of resting activity decreased ipsilaterally following 10 Hz and bilaterally following 1 Hz stimulation
and BHFS, and increased ipsilaterally following cTBS; (ii) the motor cortex showed bilateral changes following 1 Hz and 10 Hz stimulation, a contralateral decrease in synchrony following BHFS, and an ipsilateral increase following cTBS; and (iii) hippocampal synchrony decreased ipsilaterally following 10 Hz, and bilaterally following 1 Hz stimulation and BHFS. The present findings demonstrate that LI-rTMS modulates functional links within the rat RSN with frequency-specific outcomes, and the observed changes are similar to those described in humans following rTMS.

Daniel Tan – UNSW Sydney

**GENERATION OF A NEW TAU KNOCKOUT MOUSE MODEL BY CRISPR/CAS9 GENOME EDITING**

Tan CSD1, Ittner LM1,2,3, Delerue F1,2.
1. Dementia Research Unit, Department of Anatomy, School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia. 2. Transgenic Animal Unit, Mark Wainwright Analytical Centre, UNSW, Sydney, New South Wales, Australia. 3. Neuroscience Research Australia, Sydney, Australia

The microtubule-associated protein tau (MAPT) becomes hyperphosphorylated and aggregates in neurodegenerative diseases commonly referred to as tauopathies. To understand the roles of tau in physiology and disease, several tau knockout (tau−/−) mouse lines have been generated, using different gene targeting strategies in embryonic stem cells by homologous recombination, as well as different genetic backgrounds to establish these lines. As a result, findings from these tau−/− lines has produced inconsistent results. Here we present a new tau−/− mouse generated using the CRISPR/Cas9 genome editing technology, introducing a minimal deletion comprising the transcriptional start codon in exon one of MAPT. Consequently, expression of tau should be abolished. We characterized our new mouse model using DNA sequencing, western blot analysis and induced excitotoxic seizures. DNA sequencing results confirmed successful target site deletion in the MAPT gene, as well as the absence of potential off-target mutations, while western blotting revealed complete loss of tau expression. Furthermore, our new tau−/− mice display reduced susceptibility to induced excitotoxic seizures, similar to previous tau−/− strains. Taken together, we report successful generation of a new tau−/− with minimal genome alteration on a pure C57Bl/6j background. Our line will be made freely available to facilitate studying the broad functions of tau.

Esni Zajaczkowski - Queensland Brain Institute

**NEW APPROACHES TOWARDS SPATIOTEMPORAL CONTROL OF NASCENT RNA LABELLING IN NEURONS.**

Zajaczkowski EL1, Li X1, Wei W1, Spitale RC2, Bredy TW1.
1. Department of Pharmaceutical Sciences, The University of Queensland, Brisbane, QLD, Australia 4072
2. Transgenic Animal Unit, Mark Wainwright Analytical Centre, University of New South Wales, Sydney, NSW, Australia

Despite significant advances in sequencing homogenous cell populations and single cells, current techniques for profiling activity-induced molecular changes fail to differentiate between steady-state RNA and activity-induced nascent RNA. Here, we present the utility of a new method that allows for selective enrichment of nascent RNA. To accomplish this, the method employs the use of uracil phosphoribosyltransferase (UPRT), an enzyme derived from *Toxoplasma gondii*, that drives the incorporation of a chemically-modified nucleobase, 5-ethynyl-uracil (5EUracil), into RNA that is being transcribed. UPRT overexpression within a restricted population of cells enables spatial control over RNA tagging whilst the addition of the analog 5EUracil provides temporal control. Nascent RNA that contains 5EUracil can then be visualised or isolated using the well-characterised Cu(I)-catalysed alkynyl-azide cycloaddition reaction, which can append either a fluorophore or biotin group onto the nascent RNA, respectively. We demonstrate the feasibility of this approach in mouse primary cortical neurons (PCNs) and are currently in the process of optimising these methods for *in vivo* RNA labelling during behavioural tasks. We expect that our work will contribute toward better understanding the differential contributions of steady-state versus nascent RNA in various activity-related scenarios, especially those during learning and memory formation.

Mark Hackett – Curtin University

**MULTI-MODAL IMAGING TECHNIQUES TO STUDY THE CHEMICAL BIOLOGY OF BRAIN DISEASE**

Hollings A1,3, Fimognari N1,4, Tidy R1,2,4, Lam V1,5, Takechi R1,5, Mamo JC1,5, Hackett MJ1,3
Numerous methods of microscopy that probe cellular structure and chemical composition are available to the modern neuroscientist. However, there has long been an unfilled niche for techniques capable of direct biochemical imaging and elemental mapping at the cellular or sub-cellular level.

Conventional microscopies reveal important information about cellular and sub-cellular structure, as well as the distribution of “stainable” targets, such as individual proteins. However, many biochemical parameters cannot be studied with these techniques. For example, no imaging method exists for small and mobile molecules such as taurine, or diffusible ions such as K+ and Cl−. There has been much improvement in the development of fluorescence probes for Ca2+ and labile metal pools (Fe, Cu, Zn). However, although compatible with cell culture studies, the probes are often incompatible with animal models. In addition, direct imaging of markers of oxidative stress, such as lipid oxidation, altered thiol redox and protein aggregation, is notoriously difficult.

This presentation will discuss advances in recent direct spectroscopic imaging techniques such as Fourier transform infrared spectroscopic imaging (FTIRI), X-ray fluorescence microscopy (XFM), and X-ray absorption spectroscopy (XAS). These techniques allow direct imaging of important biochemical parameters such as: lactate, lipids, and protein aggregates (FTIRI)1,2; thiol redox and taurine (XAS)2,3,4; Cl−, K+, Ca2+, Fe, Cu, Zn (XFM).5,6

Applications of these techniques has revealed statistically significant biochemical and elemental alterations (p < 0.05, animal groups n > 5), which occur within the hippocampus during memory loss following cerebral ischemia and during ageing induced dementia, in rodent models.

Using machine learnability of dorsal column nuclei surface potential signal features to quantify relevant information for decoding sensory inputs from the limb

Potas JR1,2 and Loutit AJ1,2
1. Translational Neuroscience Facility, School of Medical Sciences, The University of New South Wales, Sydney, Australia.
2. Eccles Institute, John Curtin School of Medical Research, The Australian National University, Canberra, Australia.

In the context of neuroprosthetic feedback, the dorsal column nuclei (DCN) may offer advantages compared to the primary somatosensory cortex. Advances in this field require robust decoding of electrical events arising from these sensory nuclei. We aimed to rank information importance, delivered by electrode combinations and signal features extracted from the DCN surface, into their capacity to relay relevant information for predicting peripheral nerve input. Four hindlimb nerves, from 7 male adult urethane anaesthetised rats, were electrically stimulated (0.01 ms, 0.7 mA) whilst recording evoked surface potentials from an array of 7 platinum electrodes on the surface of the DCN. A gold-standard supervised back-propagation artificial neural network (ANN) configuration, achieving an overall 96.8 ± 0.8% classification accuracy, was established from an input data set comprising 5 salient signal features extracted from all 7 electrodes (35 inputs). We ranked individual electrode and feature inputs into their capacity to deliver relevant information to the ANN, then combined high-performing input combinations. This approach enabled a vast reduction in electrode and signal feature inputs (4 inputs) without significantly reducing learnability (92.8 ± 2.6% classification accuracy, p = 0.19). Midline electrodes had the greatest prediction accuracy, and thus, the capacity to resolve left/right nerve afferent signals, indicating asymmetry of DCN surface potentials. These findings demonstrate machine learnability is a powerful tool for evaluating salient information which can provide insights into electrode/signal feature relevance for resolving nerve input. Our findings also challenge the notion of functional symmetry in the DCN.

Sarawut Lapmanee - Mahidol University

CHARACTERIZATION OF PHENOTYPE MELATONIN RECEPTOR EXPRESSING CELLS IN THE BETA-GALACTOSIDASE KNOCK-IN REPORTER MICE.

Lapmanee S1,2,3, Krishnamra N1, Felder-Schmittbuhl MP2, Schuster-Klein C4, Guardiola B5, Pévet P5, Klosen P5.

1. Curtin Health Innovation Research Institute, Curtin University, Bentley, Western Australia 6102, Australia
2. Curtin Institute for Functional Molecules and Interfaces, Curtin University, Bentley Western Australia 6845, Australia
3. Department of Chemistry, Curtin University, GPOBox U1987, Bentley Western Australia 6845, Australia
4. School of Biomedical Sciences, Curtin University, Bentley, Western Australia 6102, Australia
5. School of Public Health, Curtin University, Bentley, Western Australia 6102, Australia
Melatonin is an important regulator of circadian rhythms and seasonal physiology via G protein-coupled receptors (MT1 and MT2 melatonin receptors). So far, the cellular localization of these receptors has not been identified clearly despite the use of 125I-lodomelatonin binding, in situ hybridization, and some antibodies for immunocytochemistry (ICC). This study aims to characterize the localization of melatonin receptor expressing cells using MT1 and MT2 melanotin receptor beta-galactosidase (LacZ) knock-in reporter mice with the X-gal histochemistry and ICC. MT1-LacZ expressing neurons were restricted to only a few areas such as the suprachiasmatic nucleus (SCN) and the pars tuberalis of the anterior pituitary. MT2-LacZ expressing neurons were more widely distributed and present in the olfactory bulb, the forebrain, the SCN, the hypothalamus, the paraventricular nucleus of the hypothalamus (PVN), the amygdala, the cerebral cortex and the hippocampus. Double staining ICC and ICC combined with non-radioactive in situ hybridization were used to phenotype MT1-/MT2-LacZ expressing neurons. MT1-LacZ expression was detected in some gastrin releasing peptide neurons and some vasoactive intestinal polypeptide neurons in the SCN. MT2-LacZ expression was identified in corticotropin-releasing hormone neurons in the PVN and some glutamate decarboxylase interneurons in the hippocampus. In the CA2 of the hippocampus, MT2-LacZ was present in pyramidal neurons, which are most probably glutamatergic. The mapping and characterization of MT1 and MT2 receptors could identify possible sites of action of melatonin or melatonin derived antidepressant and anxiolytic drugs.

Bruce Ngo - Florey Institute Of Neuroscience And Mental Health

**DEVELOPMENT OF A NOVEL PEPTIDE-BASED SYSTEM TO MODULATE NEURONAL FUNCTION**

Ngo BN1, Bathgate RAD1,2, Allen AM1,3
1Florey Institute of Neuroscience and Mental Health, 2Department of Biochemistry and Molecular Biology, 3Department of Physiology, University of Melbourne, Victoria, Australia

In recent years several novel approaches, such as opto- and chemo-genetics have been developed to examine the role of specific neuronal pathways driving behaviours. Whilst these approaches enable understanding of the role of a particular neuronal group in a function, they do not allow understanding of the presynaptic pathway driving their activity. To address this, we have developed a novel viral-based system to drive the expression of the insect-derived allatostatin-3 peptide in neurons. In combination with expression of the inhibitory g protein-coupled allatostatin receptor in the postsynaptic neurons we will be able to examine the involvement of pathways in particular functions. The allatostatin-3 peptide sequence was cloned into an adeno-associated viral (AAV) vector utilizing the rat neuropehysin (NP) precursor (AAV-[Allatostatin-3]-NP-IRES-mCherry) to ensure peptide amidation (essential for bioactivity) and effective neuronal trafficking. The ability of AAV-[Allatostatin-3]-NP-IRES-mCherry to produce bioactive allatostatin-3 was tested by transfection into HEK-293T cells. Cell media was tested in parallel with synthetic allatostatin-3 for its ability to inhibit cAMP activity in HEK293T and CHO-KI cells stably expressing the allatostatin receptor. Cell media inhibited cAMP activity in parallel with synthetic allatostatin-3 highlighting that AAV-[Allatostatin-3]-NP-IRES-mCherry directed the expression of amidated peptide. In vivo expression was tested by microinjection of AAV-[Allatostatin-3]-NP-IRES-mCherry into the rat brain. Immunohistochemistry demonstrated co-localization of Allatostatin-3, NP and mCherry in transduced neurons and trafficking of the allatostatin-3 peptide to the presynaptic site. This system will add another level to our ability to dissect neuronal pathways contributing to specific behaviours adding significantly to the chemogenetic toolbox.

Sebastian Stefani – The University of Sydney

**A CELLULAR MODEL FOR STUDYING INHIBITORY NEUROTRANSMISSION IN THE SPINAL CORD OF C57BL/6 WILD-TYPE MICE**

Stefani S1,2 and Aubrey K1,2
1. Discipline of Pharmacology and Bosch Institute, University of Sydney. 2. Pain Management and Research Institute, Kolling Institute of Medical Research Royal North Shore Hospital, University of Sydney.
Previous studies in primary cell cultures and brains slices of mice and rats, determined that inhibitory transmission within the brainstem and spinal cord is mediated by both γ-aminobutyric acid (GABA) and glycine. Inhibitory transmission is reduced in disease states such as chronic pain, due to dysregulation of the concentrations of these neurotransmitters in the presynaptic neuron. This study aims to characterise a new cellular model to study mixed inhibitory transmission in paired spinal cord neurons. Pregnant adult female C57BL/6 wild-type mice were time-mated and embryos were removed by caesarean section between 13-14 days old. Embryonic spinal cord neurons were cultured and left to grow for two weeks. At 15-22 days in vitro, electrophysiological recordings were taken from pairs of connected inhibitory neurons. Evoked and miniature inhibitory postsynaptic currents (eIPSC and mIPSC) were recorded from control and ORG25543 (5 µM) treated neurons. Mixed inhibitory neurotransmission was common within this cellular model, which was dominated by glycine. ORG25543 treated neurons exhibited a switch from glycine to GABA-dominated eIPSCs and a reduced glycinergic mIPSC amplitude. However, mIPSC frequency remained unchanged for both GABA and glycine. These findings indicate that inhibitory neurotransmission in the spinal cord of C57BL/6 wild-type mice contain a dominant glycinergic and a GABAergic component, consistent with previous studies. Thus, this cellular model can be used for future experiments investigating the presynaptic balance of mixed inhibitory neurotransmission.
### Development and regeneration

<table>
<thead>
<tr>
<th>Speaker</th>
<th>Institution</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hitomi Aoki</td>
<td>Gifu University</td>
<td><strong>INDUCED HAPLOINSUFFICIENCY OF KIT IMPAIRS DEVELOPMENT OF NEURAL CELLS</strong></td>
</tr>
<tr>
<td>Anita Balázs</td>
<td>Department Of Anatomy, Histology And Embriology, University Of Debrecen</td>
<td><strong>DEVELOPMENT OF GABA-ERGIC NEURONS IN THE MOUSE SUPERFICIAL SPINAL DORSAL HORN</strong></td>
</tr>
<tr>
<td>Robert Gasperini</td>
<td>University of Tasmania</td>
<td><strong>STIM1 IS NECESSARY FOR AXON GUIDANCE AND SENSORIMOTOR CIRCUIT FUNCTION IN DEVELOPING ZEBRAFISH.</strong></td>
</tr>
</tbody>
</table>

#### INDUCED HAPLOINSUFFICIENCY OF KIT IMPAIRS DEVELOPMENT OF NEURAL CELLS

Kit receptor tyrosine kinase is highly expressed in the developing mammalian brain, yet little is known about its contribution to neural cell development and function. Here we introduced a brainspecific conditional Kit loss-of-function mutation in mice and observed severe hypoplasia of the central nervous system. This was accompanied by an increase in apoptotic cell death in the early embryonic brain *in vivo* and the gradual loss of the self-renewal capacity of neuronal stem/precursor cells *in vitro*. A single copy of the brain-specific conditional Kit loss-of-function allele resulted in the observed phenotype, including impaired *in vitro* differentiation of neural cells from Kit-haploinsufficient embryonic stem cells. Our findings demonstrate that Kit signaling is required for the early development of neural cells. This potentially novel Kit-haploinsufficient lethal phenotype may represent an embryonic lethal phenomenon previously unobserved because of its dominantly acting nature.

#### DEVELOPMENT OF GABA-ERGIC NEURONS IN THE MOUSE SUPERFICIAL SPINAL DORSAL HORN

The first relay station of pain processing is the superficial spinal dorsal horn, where the inhibitory neurons control the modality and intensity of pain signals transmitted to higher brain centers. Earlier studies suggest that the construction of pain processing spinal neural circuits including the GABAergic components should be completed by birth, although major chemical refinements may occur postnatally. Because of their utmost importance in pain processing, we intended to provide a detailed knowledge concerning the development of GABAergic neurons in the superficial spinal dorsal horn. Thus, we studied the developmental changes in the distribution of neurons expressing GABAergic markers like Pax2, GAD65 and GAD67 in the superficial spinal dorsal horn of wild type as well as GAD65-GFP and GAD67-GFP transgenic mice from embryonic day 11.5 (E11.5) till postnatal day 14 (P14). We found that GABAergic neurons populate the superficial spinal dorsal horn from the beginning of its delineation at E14.5. We also showed that the numbers of GABAergic neurons in the superficial spinal dorsal horn continuously increase till E17.5, but there is a prominent decline in their numbers during the first two postnatal weeks. Our results indicate that the developmental process leading to the delineation of the inhibitory and excitatory cellular assemblies of pain processing neural circuits in the superficial spinal dorsal horn of mice is not completed by birth, but it continues postnatally.

#### STIM1 IS NECESSARY FOR AXON GUIDANCE AND SENSORIMOTOR CIRCUIT FUNCTION IN DEVELOPING ZEBRAFISH.

The formation and precision of neuronal circuitry is accomplished through the process of axon guidance. Growth cones detect and transduce guidance cue signals via receptor-mediated calcium transients. The mechanisms by which calcium transients control axon guidance *in vivo* is presently unclear. Stromal interaction molecule (STIM1) regulates store operated calcium entry (SOCE) in response to both calcium-dependent and independent guidance cues *in vitro* and regulates calcium transient activity *in vivo*. In this study, we asked whether STIM1 is important for axon guidance and fidelity of functional circuits in the developing zebrafish. We determined that STIM1 regulates guidance of neurons in zebrafish spinal and olfactory circuits by altering growth cone filopodial dynamics and calcium transient activity, resulting in significant alterations in axon trajectories at crucial pathfinding choice-points. We subsequently show that aberrant axon pathfinding causes behavioural deficits, with STIM1-deficient larvae showing decreased startle-like activity and increases in spinal twitching during early development. Significantly, olfactory deficits seen later in development were significantly increased in larvae expressing mutant STIM1 in olfactory sensory neurons. These data illustrate that STIM1 regulation of filopodial branching and calcium dynamics is an important signaling mechanism during early neural circuit development.
Tongcui Jiang - Menzies Institute For Medical Research

**TDP-43A315T EXPRESSION ALTERS SYNAPSE DEVELOPMENT IN PRIMARY CORTICAL NEURONS**

Ing. TC, Brizuela M, Handley E.A, Dawkins E, Dickson T.C and Blizzard C.A

Amyotrophic lateral sclerosis (ALS) is a multifactorial disease characterised by the progressive death of motor neurons in the central nervous system. Aggregated proteinaceous inclusions, predominately in neurons, characterise ALS pathologically. Transactive response DNA-binding protein 43 (TDP-43) is the most frequently associated protein in these aggregations. Recent research indicates that this RNA binding protein may play an important role at the synapse, however the early pathophysiological dysfunctions causing impairment in synapse are still unknown. We utilised the YFP-TDP-43A315T mouse model expressing mutant human TDP-43A315T in cortical neurons to investigate post-synapse formation in vitro. Primary cortical neurons, derived from individual E15.5 embryos were grown to 3, 5, 10 and 15 days in vitro (DIV). Our data implicated that TDP-43A315T dendrites developed normally - total dendrite length and mean dendrite length significantly (P>0.05) increased between 3 and 15 DIV in both wild-type (WT) and TDP-43A315T cultures with no significant differences (P>0.05) in total dendrite length, mean dendrite length, dendrite branching number and branching order WT and TDP-43A315T neurons. Spine tracing of YFP positive WT and TDP-43A315T cortical neurons at 10 and 15 DIV, and YFP positive WT cortical neurons transfected mCherry tagged TDP-43WT and TDP-43A315T plasmids, demonstrated a significant decrease in dendritic spine density in the TDP-43A315T cortical neurons in comparison to WT controls (P<0.05). Furthermore, electrophysiological analysis indicated that there was a significant (P<0.05) increase in depolarisation threshold in the TDP-43A315T cortical neurons in comparison to WT controls. This work will be imperative in the pursuit of identifying novel therapeutic interventions.

Michael Lovelace - St. Vincent's Centre For Applied Medical Research

**THE KYNURENINE PATHWAY OF TRYPTOPHAN METABOLISM MODULATES NEURAL STEM CELL PROLIFERATION**

Croitoru-Lamoury J, Lamoury FMJ, Walters E, Suzuki K, Walker D, Jones SP, Lovelace MD, Taylor R and Brew BJ

1 Applied Neurosciences Program, Peter Duncan Neurosciences Research Unit, St Vincent’s Centre for Applied Medical Research and 2 Department of Neurology, St. Vincent’s Hospital, Sydney NSW, Australia

2 University of Notre Dame Australia, Sydney NSW, Australia

3 Department of Physiology, Monash University, Clayton VIC, Australia

4 Faculty of Veterinary Science, University of Sydney, NSW, Sydney, Australia

The search for molecules which critically regulate neural stem cell (NSC) proliferation is ongoing, underpinning future production of cell lineages for therapy, while helping understand why innate repair in neurodegenerative diseases fails. Our ongoing research has investigated a role of the kynurenine pathway (KP) in healthy metabolism and neurodegenerative diseases. The KP critically regulates bioavailability of the essential amino acid tryptophan. In MS the KP is dysregulated, producing high levels of metabolites like neurotoxic Quinolinic acid. We investigated if modulating the KP altered NSC proliferation. In particular, if interferons (IFNs) activate KP and drive changes in the proliferation of NSCs. Developing mouse NSCs from E14 neurospheres were cultured. Agonists, antagonists or siRNAs to KP enzymes were used to dissect the pathways. IFN-β activates indoleamine-2,3-dioxygenase (IDO-1) expression, the initial rate-limiting enzyme metabolising Tryptophan, and indeed significantly induced IDO-1 in NSCs. NSCs express all KP enzymes, and IFN-β lead to impaired proliferation and an alteration of metabolic state of NSCs including their NAD+/NADH ratio (cell energy levels) via Trp depletion (required for protein biosynthesis), rather than through effects of KP metabolites. IFN-β negligibly affected IDO-1 levels, but induced IDO-2, and significantly decreased proliferation and downstream enzyme kynurenine-3-monoxygenase. We show that KP enzymes play a specific role in the biology of NSCs and tryptophan metabolism, including the dominant regulation of the KP by interferons e.g. IFN-β and IFN-β. Selective KP inhibition could minimize cell death during inflammatory episodes and optimize NSC proliferation and differentiation with direct therapeutic applications.

Shohreh Majd – Flinders University

**A COMPARISON OF LKB1/AMPK/MTOR METABOLIC AXIS RESPONSE TO GLOBAL ISCHAEMIA IN BRAIN, HEART, LIVER**
Cellular energy failure in high metabolic rate organs is one of the underlying causes for many disorders. Numerous studies have discovered the cellular axis of LKB1/AMPK/mTOR as an essential modulator of cell energy homeostasis in response to energy stress. Energy stress, however, could be sensed at different levels in various tissues, leading to applying different strategies in response to hypoxia. Here the immediate strategies of high metabolic rates organs to time-dependent episodes of ischaemia were studied by using a rat model of cardiac arrest (CA). Using western blot, we examined the responses of LKB1/AMPK/mTOR in brain, heart, liver and kidney from 15 seconds up to 8 minutes of ischaemia. The ratio of ADP/ATP was assessed in all groups. Brain, followed by kidney showed early dephosphorylation in AMPK (Thr172) and LKB1, in the absence of ATP decline. Dephosphorylation of AMPK was followed by rephosphorylation and hyperphosphorylation which was associated with a significant ATP decline. While heart’s activity of AMPK and LKB1 remained at the same level, liver’s LKB1 was dephosphorylated after 2 min ischaemia. AMPK response to ischaemia in liver was based on an early alternative and a late constant hyperphosphorylation. No significant changes was observed in mTOR activity in all groups. Together our results suggest that early AMPK dephosphorylation followed by late hyperphosphorylation is the brain’s and kidney’s strategy in ischaemia. While the liver seemed to get benefit of its AMPK system, possibly to stabilize ATP, the level of LKB1/AMPK activity in heart remained unchanged in short ischaemic episodes.
**University of Sydney, NSW 2006, Australia.**

Obstructive sleep apnea (OSA) and the prone sleeping position, are risk factors for the Sudden Infant Death Syndrome (SIDS), and believed to be due to the intermittent hypercapnic hypoxic (IHH) environment they produce. Using a postnatal piglet model of IHH, we investigated the effects of acute (1 day) vs repeated (4 day) IHH exposure on the immunohistochemical expression of nicotinic acetylcholine receptor (nAChR) subunits α2, α3, α4, α5, α7, α9, β1 and β2 in the hippocampus and brainstem medulla. Piglets were randomly assigned to one of the following four groups: 1 day IHH (1D IHH, n = 9), 4 days IHH (4D IHH, n = 8), controls exposed only to air cycles for 1 day (1D Air, n = 6) or 4 days (4D Air, n = 5). The exposure was a switch from 6 min of HH (8%O2, 7%CO2, balance N2) to 6 min of air over 48 min, while controls were interchanged from air-to-air. Results demonstrated that changes were more pronounced due to repeated IHH than acute IHH in both regions. CA3, CA2 and the DG were most affected in the hippocampus with an increased α2 and β2 expression. Hypoglossal and the nucleus of the solitary tract were most affected in the brainstem with predominant decrease for α2, α5, α9 and β2. The findings suggest that alterations in nAChRs due to repeated IHH in vital hippocampal and medullary nuclei could be causally related to the previously demonstrated functional compromises in these piglets.

Elaine Y.M. Wong - Ear Science Institute Australia

**THE ROLE OF HEDGEHOG RECEPTOR CDO IN CONTROLLING COCHLEAR HAIR CELL FORMATION**

Elaine Y.M. Wong1,2,3, Helena Cara2, Yujien Liu2, Marcus Atlas1,4 and Rodney Dilley1,4

1 Ear Science Institute Australia, Nedlands, Australia
2 School of Biomedical Sciences, The University of Hong Kong, Hong Kong SAR, China
3 Curtin University, Bentley, Australia
4 Ear Sciences Centre, The University of Western Australia, Nedlands, Australia

*Cdo* (Cell adhesion molecule-related, down-regulated by oncogenes) is a novel receptor of the Hedgehog (Hh) pathway. Mutations in *Cdo* cause holoprosencephaly, a human congenital anomaly defined by forebrain midline defects prominently associated with diminished Hedgehog pathway activity. *Cdo* functions as a receptor of the Hh signalling and feedback network. *Cdo* enhances Shh signalling by acting as co-receptors with *Ptch1*, or via regulation of *Gli* transcription factors. A proper balance of *Gli* repressor and activators is required to mediate Hh signalling during inner ear morphogenesis. *Cdo* homozygous knockout mice have profound hearing loss. However, the role of *Cdo* in inner ear development is still unknown. To understand the function of *Cdo* receptor in the modulation of Hh signaling in mammalian inner ear development, we present the differential expression pattern of *Cdo* in the developing mouse inner ear. We found that the expression of *Cdo* at E12.5 marks the prospective organ of Corti, but by E16 *Cdo* is down-regulated in hair cells and becomes restricted to supporting cells, suggest that *Cdo* may have distinct roles in molecular pathways that direct cells towards different cell fates in cochlea. Besides, the otic vesicle-derived inner ear structures are under-developed, with reduced proliferation and premature cell cycle exit during prosensory specification and ectopic hair cells formation in the *Cdo* mutants. It is possible that *Cdo* in Hh signaling is required for inhibiting cells from differentiating into hair cells and specifying progenitor cells to generate the distinctly fated cell populations in the inner ear.

Chanchanok Chaichim – UNSW Sydney

**EFFECT OF TPM3.1 OVEREXPRESSION ON SYNAPTIC FUNCTION AND STRUCTURE**

Chaichim C1,2, Fath T3, Power JM1

1. Translational Neuroscience Facility, School of Medical Sciences, UNSW Sydney, Sydney, NSW 2052, Australia. 2. Neurodegeneration and Repair Unit, School of Medical Sciences, UNSW Sydney, Sydney, NSW 2052, Australia.

The neuronal actin cytoskeleton is crucial for the formation, modulation and maintenance of synaptic connections. Actin fibres are dynamically regulated by several actin-associated proteins, including tropomyosins, which control access of other binding proteins to actin filaments. We have previously shown that expression of tropomyosin isoform Tpm3.1 is increased in the synapses of mice, modelling Alzheimer’s disease pathology. Since Tpm3.1 is thought to limit the binding of the actin filament-severing protein coflin to actin filaments, thereby increasing stability, we theorized that its overexpression
compensates for Aβ-induced synaptic disruption. Here, we used transgenic mice, expressing human Tpm3.1 to determine the impact of increased Tpm3.1 expression on synaptic function. Hippocampal field excitatory postsynaptic potentials (fEPSPs) were recorded in acute brain slices prepared from 42-56 d male transgenic mice and littermate controls. Baseline function, as measured by stimulus-response curve and paired pulse ratio, was unaltered. Synaptic potentiation induced by high frequency stimulation (100 Hz 1s) was greater (p = 0.04; RM-ANOVA) in Tpm3.1 transgenic mice (n = 16) than controls (n = 15), suggesting that Tpm3.1 overexpression facilitates synaptic plasticity. Whole-cell patch clamp recordings of mEPSCs in cultured hippocampal neurons revealed no difference in amplitude or frequency between wild-type and Tpm3.1 overexpressing cells. Morphological analysis showed that dendritic spine number was decreased in Tpm3.1 overexpressing cells (p = 0.02; unpaired t-test, Tpm3.1 Tg n = 33, WT n = 28), but the proportions of spine types was unaltered. These results suggest that overexpression of Tpm3.1 can improve synaptic plasticity without compromising normal function.

Huling Hu · Institute Of Basic Medical Sciences, Chinese Academy Of Medical Sciences & Peking Union Medical College

OVEREXPRESSION OF MIR-130B VIA IN UTERO GENE TRANSFER ACCELERATES NEURONAL MIGRATION IN EMBRYONIC CORTEX AND LEADS TO ADULT BEHAVIORAL DEFICITS

Huling Hu, Hui Wei, Qi Xu
National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences & Neuroscience Center, Chinese Academy of Medical Sciences & Peking Union Medical College

MiR-130b, previously known as an onco-miRNA involved in tumorigenesis, was recently found to be dramatically up-regulated in schizophrenia patients in a state-independent manner, implicating a crucial role as a modulator of complex regulatory networks in brain pathologies. To verify its functional role in neuropsychiatric pathology, we made a first attempt to carry out a temporal profiling of miR-130b coupled with in utero electroporation for comprehensive in vivo phenotypic exploration. We performed TaqMan-based quantitative measurement to characterize miR-130b expression pattern in cerebral cortex of normal mice and two established schizophrenia mouse models at various developmental stages. MiR-130b level in mouse embryos was then manipulated via in utero electroporation for subsequent neuronal migration analysis and a spectrum of schizophrenia-related behavioral tests. MiR-130b was progressively increased as brain developed throughout the embryonic stage and gradually decreased after birth. Further comparative analysis in poly I:C prenatal immune challenge mice and DISC1-cc transgenic mice, demonstrated that miR-130b was up-regulated evidently in embryonic cortex in schizophrenia, with a timeline concordant with neuronal migration trajectory. Transient overexpression of miR-130b in the pre- and peri-natal stages led to accelerated neuronal migration during embryonic brain development and schizophrenia-like behavioral abnormalities, including greater response in methamphetamine-induced hyperactivity and deficits in prepulse inhibition after puberty, indicating a state of dopaminergic hyperfunction and impaired sensorimotor gating in miR-130b-overexpression mice. Our work presents the distinct expression profile of miR-130b in neurogenesis, and demonstrates a novel role for miR-130b in the stage-specific modulation of cortical development and high brain functions in adulthood.

Hanjun Kim · Konkuk University, College Of Veterinary Medicine

INVOLVEMENT OF ENDOCANNABINOID SYSTEM IN NEUROPATHIC PAIN

Kim HJ, Choi EJ, Sah KH, Kim HS, Do SH
Department of Veterinary Clinical Pathology, College of Veterinary Medicine, Konkuk University, Seoul, Republic of Korea

The endocannabinoid system (ECS) is distributed in the nervous system and could be modulated in response to extrinsic stress by altering the expression of cannabinoid receptor (CB) 1 and 2. The aim of this study was to analyze ECS regulation in neuropathic animal models. For this purpose, both CB1 knockout (KO) mice and wild-type (WT) mice were underwent bilateral sciatic nerve crushing injury that the sequential alteration of clinical, histological and genetical expression pattern changes were analyzed. The withdrawal threshold from the electronic von Frey mechanical stimulus decreased from 4 weeks in the KO group than WT group to the endpoint of experiments (p<.01). In addition, histological examination showed that neurodegenerative features such as hydropic changes, inflammatory cell infiltration, and axonal myelin damage were reduced in the KO group. Furthermore, KO group showed upregulation of the S100B, NCAM, MBP, FAAH, MAGL at 2 weeks (p<.01) and showed a relatively constant expression levels until 6 weeks. However, in the WT group, nerve regeneration-related gene expression level alterations were delayed that S100B, MBP, MAGL levels were observed at 6 weeks.
Immunofluorescence staining results also showed that the number of S100, MBP and FAAH positive axons were increased from 2 weeks in the KO groups to the 6 weeks post-operation. The result indicates that deficiency of CB1R could contribute to attenuate hyperalgesia in mechanical sensitivity as well as the upregulation of neuro-regeneration and endocannabinoid biodegradation enzymes. Importantly, our findings provide preliminary evidence of the role of the CB1R to regulate the neuropathic pain. (250 words)

Acknowledgements: This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2017R1A2B3006213)

Gabriela Visini – University of Otago

**LONG TERM BEHAVIOURAL DEFICITS IN THE LONG EVANS RAT FOLLOWING A SINGLE BINGE EXPOSURE TO ETHANOL DURING LATE FETAL BRAIN DEVELOPMENT.**

**Visini G, Napper RMA.**

Anatomy Department, School of Biomedical Sciences, and Brain Health Research Centre, University of Otago, Dunedin, New Zealand.

Exposure to alcohol prenatally may result in Fetal Alcohol Spectrum Disorder (FASD), which is the leading preventable cause of central nervous system dysfunction worldwide. The current study used a rat model of alcohol binge exposure. On postnatal day 6, Long-Evans rat pups were exposed to either alcohol, at a daily dose of 6.0g/kg ethanol, via gastric intubation - alcohol-exposed condition, or a sham intubation - intubation control condition, or received no treatment - suckle control condition. This alcohol regimen results in a permanent loss of neurons in the CA1 region of the hippocampus and other brain regions such as the prefrontal cortex. At 15-21 months of age, rats were tested on the reference memory version of the radial arm maze (RAM) and the spontaneous alternation version of the T-maze. Animals were housed under reversed lighting and all testing was carried out in low light, during the dark phase of the light cycle. Alcohol-exposed (AE) animals took significantly longer to reach criterion than controls (P<0.05), and made more errors in testing on the RAM. In the T-maze, AE animals alternated significantly less than controls on the first day but there was no difference in total errors over the 8 days of testing. Alcohol as a single binge, during a period equivalent to the mid third trimester of human fetal brain development, can result in long-lasting learning and memory deficits in rats. This data supports the public health recommendation for total abstinence from alcohol throughout pregnancy to reduce the prevalence of FASD.

Yunan Ye - Queensland Brain Institute

**NFIB REGULATES RADIAL GLIAL CELL PROLIFERATION AND DIFFERENTIATION BY REPRESSING HMGA2 DURING CORTICAL DEVELOPMENT**

**Yunan Y1, Lim JWC1, Bunt J1, Richards LJ1,2. 1Queensland Brain Institute, the University of Queensland, Brisbane Australia. 2School of Biomedical Sciences, the University of Queensland, Brisbane, Australia.**

The Nuclear Factor One B (Nfib) is a transcription factor important for maintaining balanced radial glial cell proliferation and differentiation during corticogenesis. Nfib knockout embryos exhibit an enlarged and immature ventricular zone, accompanied by a delay in the production of intermediate progenitors and neurons. We investigated the downstream effectors of NFIB during radial glial proliferation and differentiation. Gene expression analyses using both RNA sequencing and real-time qPCR revealed robust upregulation of another transcriptional regulator, Hmga2, in Nfib knockout cortices. In vivo, NFIB and HMGA2 are expressed in reciprocal gradients throughout the developing cortex, and are co-expressed in the ventricular zone. Quantification of HMGA2 protein expression using immunofluorescence also revealed a concomitant increase in HMGA2 protein in Nfib knockout ventricular zones compared to their wildtype littermates. These results suggested that NFIB may repress Hmga2 during normal embryonic development. ChIP-qPCR further validated HMGA2 as a direct bona fide downstream target for NFIB. Our results expand on the regulatory network underlying cortical development by demonstrating a direct inhibitory effect of the transcription factor NFIB on Hmga2. During normal development, NFIB drives self-renewing radial glial cells down the neurogenic and gliogenic pathways. When this inhibitory regulation is removed by Nfib deletion, HMGA2 level increases and favours self-renewal of radial glial cells.

Yu Shen Yin – University of Sydney
CHARACTERISATION OF HIPPOCAMPAL NEURONAL DEGENERATION AND REPOPULATION FOLLOWING ACUTE KAINIC ACID-MEDIATED EXCITOTOXIC INJURY

Authors: Yin, Y\textsuperscript{1,2}, Konen, L\textsuperscript{2}, Vaughan, CW\textsuperscript{1}, Vissel, B\textsuperscript{2}.

1. Kolling Institute of Medical Research, University of Sydney, SYDNEY AUSTRALIA. 2. Faculty of Science, University of Technology Sydney, SYDNEY AUSTRALIA.

Excitotoxicity is a contributing factor to a variety of neurodegenerative disorders and acute brain injuries. Additionally, neuronal repopulation outside of well-established neurogenic regions is highly controversial. The assumption that the CNS has a poor capacity for recovery after injury has led to the view that only exogenous interventions can drive CNS repair. The present study aims to characterise the kainic acid (KA) model of acute excitotoxic injury to investigate the extent to which spontaneous neuron repopulation of the CA3 dorsal hippocampal regions is possible. Significant loss of neurons was observed in the CA3 region ipsilateral to the site of injury following intracerebroventricular (ICV) injection of kainic acid (KA) at 1μg/μl (p<0.01), 1.2μg/μl (p<0.01), and 1.5μg/μl (p<0.0001), when compared to vehicle-treated controls at 2 weeks post-injection (wpi). At 8wpi, CA3 neuronal population was significantly different from vehicle-treated controls following 1.2μg/μl (p<0.0005) and 1.5μg/μl (P<0.0001) of KA. However, the CA3 neuronal population following 1μg/μl of KA was comparable to vehicle-treated controls at 8wpi, suggesting repopulation of the CA3 region. The results from the present study suggest the existence of intrinsic mechanisms for spontaneous repair and recovery within the brain in the absence of exogenous interventions. By providing the framework for understanding the extent to which spontaneous repopulation can occur in the brain unassisted, we hope to inform current and future therapies for treatment of acute brain injuries and disorders.

Fatemeh Zanganeh - Florey Institute Of Neuroscience and Mental Health

CHARACTERISING THE SPECIFICITY OF GFP LABELLED MOTOR NEURONS IN THE HB9::GFP REPORTER MOUSE LINE DURING DEVELOPMENT

Zanganeh F\textsuperscript{1}, Bye CR\textsuperscript{1}, Turner BJ\textsuperscript{1}

1. Motor neuron disease laboratory, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Victoria, Australia

The spinal cord motor neuron (MN) population consists of three different subtypes; α, β and γ-MNs which are classified based on morphology and the muscle fibers they innervate. A transgenic reporter mouse expressing GFP driven by the HB9 promoter (HB9::GFP), labels dendrites, axons and somas of MNs. Here we have investigated the identity and specificity of GFP labelling in MNs throughout different developmental stages of this HB9::GFP mouse.

Spinal motor neurons were isolated from HB9::GFP mice (n=6) using FACS followed by qRT-PCR to identify specific cell markers. The isolated cell population showed high expression of MN specific gene markers while β-MN (Err3) and interneuron gene markers were absent during embryonic and perinatal ages. Immunohistochemistry showed the majority of HB9::GFP positive MNs did not co-express with commonly used mature MN markers (ChAT) during embryonic development, however, from postnatal day 20 this expression appears localised to α motor neurons. These observations suggest that during embryonic and perinatal ages HB9::GFP expression is observed in immature α MNs which do not yet express many of the mature motor neuron markers. A significant reduction in GFP-expressing MNs is seen over this time and most likely reflects network maturation within the spinal cord.

The ability to specifically label α MNs has broad applications in studying MN development and identity as well as vulnerability to neurodegeneration. For instance, determining how and when different MN subtypes degenerate in motor neuron disease may give clues to better understanding of disease pathogenesis and lead to more effective treatment interventions.

Sean Coakley - Queensland Brain Institute, The University Of Queensland

THE EPIDERmis PROTECTS SENSory AXONS FROM DEGENERATION

Sean Coakley, Fiona Ritchie and Massimo A. Hilliard.
Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Australia.
Maintenance of neuronal integrity is essential for the preservation of correct neuronal function. Sensory neurons and their axons are subject to continuous mechanical stress due to their location within the skin, muscles, and moveable joints. Despite the strong forces experienced, these neurons are able to maintain their structure and functional circuitry. The ability to resist strain has been shown to be a combination of intrinsic and extrinsic protection mechanisms, but the precise interplay between cell autonomous and cell-non-autonomous stress resistance is not known. Mutations in C. elegans β-spectrin/unc-70 cause spontaneous axonal breakage due to mechanical strain. Through an unbiased forward genetic screen using unc-70 mutants as a sensitised background, we have identified a novel mutant allele of the conserved gene tbc-10, which results in enhanced axonal damage to the PLM mechanosensory neuron. TBC-10 is a Rab-GTPase-activating protein that we demonstrate localises to the membrane of the hypodermis surrounding the PLM axon and functions non-cell-autonomously within this tissue to exert an axonal protective effect via inactivation of the conserved small GTP-ase RAB-35. Inactivation of RAB-35 within the hypodermis, by either expression of a GDP-locked RAB-35 or a loss of function mutation, is sufficient to rescue the enhanced axonal breakage phenotype in tbc-10 mutants. We show that in C. elegans the epidermis acts to protect the axons of mechanosensory neurons from spontaneous degeneration induced by disruption of the spectrin network, demonstrating a crucial role for non-neuronal support cells in maintaining an intact and functional nervous system.

Robert Gasperini – University of Tasmania

STIM1 REGULATES THE REMODELLING OF ENDOPLASMIC RETICULUM AND MOTILITY IN STEERING GROWTH CONES

The spatial control of calcium signals is crucial for axon guidance, however it is unclear how calcium signalling regulates cytoskeletal reorganisation to alter growth cone motility and steering. We have previously shown that stromal interaction molecule (STIM1) regulates store operated calcium entry (SOCE) in response to both calcium-dependent and independent guidance cues. In this study, we sought to understand how STIM1, an ER-lumenal protein, could regulate growth cone steering in response to such a diverse range of guidance cues. Given that STIM1 interacts with microtubules through interactions with end-binding (EB) proteins, we asked whether STIM1 couples spatiotemporal dynamics of ER membranes with cytoskeletal reorganisation in response to guidance cues in vitro. We demonstrate that STIM1 is required to steer rodent sensory growth cones and that STIM1-EB interactions are required to target microtubule polymerisation and ER into filopodia. These data suggest that STIM1 and the ER act to stabilise the microtubule cytoskeleton, thus providing a mechanism for the dynamic translocation of ER to the turning, or protruding side of the growth cone. Significantly, STIM1 was necessary for microtubule and ER protrusion into filopodia, suggesting that STIM1-EB interactions represent a direct connection between guidance cue-derived calcium signals and the growth cone cytoskeleton, thus providing a new model for regulating calcium signals in during axon guidance.

Patricia Jusuf – University of Melbourne

REGENERATION OF RETINAL NEURONS IN THE ABSENCE OF THE MÜLLER GLIA STEM CELL POPULATION IN ZEBRAFISH

Krylov A1, Ng Chi Kei N2, Jusuf PR1–2

1School of Biosciences, University of Melbourne, Australia
2Australian Regenerative Medicine Institute, Monash University, Australia

Neural regeneration in the central nervous system including the retina is relatively limited in mammals, whilst being highly efficient in lower vertebrates. Across vertebrates, the same five retinal neuron types and Müller glia are generated developmentally by conserved gene expression patterns, suggesting a common foundation for neurogenesis.

Using retinal injury models in zebrafish, regenerative process can be tracked, and contributing cells and genes identified. Following a variety of retinal injuries, resident Müller glia have been shown to de-differentiate into stem-cell like progenitors, proliferate and regenerate neurons. However, whether other cells can contribute remains ill-defined.

This study investigates regenerative processes in the absence of Müller glia to identify and quantify relatively contributions of other cells. A Notch inhibitor (swimming exposure) was used to block Müller glia development. Using a nitroreductase enzyme expressed only in horizontal and amacrine interneurons, metronidazole (swimming exposure) was converted into a cytotoxin specifically ablating these neurons.

We identified an early stage, in which neuronal regeneration occurred at a comparable rate in the absence of Müller glia,
suggestive of contributions of alternate cells. At later stages regeneration did not reach the same efficiency, demonstrating Müller glia contributions. Early regenerated cells arose from non-proliferative cell sources. Thus, novel endogenous cells may contribute to efficient neural regeneration. If these are common to vertebrates, they represent a new target that could be activated to improve mammalian neural regeneration.

Il-Soo Moon - Depart Of Anatomy, Dongguk Medical School

MOONLIGHTING FUNCTION OF GlcNAc KINASE: NEURITOGENESIS AND REMOVAL OF AGGREGATES

Il Soo Moon

Department of Anatomy, Dongguk Univ Graduate School of Medicine, Gyeongju 38066, Republic of Korea

*N*-acetylglucosamine kinase (GlcNAc kinase or NAGK) is a *N*-acetylhexosamine kinase that belong to the sugar kinase/heat shock protein 70/actin superfamily. NAGK produces GlcNAc-6-phosphate, the main intermediate for UDP-GlcNAc production, in a salvage pathway, and so produced, energized GlcNAc moiety of UDP-GlcNAc is used in the synthesis of O-/N-glycans, sialic acids, and O-GlcNAc. Data from my lab, however, showed moonlighting functions of NAGK. NAGK was expressed at high level in neuron. Overexpression upregulated the cytoarchitectural complexity, whereas knockdown of NAGK by shRNA resulted in degeneration of axon and dendrites. Interestingly, mutant NAGK, which completely lost kinase activity, also promoted cytoarchitecture. The small domain exhibited a dominant-negative effect, similar to shRNA. These phenomena indicate a moonlight structural role, irrelevant of its kinase activity. We further found by yeast two-hybrid, immununocytochemistry and PLA that NAGK interacts with dynine light-chain roadblock type 1 (DYNLRB1). Indeed, interruption of this interaction by the ectopic introduction of a small peptide derived from the C-terminal amino acids of DYNLRB1 resulted in the stunting of dendrites and axons of hippocampal neurons in culture. NAGK-dynein interaction also promoted diverse functions of dynein motor such as cell division, neuronal and non-neuronal cell migration. Importantly, we found that NAGK effectively remove intracellular aggregates such as htt and a-synuclein. Our data indicate that NAGK is an accelerator of dynein motor and suggest its application in the prevention and cure of neurodegeneration.

Pavitha Parathan – The University of Melbourne

CHANGES IN SUBMUCOSAL NEURONAL SUBTYPES BETWEEN WEANING AND ADULTHOOD IN MICE

Parathan P1, Wang Y1, Foong JPP3

3. Department of Physiology, The University of Melbourne, Parkville, Victoria

The enteric nervous system (ENS) of the gut consists of two major plexuses (myenteric plexus, MP; submucosal plexus, SMP). Development of the MP has been extensively studied and several properties of myenteric neurons are still developing during early postnatal stages. While there is evidence for development to continue over a protracted time, postnatal development of both plexuses, especially the SMP is poorly understood. Here, we used standard immunohistochemistry techniques to compare the ENS of mice just before weaning (pre-weaner, postnatal day, P14-21) and adulthood (P42-47). MP and SMP preparations were acquired from the duodenum and colon. The proportions of Hu+ neurons that contained several subtype markers were determined. Submucosal neurons of both gut regions were mainly immunoreactive for vasoactive intestinal peptide (VIP) or choline acetyltransferase (ChAT). Some ChAT+ neurons were also calcitonin gene-related peptide+ (CGRP+). Neurofilament M (NFM)-immunoreactive cell bodies in SMP rarely contained ChAT and were found in the colon only. There were significantly more duodenal CGRP+ neurons (n=3-4, p<0.02), colonic VIP+ (n=3-4, p<0.02) and NFM+ (n=3, p<0.02) submucosal neurons in adults than in preweaners. In the myenteric plexus, while there is a significant decrease in neuron density (n=5-7, p<0.001), there were no changes in neuronal nitric oxide synthase+ and/or NFMT+, Calbindin and/or Calretinin+ and ChAT+ neuronal proportions between the two age groups. Overall, we found significant changes in the chemical coding of submucosal but not myenteric neurons between weaning and adulthood.

Xin-fu Zhou – University of South Australia

CONVERSION OF HUMAN FIBROBLASTS INTO NEURAL STEM CELLS AND NEURON-LIKE CELLS BY SMALL MOLECULES

Dong-Hui Liu1-2, Nimshitha Pavathuparambil Abdul Manaph1, Mohammed Al-Hawaas1, Larisa Bobrovskaya1, Fiona H
Neural stem cells (NSC) and neurons are potential therapeutic cells useful for the regeneration of damaged nervous system. NSC can be generated by differentiation from embryonic stem cells or induced pluripotent stem cells, or by reprogramming somatic cells with transcription factors. However, these cells may be associated with mutagenesis and tumour formation due to incomplete differentiation or viral infection-induced genome instability. In a previous study, we have successfully generated mouse neural stem cells (iNSC) with only small molecules. In the present study, we aim to convert human fibroblasts into iNSC or neuron-like cells (iN) by reprogramming of small molecules with high efficiency. In 2-3 weeks, human fibroblasts treated with 5-6 small molecules displayed Sox-2 positive NSC like spheres in a suspension culture or rosette-like NSC morphology in adhesive monolayer culture. These cells could differentiate into GFAP-positive astrocytes, oligo-2 positive oligodendrocytes or NeuN positive neuron like cells autonomously or under the differentiation conditions. In addition, fibroblasts can be reprogrammed into neuron-like cells (iN) with neuronal morphology expressing Tuji 1 and NeuN by a group of small molecule affecting different signal pathways within a week. On-going experiments will test whether these cells can survive in vivo and have physiological functions. Therefore, iNSC and iN will become safer cells for repairing injured nervous system utilized in various applications as a part of precision medicine.

The ubiquitin proteasome system (UPS) is an essential post-translational modifying mechanism which is increasingly implicated in both development and disorders such as motor neuron diseases. We investigated the susceptibility of induced pluripotent stem cell (iPSC)-derived motor neurons and derivative cells stages including iPSCs and fibroblasts to UPS stress, and subsequently, UBA1 inhibition via PYR41 treatment. UBA1 inhibition at a high concentration (10 µM PYR41) significantly decreased early stage neurite outgrowth in motor neuron precursors and was cytotoxic upon repeated doses; Long-term low level UPS stress (1 µM PYR41) was found to significantly decrease motor neuron viability over a four week treatment. Surprisingly, iPSC were extremely susceptible to UBA1 inhibition which caused total cell death in less than 16 H at 1 µM PYR41. Together these results indicated the UPS is fundamental in the generation of iPSC-derived motor neurons.

We therefore sought to map the changing ubiquitinated proteome (ubiquitome) in cultured motor neurons and these same derivative cell stages. We have identified ~1500 ubiquitinated proteins across various functional pathways including neuron differentiation, cell cycle and the UPS itself. These findings indicate a significant role for ubiquitin in the regulation of a wide variety of cellular mechanisms essential to these cell types. Studying these changes could further improve motor neuron modelling, an essential task for therapeutic development for a range of devastating motor neuron diseases.

In the rat visual system, previous studies have provided evidence that developing RGCs depend on local neurotrophins for their survival until innervation of central targets, at which time there is a switch to target-derived neurotrophin dependence. Our study investigated if this switch was reliant on the action of the major receptor for brain-derived neurotrophins.
neurotrophic factor (BDNF), the tropomyosin receptor kinase B (TrkB). Time mated pregnant Wistar rats were injected with 5-Bromo-2’-deoxyuridine (BrdU) at embryonic day 18 (E18) of gestation, to pre-label RGCs born on that day. Pups were euthanised at birth and their RGCs isolated, pooled and seeded into culture wells containing growth media with or without BDNF and with or without a TrkB-Fc chimera (biosensis), which reduces neurotrophin binding to the receptor. Each set of cultures was incubated for 24hrs before fixing and immunohistochemistry to label RGCs (βIII-Tubulin) and E18 cohort (BrdU). βIII-Tubulin+ RGCs were quantified as BrdU positive (+) or negative (−), and with or without visible processes. After 24hrs, the proportion of surviving BrdU+ RGCs was least in the BDNF/TrkB-Fc+ condition. The percentage of double-labelled RGCs with processes was increased in the BDNF+/TrkB-Fc− (71%) wells when compared to the BDNF+/TrkB-Fc+ (57%) and BDNF−/TrkB-Fc+ (45%) groups, and significantly increased compared to the BDNF+/TrkB-Fc− (42%; p=0.05, Mann-Whitney U) condition. These data support the proposal that late-born RGCs with axons not yet in central targets rely on endogenous BDNF for viability, and when TrkB action is blocked, maturation of these neurons is also affected.

Zarina Greenberg – University of South Australia

**14-3-3ζ REGULATES NON CANONICAL SHH SIGNALLING TO CONTROL CORTICAL INTERNEURON DEVELOPMENT.**

**Greenberg Z, Ramshaw H, Xu X, Schwarz Q**

Centre for Cancer Biology, University of South Australia, Adelaide, South Australia

Dysfunction in the formation and function of GABAergic cortical interneurons has been implicated as a central pathogenic mechanism in schizophrenia. 14-3-3ζ is part of a family of highly conserved intracellular proteins, that bind to the phosphoserine/threonine sites on target proteins and is highly expressed in the brain. Several findings in recent years implicate 14-3-3ζ as a candidate risk factor for schizophrenia including: 1) 14-3-3ζ is downregulated in post-mortem schizophrenic brain samples at the mRNA level; 2) 14-3-3ζ is downregulated across multiple neuroproteomic studies on schizophrenia patient samples; 3) linkage studies have implicated 14-3-3 family proteins in numerous neurodevelopmental disorders, and 4) genetic mutations in the gene encoding 14-3-3ζ have been found in schizophrenia patients. Previous studies have shown that 14-3-3ζ KO mice exhibit anatomical and behavioural traits akin to those seen in schizophrenia. Here we expand on previous findings by identifying a novel role for 14-3-3ζ in interneuron development. A key observation of this study was a subtype specific reduction in parvalbumin expressing interneurons throughout the cortex of 14-3-3ζ KO mice. Through a series of molecular, biochemical and morphological studies and analyses of unique mouse mutants I identified defects in the specification and formation of interneurons during early brain development. Furthermore, my data fits with the notion that 14-3-3ζ regulates the non-canonical Shh signalling pathway via Rac1 to control interneuron development. Taken together, this work provides novel insight into the role of 14-3-3ζ in controlling interneuron development and hence identifies a novel role of 14-3-3ζ in the pathogenesis of schizophrenia.

Sarah Kerwin - School Of Biomedical Sciences, The University Of Queensland

**REGULATED ALTERNATIVE SPlicing OF DROSOPHILA DSCAM2 IS NECESSARY FOR ATTAINING THE APPROPRIATE NUMBER OF PHOToreceptor SYNAPses.**

**Kerwin SK1, Li JSS1,2, Shin GJ1,2, Noakes PG1,2, Millard SS2.**

1. School of Biomedical Sciences, Faculty of Medicine, The University of Queensland, Brisbane, QLD 4072, Australia. 2. Queensland Brain Institute, The University of Queensland, Brisbane, QLD 4072, Australia. 3. Current address: Physiology and Cellular Biophysics, Columbia University Medical Center, New York, NY 10032, USA. 4. Co-first authors.

Neuronal cell-specific alternative splicing increases the diversity of brain wiring proteins and plays a role in both the organization and the function of neural circuits. Whether cell-specific isoform expression functions in the context of synapse formation, however, is not known. In Drosophila, photoreceptor terminals synapse with four postsynaptic neurons, two of which are invariably L1 and L2. These neurons express distinct isoforms of Dscam2 that mediate isoform-specific homophilic repulsion. We previously proposed that this expression pattern is necessary for preventing repulsive interactions between L1 and L2 that could otherwise disrupt the synapse. Here, we find that the number of synapses and the complexity of postsynaptic dendrites were reduced in flies that express only one isoform. This reduction in synapses was most apparent upon observing a decrease in presynaptic T-bar structures via electron microscopy (p<0.0001). Our data support that these defects result from inappropriate interactions between L1 and L2 dendrites. We conclude that cell-type specific Dscam2 alternative splicing is necessary for the proper assembly of photoreceptor synapses.

Min Kim – Monash University
TARGETING EAE-INDUCED DEMYELINATION AND AXONAL PATHOLOGY BY TRANSPLANTING HAEMATOPOIETIC STEM CELLS THAT OVEREXPRESS NGR(310)ECTO-FC FUSION PROTEIN

Kim MJ1, Lee JY1, Thomas S1, Kang JH1, Bedford T1, Petratos S1.

1Central Clinical School, Monash University, Prahran, Melbourne, Australia

Haematopoietic stem cell (HSC) transplantation is currently being trialed to treat multiple sclerosis (MS) as a means of modulating autoimmuno-mediated inflammation and neurological disability. As MS is an immune-mediated neurodegenerative disorder, immunomodulation may not be effective for progressive neurodegeneration. Nogo receptor 1 (NgR1) is a high affinity receptor for myelin-associated inhibitory factors (MAIFs) that block for neurite outgrowth and may potentiate axonal degeneration in an animal model of multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE). As MS and EAE exhibit large numbers of inflammatory cell infiltrates within central nervous system (CNS) lesions, we utilised transplantable HSCs as a cellular delivery method of the NgR(310)ecto-Fc fusion protein. We have shown that we can deliver the specific NgR(310)ecto-Fc fusion protein through the transplantation of lentivirus (LV)-transduced HSCs that encode the NgR-Fc protein to sites of EAE pathology. We exclusively identified macrophages that were positive for the myc-tag (NgR-Fc-positive) occupying significant areas of inflammation and demyelination (2.0 x 10^3 ± 0.5 x 10^3 cells/mm², p<0.0001), signifying the engulfment of NgR-Fc protein-MAIF complex by activated macrophages, which may increase the phagocytic activity of these populations and enhance repair. Importantly, n=3 animals transplanted with therapeutic NgR(310)ecto-Fc overexpressing HSCs, were rescued from symptoms associated with EAE. These results suggest that HSCs can be utilised as carriers of the therapeutic protein for specific delivery into EAE lesions and can potentiate neurological recovery.

Hannah Leeson – Griffith University

P2X7 RECEPTOR REGULATION OF ADULT HIPPOCAMPAL NEURAL PROGENITOR CELLS

Leeson HC1, Kasherman MA1, Chan-Ling T2, Lovelace MD2, Brownlie JC3, Toppinen KM1, Gu BJ4, Weible MW1,2,3

1. Griffith Institute for Drug Discovery, Griffith University, Nathan, QLD. 2. Bosch Institute, The University of Sydney, Camperdown, NSW. 3. School of Natural Sciences, Griffith University, Nathan, QLD. 4. Florey Institute of Neuroscience and Mental Health, Melbourne University, Parkville, VIC.

Identifying the signalling mechanisms that regulate adult hippocampal neurogenesis is an essential step towards understanding how new neurons are generated and integrated into existing cytoarchitecture. Here we examine the roles of the P2X7 receptor, a purinergic calcium channel, in regulating both neural progenitor proliferation and phagocytosis of apoptotic immature neurons. Primary cultures of hippocampal neural progenitor cells were characterised using immunocytochemistry, and functional activity of P2X7 receptors was demonstrated using calcium influx and ethidium bromide uptake assays, both canonical functions of this receptor. Live cell confocal microscopy revealed hippocampal neural progenitors as capable of phagocytosing fluorescent latex beads, and flow cytometry in conjunction with specific inhibitors indicated P2X7 receptors as capable of facilitating this phagocytosis. Finally, P2X7 receptors were activated with bzATP and the thymidine analogue EdU was used to observe a significant dose-dependent relationship between concentration and proliferation. Evidence presented here demonstrate that P2X7 receptors can function as scavenger receptors in the absence of ATP allowing neural progenitors to phagocytose their apoptotic peers during neurogenesis as well as governing rates of proliferation, possibly by regulating calcium dependent transcription factor activation. Taken together, these data present a dual role for P2X7 receptors during adult neurogenesis. Given the crucial role neurogenesis plays in the hippocampus, dysregulation may lead to memory deficits and both neurological and psychological disorders. Our research is the first to fully characterise the dichotomous signalling roles of P2X7 receptors in adult hippocampal neural progenitor cells.

Joshua Li – The University of Queensland

NEURONAL CELL-TYPE-SPECIFIC ALTERNATIVE SPlicing OF DROSOPHILA DSCAM2 IS REGULATED BY MUSCLEBLIND

Li JS, Millard SS.
**The University of Queensland, School of Biomedical Sciences, Brisbane, Australia.**

Alternative splicing (AS) generates proteome diversity much needed in the nervous system, where a limited set of genes specifies more than 10^{15} neuronal connections. If AS plays a role in specifying neuronal connections, it needs to be regulated in a cell-type-specific manner. Recent work from our lab has shown that this is the case for the cell recognition molecule, Dscam2. We demonstrated that two alternative isoforms of Drosophila Dscam2 are expressed in distinct neuronal cell types and that this expression pattern is crucial for proper neuronal wiring. In our current work, we sought to identify the mechanism underlying Dscam2 cell-type-specific AS. In an RNAi screen, we knocked-down ~160 RNA binding proteins and identified muscleblind (mbl) as a regulator of Dscam2 AS. Mbl loss of function derepresses one Dscam2 isoform (p<0.001), whereas mbl overexpression activates the other (p<0.001). Analysis of mbl expression suggests that it is regulated in a cell-specific manner that correlates with the repressed Dscam2 isoform. Understanding the mechanism underlying cell-type-specific Dscam2 AS could give us insights into how functional diversity is achieved by the proteome.

Sonja Meier – The University of Queensland

**P75 NEUROTROPHIN RECEPTOR FUNCTION IN CORTICAL NEUROGENESIS**

Meier S¹, Alfonsi F², Coulson EJ¹-²

¹Queensland Brain Institute, ²School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, Australia

During development, the p75 neurotrophin receptor (p75NTR) is widely expressed in the nervous system where it regulates neuronal differentiation, migration and axonal outgrowth, and mediates the survival and death of newly born neurons. Activation of p75NTR by neurotrophin binding and association with co-receptors can induce a variety of different downstream signalling pathways with functional outcomes being dependent on both timing and cellular context. To date, most studies investigating p75NTR focussed on the peripheral nervous system leaving its function in the developing brain largely unexplored. We show that p75NTR knockout in neural progenitors in K nestin-Cre; p75NTR flox/flox mice causes a severely impaired brain phenotype with significantly thinner cortices and reduced number of cortical interneurons. During embryonic development, apoptosis of neuronal progenitors is greatly increased suggesting that p75NTR functions in the survival of these cells. Using two novel conditional knockout strains, we show that progenitors of cortical parvalbumin positive interneurons are particularly vulnerable to loss of p75NTR. Furthermore, preliminary results suggest that the apoptotic cells are mainly intermediate progenitors located in the subventricular zone, and that their ability to undergo neurogenic divisions may be reduced. In order to elucidate the precise mechanism by which loss of p75NTR induces cell death, we are investigating the rate of neurogenesis, neuronal lineage progression and neuronal migration in these conditional p75NTR knockout strains. Studying the role of p75NTR in cortical progenitors will lead to a better understanding of the complex process of corticogenesis and shed light on the function of p75NTR in the developing brain.

Zan-min Song – Australian National University

**THE EFFECTS OF ENDOTHELIN RECEPTOR B DEFICIENCY ON THE DEVELOPMENT OF THE NEURAL CREST-DERIVED CAROTID BODY.**

Wooff Y¹, Crooker GDH², Song Z-M¹-³

¹. Eccles Institute of Neuroscience, John Curtin School of Medical Research, Australian National University, ACT.  2. Department of Paediatric Surgery, Canberra Hospital, ACT.  3. Medical School, Australian National University.

The carotid body is a neural crest-derived chemoreceptive organ that plays an important role in respiratory control. Endothelin receptor B (EDNRB) is known to control the development of some cell types derived from the neural crest, however, the role of EDNRB in the development of the carotid body has not been explored. Using a Wistar–Imamichi rat strain with a spontaneous null mutation of EDNRB, we characterized the major cell types of the neonatal carotid body by immunohistochemistry and investigated the effects of EDNRB mutation on cell proliferation and apoptosis. Proliferating cells in wildtype (+/+), heterozygous (+/sl) and homozygous (sl/sl) rats were labelled by intraperitoneal injection of 5-bromo-2′-deoxyuridine (BrdU) at postnatal day three, while cell apoptosis was assessed using terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay at postnatal day four. We identified a novel population of cells in the neonate, which expressed both tyrosine hydroxylase (TH) and glial fibrillar acidic protein (GFAP), typically considered to be specific markers of two separate cell types in the adult carotid body, Type-I and Type-II cells respectively.
We also demonstrated that EDNRB played a role in both the proliferation and apoptosis of cells in the carotid body, with a significantly reduced density of proliferating cells (P≤ 0.01) and a significantly increased density of apoptotic cells (P≤0.01) in sl/sl rats compared with +/- littermates. These findings show that EDNRB plays a role in the development of the carotid body and it is likely that mutations in EDNRB will have functional consequences on respiratory control.

Zan-min Song – Australian National University

INCREASED CELL DEATH IN THE BRAIN OF ADULT RAT MODEL OF HIRSCHSPRUNG’S DISEASE

Xie D1,3, Croaker GDH, Song Z-M1
1. John Curtin School of Medical Research AND Medical School, Australian National University, ACT. 2. Department of Paediatric Surgery, Canberra Hospital, ACT. 3. Beijing Friendship Hospital, Capital Medical University, Beijing, China.

Hirschsprung’s disease (HSCR) is a congenital malformation characterized by the absence of enteric ganglia in the distal intestine and subsequent gut obstruction. A subpopulation of HSCR patients is associated with neurological anomalies. Little is known about the cellular changes in the brains of HSCR patients due to the lack of human tissues. We studied a rat model of HSCR, known as spotting lethal (sl/sl) rats, which carry a null mutation in endothelin receptor B (EDNRB) and manifests a similar phenotype as human HSCR. Sl/sl rats were rescued from premature death by colostomy surgery so they lived to 5-6 weeks. The density of apoptotic nuclei was examined in several brain regions by using terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling assay in wildtype (+/+), heterozygous (+/sl) and homozygous (sl/sl) rats. The density of -positive-cells in the cerebellum of sl/sl rats was significantly increased compared to the +/- and +/sl rats. TUNEL-positive-cells were mainly distributed in the molecular layer and granular cell layers in all genotypes. Similarly, The density of -positive-cells in the hippocampus of sl/sl rats was significantly increased compared to the +/- and +/sl rats. In contrast, no significant differences in the density of TUNEL-positive-cells were revealed in the cerebral cortex. These results suggest that ENDRB mutation has long lasting effect on cell survival in the cerebellum and hippocampus of adult sl/sl rat. Our findings will lead to a better understanding of cellular changes in the brains of HSCR patients with similar congenital ENDRB mutation.

Natasha Gabay – University of Sydney

SPATIOTEMPORAL PROPERTIES OF EIGENMODES OF BRAIN ACTIVITY: A NEURAL FIELD ANALYSIS

Natasha C. Gabay1,2, Peter. A Robinson1,2, Tahereh Babaie1,2
1. School of Physics, University of Sydney, NSW, Australia. 2. Centre for Integrative Brain Function, University of Sydney, NSW, Australia

Modern neuroimaging techniques (such as fMRI and EEG) detect large-scale brain activity and are used to infer the underlying architecture and organization of the brain. Many studies rely on statistical data analysis techniques that do not take into account the physiological nature of the data. Neural field theory is a physiologically-based model that averages over brain microstructure and is ideally suited to deriving large-scale brain dynamics. These eigenmodes of brain activity (natural modes of neural activity on the cortex) are the basic building blocks of all cortical dynamics. We provide methods for deriving spatiotemporal eigenmode properties, and apply these to the 9 lowest-energy eigenmodes within a widely-verified corticothalamic neural field theory. Previous numerical results showed that the spatial eigenmodes are close analogues of natural modes of a sphere, despite heavy cortical folding. Motivated by this similarity, cortical folding was treated as a perturbation from an initially spherical geometry in order to calculate eigenmodes. We found close agreement with numerical solutions of the neural field equations, which suggests that cortical folding can be considered a first-order perturbation to a spherical topology and enables tools and insights from the well-understood theory of spherical harmonics and perturbation theory to be applied to brain dynamics. We further found that gross cortical shape, rather than finer-scale gyri and sulci, is what determines the overall orientation of dominant eigenmodes. This can explain the inter-subject consistency of resting state networks, because although relative locations of gyri and sulci between individuals differs, the gross shape of the cortex is fairly consistent.

Alastair Loutit – UNSW
Sensory Systems

Helen Beard – SAHMRI

TEMPORAL PATHOLOGICAL CHANGES IN THE EYE OF A MOUSE MODEL THE NEURODEGENERATIVE LYSOSOMAL STORAGE DISORDER SANFILIPPO SYNDROME (OR MPS IIIA).

Beard H1, Winner LK2, Douglass ML1, Trim PJ1, Snel MF1, Chidlow G2, Casson RD2, Hemsley KM1.
1 Lysoosomal Diseases Research Unit, South Australian Health and Medical Research Institute, 2 South Australian Institute of Ophthalmology, Hanson Institute Centre for Neurological Diseases.

Sanfilippo syndrome (Mucopolysaccharidosis type IIIA; MPS IIIA) is a rare, inherited, neurodegenerative lysosomal storage disorder. Normal, early development is followed by progressive neurocognitive decline resulting in a significantly shortened lifespan. There is no treatment. MPS IIIA brain pathology is well documented and while visual impairment is a common clinical feature, changes in the retina and optic nerve have not been fully explored. Using a mouse model of MPS IIIA and age-matched controls, we examined temporal-pathological and biochemical changes in the eye. Analysing stained sections of the retina at 3, 6, 9, 12, 15, 22 and 25 weeks of age (n=5-6/age/genotype), we found reduced total thickness of the MPS IIIA mouse retina by 22 weeks of age (p<0.01; c.f. unaffected). There was progressive thinning of the outer nuclear layer, first detectable at 12 weeks of age (p<0.05) and shortening of photoreceptor outer and inner segments by 15 weeks of age in the MPS IIIA mice. Other layers of the retina showed thinning by 25 weeks of age. In MPS IIIA mice aged 4, 6, or 12 weeks, immunohistochemical labelling revealed significant expansion of the endo/lysosomal system in the retina by four-weeks of age (p<0.05) and the presence of isolectin B4-stained activated microglia. Heparan sulphate, the primary substrate that accumulates in MPS IIIA cells, was significantly elevated in whole eye preparations by four-weeks of age (p=0.0001). Early administration of treatments targeting both the eye and the brain will therefore be required to optimise patient quality and length of life.

Phill Bokiniec - Max Delbruck Center For Molecular Medicine

SOMATOSENSORY DISCRIMINATION OF TACTILE AND THERMAL PERCEPTION OF THE MOUSE FOREPAW

Somatosensation is a highly integrative sense that contains a number of sub-modalities including temperature, mechanosensation, proprioception, and pain. A central question is: what are the neural mechanisms that allow sensory...
discrimination from different somatosensory modalities arising from the same sense organ? We are addressing this question with multiple approaches including electrophysiological recordings, behavioral training of head-restrained mice and optogenetic manipulations in the forepaw somatosensory system. Behavioral training has shown that mice are able to detect small temperature changes delivered to the paw (<1°C) – putting the sense of touch in a similar range as for humans. Ongoing work investigates the functional pathways of innocuous temperature and touch from the forepaw to the primary somatosensory cortex.

Alexander Burton - Neuroscience Research Australia

THE EFFECTS OF TONIC MUSCLE PAIN ON FUSIMOTOR CONTROL OF HUMAN MUSCLE SPINDLES DURING VOLUNTARY CONTRACTIONS.

Smith LJ1, Macefield VG1,2,3, Birznieks I2,4,5 and Burton AR1,2,4

1. School of Medicine, Western Sydney University, NSW 1797, Australia. 2. Neuroscience Research Australia, Sydney, NSW 2031, Australia. 3. College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, UAE. 4. School of Medical Sciences, University of New South Wales, Sydney, NSW 2031, Australia. 5. School of Science and Health, Western Sydney University, NSW 1797, Australia.

Animal studies have revealed nociceptors can excite fusimotor neurones and change the sensitivity of muscle spindles to stretch; such nociceptive reflexes have been suggested to underlie mechanisms that lead to chronic musculoskeletal pain syndromes. However, the validity of the “vicious cycle” hypothesis in humans has yielded contrasting results to that found in animals. Given spindle firing rates are generally much lower in humans than in animals, it is possible that some of the discrepancies between human experiments and those obtained in anaesthetised animals could be explained by differences in background fusimotor drive when the leg muscles are relaxed. We examined the effects of tonic muscle pain from 14 single fusimotor driven muscle spindle afferents (6 primary, 8 secondary) during intramuscular infusion of hypertonic saline in awake humans. We did not observe any significant increases in muscle spindle nerve activity during tonic pain.

Furthermore, a subjects capacity to maintain a constant level of force, while relying on proprioceptive feedback in the absence of visual feedback, was not compromised during tonic pain.

Kristen Farrell – The University of Newcastle

IN VIVO ELECTROPHYSIOLOGICAL CHARACTERISATION OF CALRETTININ-POSITIVE INTERNEURONS IN MOUSE DORSAL HORN

Farrell KE1, Smith KM1, Callister RJ1 and Graham BA1.

1. Preclinical Neurobiology Research Group, School of Biomedical Sciences and Pharmacy, The University of Newcastle, Australia.

Spinal cord dorsal horn (DH) neurons are important for the processing and integration of nociceptive, light touch, itch and thermal sensations. Importantly, there is a specific subpopulation of DH neurons that express calretinin (CR+), that are implicated in linking innocuous tactile inputs with nociceptive circuitry. Here, we use in vivo patch-clamp electrophysiology to study the functional role of DH CR+ neurons in sensory processing by examining their responses to cutaneous stimulation, as well as their functional properties. Transgenic mice (C57Bl6/J background, 8–15 weeks, M/F) with channel rhodopsin-2 expressed in CR+ neurons (CR-ChR2) were anaesthetised (isoflurane 1-3%), and patch-clamp recordings were made from L4/5 DH neurons. CR-ChR2 neurons were identified by the presence of direct photocurrents in response to spinal photostimulation (470 nm light). Von Frey filaments, light brushing and pinching of the hind paw were used to assess the responses to cutaneous stimuli. We confirmed two distinct populations of CR+ neurons could be differentiated in vivo, termed typical and atypical, which our group first identified under in vitro conditions. In addition, we observed that both CR+ neurons, and neurons with synaptic inputs from CR+ neurons respond to all forms of cutaneous stimulation applied. Importantly, in a subset of neurons with synaptic inputs from CR+ neurons, subthreshold responses were amplified to produce AP discharge after photostimulation of CR+ neurons. Our results show that CR+ neurons actively participate in sensory processing of cutaneous tactile inputs, and influence (enhance) the response of other neurons within the DH.

Brett Graham – University of Newcastle

SPINAL OPTOGENETIC STIMULATION AS A NOVEL ANALGESIC DRUG SCREEN
Australasian Neuroscience Society Annual Scientific Meeting 2017
International Convention Centre, Sydney, December 3rd – 6th 2017

Owen CG, Smith KM, Callister RJ, Dayas CV, Graham BA
Preclinical Neurobiology Research Group, School of Biomedical Sciences and Pharmacy, The University of Newcastle, Australia.

Persistent pain arises from a wide variety of diseases and injuries and imposes a significant clinical burden that is often poorly treated. In addition to the obvious patient suffering, inadequate pain treatment brings a substantial cost to the individual and community with lost productivity and reduced work participation compounding the problem. This highlights the need for better pain treatments, yet the development of new analgesic drugs can be characterised as slow, at best, and heavy reliance on opiates persists. Thus, new approaches to accelerate analgesic discovery and development are required. This study has assessed a new approach to study analgesic efficacy and site of action using spinal optogenetic stimulation. Specifically, we have recently shown that optogenetic stimulation in the spinal dorsal horn produces a distinct nociceptive response that can be quantified and studied. Importantly, this approach produces nociceptive signals without activating peripheral nociceptors and is thus a model of centrally evoked pain. To assess the viability of this preparation to distinguish between central and peripheral analgesia we have assessed the capacity of opiates (central) and non-steroidal anti-inflammatory drugs (peripheral) to block optogenetic behavioural responses. Our data confirms that the nocifensive response duration is attenuated by the centrally acting analgesic buprenorphine (37±5s vs. 6±3s, n=4, p=0.004); but not by the peripherally acting analgesic caprofen (31±5s vs. 33±8s, n=4, p=0.835). These results support the sensitivity of spinal optogenetic stimulation to assess the site of action for analgesic compounds, making it a useful addition to the analgesic screening and development pipeline.

Jason Ivanusic – The University of Melbourne

AN IN VIVO BONE-NERVE PREPARATION TO EXPLORE THE ROLE OF C-Fibre BONE MARROW NOCICEPTORS IN BONE PAIN

Wong A, Nencini S and Ivanusic JJ
Department of Anatomy and Neuroscience, The University of Melbourne

We have recently developed an in vivo bone-nerve preparation which uses monopolar hook electrodes to record the activity of single bone marrow nociceptors, and to explore their responses to noxious mechanical stimulation of bone delivered by increased intra-osseous pressure (ramp-and-hold stimulus; 300 mmHg). Using this preparation, we have been able to isolate and study the responses of bone marrow nociceptors with Aδ but not C-fibre conduction velocities. Here we report further development of the preparation, through the use of bipolar electrodes, that permits isolation of bone marrow nociceptors with C-fibre conduction velocities. We experimented with different inter-electrode distances and found that smaller distances (~1 mm) resolved single C-fibre units better than larger inter-electrode distances (~3-5 mm). During the ramp phase of the pressure stimulus the threshold for mechanical activation of single-unit C-fibre activity was between 30 to 219 mmHg (107 ± 18 mmHg; n=12). Single-unit responses were classified as phasic-tonic, phasic or tonic. The majority of C-fibre bone marrow nociceptors were phasic-tonic units (n= 9/12) that had a large initial burst of activity during the ramp phase of the stimulus, and continued to fire action potentials at a lower frequency during the hold phase. The others were phasic units that only had a brief burst of activity during the ramp phase of the stimulus (n=1/12) or tonic units that displayed little adaptation during the ramp-and-hold stimulus (n=2/12). We are currently using this preparation to determine how C-fibre bone marrow nociceptors respond to noxious stimulation and signal bone pain.

William Kwan – Monash University

THE EMERGENCE OF A COMPLEX INFERIOR PULVINAR IN THE SIMIANS RESULTED IN THE DEVELOPMENT OF DIRECT RETINAL INPUT ELIMINATING THE COLLICULAR PATHWAY

Kwan WC [1], Mundinano IC [1], Lee SC [2,3], Martin PR [2,3], Grünert U [2,3], Bourne JA [1]

1. Australian Regenerative Medicine Institute, Monash University, Melbourne, VIC, Australia
2. Save Sight Institute and Department of Clinical Ophthalmology, The University of Sydney, Sydney, NSW, Australia
3. ARC centre of Excellence for Integrative Brain Function, The University of Sydney

Numerous studies in primates have demonstrated that there are alternative routes for visual information to reach the visual cortex, independent of the primary retinogeniculostriate pathway. One such pathway is retinopulvinar-MT pathway. However, there have been conflicting reports as to whether the non-cortical visual inputs to medial subdivision of the inferior pulvinar subnucleus (Plm) arise only from retina or whether Plm is also the recipient of visual input from the...
superior colliculus (SC). Furthermore, there is limited information about the population of retinal ganglion cells (RGCs) that innervate the Pim, specifically whether they are exclusive or collaterals with retinogeniculate or retinotectal pathways.

Adult marmosets received injections of a bidirectional tracer cocktail in the Pim and SC (n=4), or Pim and the dorsal lateral geniculate (n=2). In all cases here was no colocalisation of retrograde labelled RGCs, demonstrating that the retinopulvinar, retinogeniculate and retinotectal pathways are independent. Additionally, some of the RGCs which project to the pulvinar are anatomically distinct from SC projecting cells. Finally, we found that the retinorecipient Pim receives no input from the SC. The SC does, however, project to the neighbouring inferior pulvinar subnuclei PIp and Pic.

Our results suggest that during evolution and emergence of the inferior pulvinar complex in primates, a new visual pathway to pulvinar has emerged. Identifying the functional role of this pathway will be important in understanding how nonhuman primates and humans have evolved to develop complex and high-level visual processing.

Justin Lees – Soms UNSW

MOUSE MODELS FOR IDENTIFYING NEUROPROTECTANTS IN CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

1. Neuropathic Pain Research Group, Translational Neuroscience Facility, School of Medical Sciences, the University of New South Wales, Sydney, NSW, 2051, Australia. 2. Central Clinical School, the University of Sydney, NSW, 2001, Australia. 3. Department of Medical Oncology, Prince of Wales Hospital, Australia and Prince of Wales Clinical School, University of New South Wales, Sydney, NSW, 2051, Australia.

Cancer survival rates are increasing and in parallel there is increase in the clinical burden of long term toxicity issues in survivors. Chemotherapeutics such as platinum based drugs and taxanes are neurotoxic, often leading to the common and damaging side effect known as chemotherapy-induced peripheral neuropathy (CIPN). This condition causes numbness, allodynia and abnormal sensory function and chronically effects a substantial numbers of cancer survivors. Unfortunately, there are no currently available therapeutic options for treatment or prevention. Our group has developed physiologically relevant C57BL/6J mouse models of CIPN. For oxaliplatin (platinum drug), the model is based on the FOLFOX regimen used in the treatment of colorectal cancer (12 cycles of chemotherapy); and for paclitaxel (taxanes), the model is based on breast cancer treatment (6 cycles of chemotherapy). In both models, we observed significant mechanical allodynia in the treated mice when compared to non-treated mice (p<0.05), indicating the presence of neuropathic pain symptoms. The oxaliplatin model also demonstrates significantly reduced weight gain (p<0.001), decrease exploratory behaviour (p<0.01) and increased signs of defeated behaviour (p<0.05). These mouse models are currently being used to test selected neuroprotectants for CIPN.

Richard Leibbrandt - College Of Medicine And Public Health, Flinders University

FLYFLY: VISUAL MOTION STIMULUS SOFTWARE WITH SUPPORT FOR TARGET TRACKING STUDIES

Leibbrandt RE, Henriksson J, Måhlberg T, Nordström K.
Centre for Neuroscience, College of Medicine and Public Health, Flinders University.

The Hoverfly Vision group at Flinders University and Uppsala University have developed FlyFly, a visual stimulus software system that allows researchers to create and display moving stimuli. FlyFly was designed to be simple to use and extend. It has been used as the stimulus presentation software in numerous projects1-3 and is freely available for download from our website (http://hoverflyvision.weebly.com/software.html).

We here describe recent development of the software (version 3.1) that supports research into tracking visual targets, optionally against a “cluttered” background. Newly added stimuli allow the researcher to create and display spherical target objects which can move along specified trajectories in 3-dimensional space. Targets can be embedded in a background space of other spherical objects which move coherently to simulate the type of optic flow that would be generated by the fly’s own motion through the world. The software runs with high temporal and spatial precision. By using a photodiode trigger, neural or behavioural responses to visual stimuli can be correlated on a frame-by-frame basis.

FlyFly runs on MATLAB (2009 onwards). The current version has been tested on OSX Sierra onwards and Ubuntu 16.04 onwards, displaying 5000 independent objects simultaneously at framerates up to 160Hz, with under 1% dropped frames.

In our ARC-funded work on target tracking in hoverflies, we are using these new capabilities in FlyFly to reconstruct visual
The light and dark of visual motion sensitivity.

Luo GQ1, Mazade R2, Alonso J-M2, Freeman AW1.

1. Sydney Medical School, The University of Sydney, Lidcombe, Australia. 2. College of Optometry, State University of New York, New York, USA.

Aim. It has been known for decades that motion direction selectivity in primates and carnivores arises in primary visual cortex. The mechanisms underlying this selectivity are, however, still mysterious. It has been shown recently that the relative sensitivity to light and dark is an important organizing principle in cortex. We therefore investigated the effect of contrast polarity on psychophysical and neuronal responses to moving edges and bars. Methods. Psychophysical subjects were visually normal adult humans. The neurophysiology used anaesthetised cats: multi-port electrodes were inserted parallel to the surface of primary visual cortex. Results. Human subjects were more sensitive and responded faster to light stimuli than to darks of the same contrast magnitude. The advantage for lights declined as speed increased from 1 to 10 deg/s, and reversed to an advantage for darks at 30 deg/s. These results are surprising, given that responses to stationary stimuli are stronger for darks than for lights. We sought to corroborate these findings with multi-unit recordings from cat primary visual cortex. We found that responses to light bars preceded responses to dark bars when stimuli moved at 5 deg/s but not at higher speeds. The psychophysics and neurophysiology are therefore in agreement. Conclusion. Why are light stimuli dominant for moving stimuli? The answer may lie in the receptive field structure, if the preferred direction is from an on- to an off-subfield. An alternative possible cause is intracortical inhibition: most neurons in primary visual cortex are off-dominated, and dark stimuli may therefore evoke greater inhibition.

Hamish Meffin - University Of Melbourne

Revisting feature invariance of complex cells in primary visual cortex

Meffin H 1,2, Almasi A 2,3, Sun S 1,2, Cloherty SL 1,2, Wong YT 2,3, Yunzab M 1,2, Ibbotson MR 1,2

1. ARC Centre of Excellence for Integrative Brain Function, Department of Optometry and Vision Sciences, University of Melbourne, Parkville, VIC 3010, Australia. 2. National Vision Research Institute, Australian College of Optometry, Corner Keppel and Cardigan Streets, Carlton, VIC 3053, Australia. 3. Department of Biomedical Engineering, University of Melbourne, Parkville, VIC 3010, Australia.

Cells in primary visual cortex have been investigated using oriented grating stimuli. Such studies have shown that the spike rate response of many complex cells is invariant to the phase of drifting sinuosoidal gratings presented at their preferred orientation: a form of translation invariance. By contrast, simple cells are highly sensitive to spatial phase. Here we investigated the visual features to which complex cells are invariant using stimuli that make no prior assumptions about the features they prefer, namely, pixelated Gaussian white noise. The set of features to which each cell was sensitive was found by estimating its receptive field filters from its response to the noise stimuli. The cell spike rate was plotted as a function of the degree to which those features were present in each noise sample.

Amongst the population of complex cells (n=47) there is a population with classic spatial phase invariance (10%). In addition we find complex cells with other more complicated forms of invariance (90%). There is a large population of cells that show partial phase sensitivity that is in-between classic simple and complex cell behaviour (80%). In addition there is a small population of cells that show invariant response to features with different orientations (10%). Our results challenge current theories of the encoding of visual stimuli in primary visual cortex.
Sarah Nicholas – Flinders University

PERCEIVED SELF MOTION AFFECTS THE NEURAL RESPONSE OF MALE HOVERFLIES TO SMALL TARGETS

Nicholas SJ, Nordström K.

Centre for Neuroscience, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia

Many insects, such as hoverflies, are able to track and pursue small targets, for instance during mating and territorial behaviors, even though their own motion generates background optic flow. Supporting this behavior, neurons in the hoverfly third optic ganglion, the lobula complex, respond robustly to target motion against background clutter, even without relative motion differences. In this study we performed extracellular recordings from the ventral nerve cord (VNC), which is post synaptic to the lobula complex, of male hoverflies. We first confirmed that like in dragonflies, the hoverfly VNC houses neurons that respond selectively to optic flow, and those that are tuned to target motion (target selective descending neurons; TSDNs). The TSDN size and speed tuning is strikingly similar to the tuning of the small target motion detector (STMD) neurons in the lobula complex. However, surprisingly, and as opposed to STMDs, TSDN responses are significantly and substantially affected by background motion. Indeed, both the relative speed and the direction of the optic flow affects the response to small targets. For example, when the background and the target move at the same speed and direction, the response to the target is minimal compared to the response when the background is stationary or moving in the opposite direction. We hypothesize that when the generated optic flow of the hoverfly matches that of the small target, in both direction and velocity, the responsiveness to the target is suppressed, as the target would be perceived as a stationary object.


Chamini Perera – UNSW Sydney

CHARACTERISATION OF COCHLEAR INNERVATION IN PERIPHERIN KNOCKOUT AND WILD TYPE MICE USING IMMUNOHISTOCHEMISTRY

Perera CJ1, Von Jonquieres G2, Pinyon JL1, Cederholm JME3, Parley KE1, Ryan AF2 and Housley GD1

1Translational Neuroscience Facility & Department of Physiology, School of Medical Sciences, UNSW Sydney, NSW 2052, Australia; 2University of California, San Diego and the VA Medical Center, 9500 Gilman Drive, La Jolla, CA 92093, U.S.A.

Peripherin is a type III intermediate neurofilament, known to play a role in neurite growth. In the cochlea, peripherin is uniquely expressed by type II spiral ganglion neurons (SGN) that innervate outer hair cells (OHCs). Previous findings suggest that contralateral suppression is compromised in peripherin knockout (KO) mice, with reduced amplitude of distortion product otoacoustic emissions and disrupted type II innervation to the OHCs (Froud et al., 2015). The present study further investigated the type II innervation in peripherin KO and wildtype (WT) animals. Immunohistochemistry was performed on cochlear cryosections and whole-mount tissue from adult peripherin KO and WT mice (n=3). Anti-NF200 or anti-β-tubulin antibodies were used to label inner and outer spiral bundle (OSB) fibres. Both immunofluorescence (IF) and immunoperoxidase (IP) tests were performed with anti-parvalbumin antibody to label terminals of type II SGNs on OHCs. In both cryosections and whole-mount tissue immunolabelling with either NF200 or β-tubulin demonstrated fewer OSB fibres in the mid-lower apical turns of KO mice compared to WT mice. Additionally, a markedly reduced number of OSB fibres and their terminals were seen in the KO phenotype, as detected by both IF and IP tests with anti-parvalbumin antibody. These provide key validation that peripherin KO mice have distorted sensory innervation of the outer hair cells, which likely reconciles impaired contralateral suppression.

216 words

Lauren Poppi – The University of Newcastle

**CALCIUM IN BALANCE: IN SITU GCAMP IMAGING IN THE CRISTA AMPULLARIS**

Holman H², Poppi LA¹, Lim R², Brichta AM¹, Rabbitt RD².

1. School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, NSW, Australia
2. Department of Bioengineering, University of Utah, Salt Lake City, Utah, USA.

The crista ampullaris is made up of thousands of mechanosensory hair cells that convert the mechanical energy of head movement into graded neurotransmitter (glutamate) release onto primary afferents. In addition to primary afferent neurons, the crista is also innervated by efferent neurons that contact type II hair cells, afferent terminals, and afferent fibres. The efferent innervation has a profound modulatory effect on the sensory activity of the vestibular organs, and is a promising therapeutic target for balance disorders and vestibular rehabilitation. The effect of the efferent neurotransmitter (ACh) on vestibular hair cells and afferent neurons is still not well understood in mammals, however new findings indicate that intracellular calcium (Ca²⁺), Ca²⁺ signalling pathways, and potentially intracellular Ca²⁺ stores, play a critical role in the cholinergic modulation of vestibular type II hair cells. Here, we show preliminary results from experiments in mice expressing GCaMP5g and GCaMP6, under GAD and parvalbumin promoters, respectively. Mice (both sexes, 0-12 weeks old) were overdosed with ketamine (100 mg/kg i.p.) and rapidly decapitated. The inner ears were dissected, placed in the recording bath, and imaged using a custom swept-field confocal microscope. GCaMP fluorescence in hair cells and afferents was recorded before, during, and after ACh application. Pharmacological blockade of receptors and ion channels (atropine, strychnine, nifedipine, ryanodine) was used to test putative efferent mechanisms. We were able to show significant, and contrasting changes in intracellular Ca²⁺ using GCaMP₆ fluorescence imaging, and results indicate that Ca²⁺ plays a critical role in efferent signalling in the crista ampullaris.

Kiley Seymour – Western University Sydney

**DIFFERENTIAL ORIENTATION TUNING OF NEAR AND FAR SURROUND SUPPRESSION REVEALS TWO INHIBITORY MECHANISMS IN HUMAN V1**

Seymour KJ¹,² and Wardle SG²,³.

1. School of Social Sciences and Psychology, Western Sydney University, New South Wales, Australia. 2. ARC Centre of Excellence in Cognition and its Disorders, Macquarie University, Sydney, NSW, 2109, Australia. 3. Department of Cognitive Science, Macquarie University, Sydney, NSW, 2109, Australia

It is well-established that responses to visual stimuli placed within a neuron's receptive field are modulated by stimuli placed in the surrounding regions. In primary visual cortex (V1), this contextual modulation is orientation-tuned, with greatest suppression of the neural response to stimuli inside the receptive field by iso-oriented surrounds. Findings from primate neurophysiology and human psychophysics suggest that surround suppression in V1 involves two distinct inhibitory mechanisms operating over differing spatial extents. Here we use fMRI to measure surround suppression in human V1 at the population level. To selectively probe either short or long-range inhibitory mechanisms, we varied the spatial proximity of a surrounding grating relative to a central target grating and measured suppression of the BOLD response relative to the target presented in isolation. Consistent with predictions from studies in non-human primate, the magnitude of surround suppression in human V1 was orientation-tuned for near surrounds, but not for far surrounds. Suppression of the BOLD response to a target grating was greatest for iso-oriented surrounds located in close proximity to the target stimulus. In contrast, the magnitude of far surround suppression was equivalent for parallel and orthogonal surrounds, suggesting that the orientation specificity of surround suppression in V1 is conveyed by a short-range mechanism. The results indicate that near and far surrounds engage different inhibitory mechanisms in human V1, supporting the existence of distinct tuned and un-tuned mechanisms that operate over different spatial ranges.

Natalie Zeater – University of Sydney, Save Sight Institute

**INTEROCULAR MATCHING OF RECEPTIVE FIELD PROPERTIES IN BINOCULAR CELLS OF THE MARMOSET LGN**

Zeater N¹,²,³, Cheong S.K⁴, Pietersen A.N. J¹,²,³, Dreher B³, Solomon S.G⁵, Martin P.R¹,²,³
CR assays were carried out on the cristae, the silon RI signalling, viral

The peripheral vestibular organs play a critical role in posture, balance and gaze stability. Vestibular function is affected by age-related degenerative changes that likely contribute to falls in the elderly. However, the molecular mechanisms underlying aging of the vestibular organs are not well understood. Therefore, we have begun to characterise the molecular changes that may contribute to age-related balance disorders. RNA was extracted from young (3.5 months), middle (14 months), and old age (>28 months) mouse peripheral vestibular organs for microarray analyses. Differentially expressed genes were determined using Affymetrix GeneArrays, and gene lists analysed by DAVID for identification of enriched biological pathways. Raw array data was also used for gene set enrichment analysis (GSEA). Expression changes for genes that are components of pathways identified by both bio-informatics analyses were confirmed using qPCR. For a systematic analysis of the different major components of the vestibular organs, the qPCR assays were carried out on the cristae, the otolith organs, and the membranous epithelial cells. Significant age-related changes in stress, inflammation, and immune response pathways were indicated by both methods: e.g. B cell receptor signalling, Fc epsilon RI signalling, viral myocarditis, Fc gamma R-mediated phagocytosis, natural killer cell mediated cytotoxicity, focal adhesion, Parkinson’s disease, ribosome, ECM receptor interaction, spliceosome, and systemic lupus erythematosus. These data suggest immune cell infiltration, activation, and inflammation, are involved in age-related degeneration of the peripheral vestibular apparatus. Further evidence suggests these changes may be triggered by breakdown of the blood-labyrinth barrier as seen in other ageing tissue barriers.

Stimulation and sensitization of nociceptive neurons with cell bodies in dorsal root ganglia (DRG) play a critical role in the
NAv1.1 CONTRIBUTES TO ACTIVATION AND ALTERED EXCITABILITY OF Aδ BONE MARROW NOCICEPTORS

Gronert SF, Nencini S, Chow CY, King GF and Ivanusic JJ
Department of Anatomy and Neuroscience, The University of Melbourne

Voltage-gated sodium channels (VGSCs) are a highly conserved family of ion channels required for generation of action potentials in neurons including nociceptors. The role of specific VGSC subtypes in peripheral bone pain is unclear, and has been difficult to establish because of a lack of subtype specific agonists. Here we investigated responses of peripheral bone marrow nociceptors to a spider venom peptide (Hm1b) that is known to selectively inhibit fast inactivation of Nav1.1 and increase sensitivity to mechanical stimulation in the footpad of mice. We used an in vivo electrophysiological bone-nerve preparation to record the activity of single Aδ-fibre bone marrow nociceptors in response to a standardised noxious mechanical stimulus (increased intra-osseous pressure), before and after application of Hm1b to the marrow cavity. Application of Hm1b sensitized each of the single mechanically activated Aδ bone marrow nociceptors we isolated in our bone-nerve preparation. It also resulted in a transient increase in ongoing activity of at least some of the Aδ bone marrow nociceptors we have been able to isolate in the absence of mechanical stimulation. The results indicate that Nav1.1

Samuel Evans – University of Adelaide

(-)-NALTREXONE MAINTAINS CAPSAICIN INDUCED ALLODYNIA IN A BALB/C PAIN MODEL

Evans S1, Mustafa S1, Buisman-Pijlman F1, Hutchinson M1,3.
1. Discipline of Physiology Research Laboratory, University of Adelaide, Adelaide, AUS.
2. Discipline of Pharmacology, University of Adelaide, Adelaide, AUS.
3. ARC Centre of Excellence for Nanoscale BioPhotonics, Adelaide, AUS

Intraplantar injection of capsaicin into the mouse hind paw produces a robust acute allosthenia lasting 45-60 minutes in a Balb/c model. The model uses a von Frey test to measure tactile allodynia following a capsaicin challenge (0.8ug/paw). We observed that this response can be attenuated by intraplantar injection of vehicle 15 minutes prior to the noxious insult. We used opioid receptor antagonist (-)-Naltrexone in order to understand a potential endogenous opioid involvement in this effect. (-)-Naltrexone administered 30 minutes pre-capsaicin dose dependently reversed the attenuation (p < 0.0001); and at high doses (60mg/kg) allosthenia remained significantly elevated up to 135 minutes (p < 0.0001). At a medium dose (6mg/kg) (-)-Naltrexone has no effect on capsaicin allosthenia compared to vehicle. Administration of the same dose 30 minutes following capsaicin causes significant increases in duration of allosthenia (p < 0.0001). However, the opioid dependency of this effect is called into question as the same effect is observed when using the opioid inactive stereoisomer (+)-Naltrexone (p < 0.0001). The results suggest Naltrexone effects Capsaicin-induced allosthenia only after initiation of a nociceptive response at lower doses. Naltrexone potentially interferes with capsaicin receptor TRPV1 however the mechanisms behind this selective extended allosthenia are unknown.

Sebastian Gronert – The University of Melbourne

development of chronic and neuropathic pain. Investigations of DRG neurons are limited by the absence of a suitable high-throughput model representing individual cell types found in the DRG. Our aim was to determine whether an immortalized DRG cell line (S0B11), could be selectively differentiated into the two basic nociceptor subtypes, nerve growth factor (NGF)-dependent peptidergic and glial derived growth factor (GDNF)-dependent non-peptidergic cells functionally responding to selective stimulants and pro-inflammatory mediators. q-RT-PCR (n = 5) and Western blotting (n = 5) were used to characterize the phenotypes of S0B11 cells differentiated with forskolin, NGF and GDNF. Calcium imaging was used to characterise functional responses of the S0B11 cells to adenosine tri-phosphate (ATP) and capsaicin under control and a prostaglandin E2 (PGE2)-sensitized state (n = 5). Our results show that NGF and GDNF differentially regulate S0B11 cells to phenocopy peptidergic (TRPV1 expressing, capsaicin-responsive) or non-peptidergic (P2X3 expressing, ATP-responsive) nociceptor subtypes respectively. Importantly, when compared to controls, peptidergic S0B11 cells showed increased responses to reduced concentration of capsaicin indicating sensitization with PGE2 (n = 4). Our data show that the S0B11 cells have the ability to differentiate into functionally distinct peptidergic and non-peptidergic phenotypes, and demonstrate the ability to be sensitized by a pro-inflammatory mediator. The S0B11 cells may provide a suitable alternate to cultured primary DRG neurons for investigations of nociceptive pathways for peptidergic and non-peptidergic neuronal subtypes.

1. Discipline of Physiology Research Laboratory, University of Adelaide, Adelaide, AUS.
2. Discipline of Pharmacology, University of Adelaide, Adelaide, AUS.
3. ARC Centre of Excellence for Nanoscale BioPhotonics, Adelaide, AUS

Gronert SF, Nencini S, Chow CY, King GF and Ivanusic JJ
Department of Anatomy and Neuroscience, The University of Melbourne

Voltage-gated sodium channels (VGSCs) are a highly conserved family of ion channels required for generation of action potentials in neurons including nociceptors. The role of specific VGSC subtypes in peripheral bone pain is unclear, and has been difficult to establish because of a lack of subtype specific agonists. Here we investigated responses of peripheral bone marrow nociceptors to a spider venom peptide (Hm1b) that is known to selectively inhibit fast inactivation of Nav1.1 and increase sensitivity to mechanical stimulation in the footpad of mice. We used an in vivo electrophysiological bone-nerve preparation to record the activity of single Aδ-fibre bone marrow nociceptors in response to a standardised noxious mechanical stimulus (increased intra-osseous pressure), before and after application of Hm1b to the marrow cavity. Application of Hm1b sensitized each of the single mechanically activated Aδ bone marrow nociceptors we isolated in our bone-nerve preparation. It also resulted in a transient increase in ongoing activity of at least some of the Aδ bone marrow nociceptors we have been able to isolate in the absence of mechanical stimulation. The results indicate that Nav1.1

Sebastian Gronert – The University of Melbourne

Gronert SF, Nencini S, Chow CY, King GF and Ivanusic JJ
Department of Anatomy and Neuroscience, The University of Melbourne

Voltage-gated sodium channels (VGSCs) are a highly conserved family of ion channels required for generation of action potentials in neurons including nociceptors. The role of specific VGSC subtypes in peripheral bone pain is unclear, and has been difficult to establish because of a lack of subtype specific agonists. Here we investigated responses of peripheral bone marrow nociceptors to a spider venom peptide (Hm1b) that is known to selectively inhibit fast inactivation of Nav1.1 and increase sensitivity to mechanical stimulation in the footpad of mice. We used an in vivo electrophysiological bone-nerve preparation to record the activity of single Aδ-fibre bone marrow nociceptors in response to a standardised noxious mechanical stimulus (increased intra-osseous pressure), before and after application of Hm1b to the marrow cavity. Application of Hm1b sensitized each of the single mechanically activated Aδ bone marrow nociceptors we isolated in our bone-nerve preparation. It also resulted in a transient increase in ongoing activity of at least some of the Aδ bone marrow nociceptors we have been able to isolate in the absence of mechanical stimulation. The results indicate that Nav1.1
**IMPACT OF VISUAL STIMULI ON CERVICAL VESTIBULAR EVOKED MYOGENIC POTENTIALS (cVEMPS)**

**Hesselstrand E**, Mohammed Ali F, Camp AJ

1. The Faculty of Medicine and Health Sciences, Linköping University, Sweden. 2. Discipline of Anatomy and Histology, Sydney Medical School, University of Sydney, NSW, Australia. 3. Discipline of Biomedical Science, Sydney Medical School, University of Sydney, NSW, Australia.

The vestibular evoked myogenic potential (VEMP) is a neurophysiological technique used to assess vestibular function. Conventionally, cervical VEMPs (cVEMPs) are obtained from the sternocleidomastoid (SCM) muscle of the neck. Several studies have also recorded cVEMPs from dorsal neck muscle splenius capitis (SPL). This study investigated the impact of visual input on cVEMP recordings in young healthy subjects from the SCM and SPL in a seated head turned position. Given the interactions between the visual and vestibular systems, it is hypothesised that there will be differences in cVEMPs recorded during different visual stimuli. Among 23 subjects, the results indicated the following: there was a significant difference in cVEMPs between those recorded during the control stimulus when compared with those of the visual stimuli where direction influenced cVEMP parameters. Specifically, the latency and positive-negative ratio of cVEMPs recorded during horizontal stimuli presentations showed delayed latencies and larger amplitudes. For the first time, this study examined the impact of visual stimuli on cVEMPs. These findings reflect the known interactions between the visual and vestibular systems and importantly, provide diagnostic utility for the cVEMP. Currently, cVEMPs are used in the diagnosis of vestibular disorders however these results may lead to cVEMPs being used in the diagnosis of visually-mediated vestibular disorders.

**PHARMACOLOGICAL SIMULATION OF STARVATION IN DIPTERAN FLIES**

**Holden M, Petersen J, Nordström K.**

Centre for Neuroscience, Flinders University, Adelaide, Australia.

The cellular regulation of appetite is still not completely understood. The mechanistic target of rapamycin (mTOR) protein detects nutritional availability and operates within two signaling complexes (TORC1 and TORC2). These complexes signal to the body when nutritional needs have been satisfied. Blocking these pathways has previously been shown to increase appetite in the rat. To investigate if a similar state of starvation can be induced in invertebrates, we here administered the selective mTOR inhibitor Torin1 to dipteran flies. We found that drug affected flies showed an immediate gain in body weight (up by 60% within 2 hours of a Torin1 injection) compared to flies administered with a vehicle control. This finding suggests that Torin1 indeed holds the ability to increase appetite in dipteran flies. We further aim to assess the effects that this pharmacologically induced state of starvation has on fly visual processing. Our results not only highlight the similarity in TORC signaling between mammalian and invertebrate systems, but additionally puts forward a novel pharmacological method to systematically investigate how perceived starvation in the fly affects neural processing. This finding also provides new insight regarding the cellular regulation of appetite in an invertebrate model.

**A QUANTITATIVE EVALUATION OF THE LIMITS OF CORTICAL AND SUB-CORTICAL MECHANISMS UNDERLYING ORIENTATION SELECTIVITY**

**Lloyd EKJ, Vidyasagar TR**

Department of Optometry and Vision Sciences, The University of Melbourne, Parkville, Victoria 3010, Australia

How neurones in Primary Visual Cortex (V1) attain their selectivity to a particular stimulus orientation persists as a major
and archetypal question of systems neuroscience. Hubel and Wiesel’s original model of spatial convergence, where V1 neurons sum inputs over an elongated region of visual space to derive their selectivity, is the theoretical starting point. However, recent data places a constraint on the extent of the elongation of the inputs to V1 neurons, and therefore limits the viability of the spatial convergence mechanism. The alternative origin of orientation tuning is the broader selectivity known to exist in the retina and visual thalamic “relay”, the LGN.

Generated by a process that is independent of cortical development, a model based on this ‘sub-cortical’ tuning may be attractive for its implications on developmental questions. Given the constraints that recent data place, our concern was to numerically evaluate what room is left for this sub-cortical mechanism to be disregarded in favour of spatial convergence. Using both linear and non-linear spiking simulations of the LGN to V1 system in cats, and the existing data on both mechanisms as constraints, the relative contributions of each mechanism to the final preferences of V1 were evaluated. Under most conditions, the sub-cortical mechanism was always, at least, contributory, and, the majority of the time, determined V1 selectivity (Randomly resampled, Chi-squared test, p<0.05). These results indicate that sub-cortical selectivities ought to be included in all investigations of the mechanisms of orientation selectivity.

Fatema Mohammed Ali – University of Sydney

**SPLENIUS CAPITIS AS A TARGET FOR THE cVEMP IN OLDER AND NEURODEGENERATIVE PATIENTS**

Mohammed Ali F1, Westling M2, Zhao L1, Corneil BD1, Camp AJ3

1. Discipline of Anatomy and Histology, Sydney Medical School, University of Sydney, NSW, Australia. 2. The Faculty of Medicine and Health Sciences, Linkoping University, Sweden. 3. Department of Physiology and Pharmacology, Robarts Research Institute, University of Western Toronto, Canada. 4. Discipline of Biomedical Science, Sydney Medical School, University of Sydney, NSW, Australia.

The vestibular evoked myogenic potential (VEMP) is a neurophysiological technique used to assess vestibular function. Conventionally, cervical VEMPs (cVEMPs) are obtained from the sternocleidomastoid (SCM) muscle and requires lying in an uncomfortable clinical position to maximally activate the SCM. Several studies have examined the utility of dorsal neck muscle splenius capitis (SPL) for cVEMPs and show it is a reliable target in young, healthy subjects. It is unclear whether
SPL-cVEMPs may also be a useful clinical addition for patient populations. This study investigated cVEMPs in two positions, the clinical posture and seated head turns, across young subjects (n=13), older subjects (n=14), and Parkinson’s disease patients (n=6). While the incidence of cVEMPs in young subjects did not significantly differ, SPL-cVEMPs consistently displayed less noise compared to SCM-cVEMPs. Out of 60 seated tests conducted on older subjects, 50 SPL-cVEMPs were present compared to 41 SCM-cVEMPs (p<.05). Further, out of 28 seated tests conducted in Parkinson’s patients, SPL-cVEMPs were present 21 times compared to 12 SCM-cVEMPs (p<0.05). In the clinical posture, 70% of tests yielded present SCM-cVEMPs in older subjects compared to 50% in PD patients. SPL-cVEMPs were therefore, more reliably elicited compared to SCM-cVEMPs in older subjects and Parkinson’s patients while the clinical position may not be ideal for these groups. Additionally, expected aging alterations in cVEMPs were not as profound in SPL-cVEMPs. Since SPL-cVEMPs are reliable and do not require an awkward posture, it is likely they will provide clinical utility in older and clinical populations.

Yamni S Mohan – The University of Melbourne

ARE SIMPLE CELLS IN THE PRIMARY VISUAL CORTEX OF THE TREE SHREW IDEAL FOURIER ANALYSERS?

Mohan YS1, Viswanathan S1, Lloyd EKJ1, Jayakumar J1,2, Vidyasagar TR1,2

1Department of Optometry and Vision Sciences, The University of Melbourne, Parkville, Australia. 2 Centre for Computational Brain Research, Indian Institute of Technology, Madras. 3 Melbourne Neuroscience Institute, The University of Melbourne.

Introduction: It has been contentious whether simple cells in the primary visual cortex (V1) perform patch-by-patch Fourier analysis on the visual scene, with cells representing a patch of visual field having a constant receptive field size, but different peak spatial frequency preferences (fpeak). When receptive field size is thus constant, an increase in fpeak leads to an increase in the number of subregions, and thus a decrease in the spatial frequency tuning bandwidth of linear filters. We hypothesised that if the tree shrew V1 simple cells are Fourier analysers, then, as the peak spatial frequency increased, the relative bandwidth of tuning would decrease proportionately.

Methods: We recorded from the V1 of anaesthetised tree shrews. Spatial frequency tuning curves were calculated by measuring the response of neurons to optimally oriented gratings of different spatial frequencies. The frequency at which the response was greatest was the fpeak. The relative bandwidth was calculated by dividing the full width at half height by fpeak.

Results: In 12 animals, a total of 29 units were classified as simple cells. All units were recorded within a restricted region close to zero azimuth (+/- 5 degrees). As the peak spatial frequency increased, there was a moderate but significant decrease in relative bandwidth (r = 0.4262, p<0.05).

Conclusions: Our results indicate that though V1 simple cells in the tree shrew are not ideal Fourier analysers (r = -1), they are far more so than it has been reported for macaques (r = -0.17).

Kevin Ng – UNSW

MULTIPLEXED INTENSITY AND FREQUENCY INFORMATION WITHIN TACTILE AFFERENT BURSTING SPIKE PATTERNS

Ng KKW1,2, Birznieks I1,2, Vickery RM1,2

1. School of Medical Sciences, UNSW Sydney, NSW, Australia. 2. Neuroscience Research Australia, NSW, Australia.

The temporal features of spike trains play an important role in the neural coding of stimulus information. Recent research has found that vibrotactile frequency perception depends on the duration of silent gaps between spike bursts, regardless of the spike count within the burst. In this study, we investigated the contribution of the number of spikes within a burst to vibrotactile perception of intensity. Six subjects aged 20-55 gave informed consent to participate in the psychophysical experiment, which was approved by the UNSW HREC (16245). Patterns of spike trains were evoked in tactile afferents using a mechanical stimulator array to deliver pulsatile stimuli to the fingertip skin. The stimuli were organised into bursts of impulses that repeated at a rate of 20.9 Hz. The number of impulses within a burst was varied from 2 to 4, being separated by between 13.05 and 4.35 ms respectively. Subjects were blindfolded and listened to white-noise through headphones to mask visual and auditory cues. The psychophysical method of magnitude estimation was used to determine subjects’ perceived intensity. We found that increasing the number of impulses in a burst from 2 to 4 resulted in an increased in subjective intensity (one-way ANOVA, DF (3, 20), P<0.0001). This represents a hitherto undescribed method of duplex encoding for frequency and intensity in a single spike pattern.
Australasian Neuroscience Society Annual Scientific Meeting 2017
International Convention Centre, Sydney, December 3rd – 6th 2017

DISTINCT IH CURRENT PROPERTIES IN DORSAL HORN INTERNEURON POPULATIONS

KM Smith1, MA Gradwell1, RJ Callister2 and BA Graham1
1Preclinical Neurobiology Research, School of Biomedical Sciences and Pharmacy, University of Newcastle, Australia.

The dorsal horn (DH) is a key region for sensory processing with the excitability of DH neurons determined by many factors, including a diverse range of voltage-gated ion channels. For example, hyperpolarization-activated cation currents (Ih) have recently been shown to contribute to the development and maintenance of chronic pain. Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels mediate Ih currents, with 4 different HCN isoforms (HCN1-4) known to influence Ih current properties. Here, we use patch-clamp electrophysiology to compare the activation kinetics of Ih currents in several DH interneuron populations. Targeted recordings were made using transgenic animals where green fluorescent protein is expressed in calretinin (CR; n=28), parvalbumin (PV; n=21) or choline acetyl transferase (ChAT; n=18) expressing DH interneurons. Ih currents were activated by a series of hyperpolarising current steps from a membrane potential of -50mV. Time to half Ih activation was significantly slower in CR neurons compared to PV and ChAT neurons, while the normalised conductance-voltage relationship, showed that V1/2 and slope were both significantly decreased in PV versus CR neurons. These data identify functional differences in Ih currents, which might reflect differential expression of HCN isoforms on each population. Previous work has reported HCN1-4 are expressed in the spinal cord however information is limited on their expression on different interneuron populations. Future immunolabelling studies will be required to compare HCN expression in CR, PV and ChAT neurons. Selective HCN subunit expression on interneuron subpopulations could inform future research into targeted analgesic therapies aimed at manipulating Ih currents for pain relief.

CHANGES IN RETINAL GANGLION CELL ACTIVITY EARLY IN DIABETIC RETINOPATHY

Wang YM1, Vessey KA2, Mills SA1, Fletcher EL1
1. Department of Anatomy and Neuroscience, University of Melbourne, Victoria, Australia.

Diabetic retinopathy is diagnosed based on vascular abnormalities in the retina. Extensive evidence indicates, however, that neuronal dysfunction in the inner retina occurs prior to vascular changes, and we aimed to elucidate if there are early changes in retinal ganglion cell (RGC) activity. Diabetes was induced in male dark agouti rats using an injection of 65mg/kg streptozotocin (n=4). Control rats (n=2) were given citrate buffer. Four weeks later, retinal ganglion cells (RGCs) were flatmounted onto a multielectrode array to record dark-adapted spontaneous activity and responses to full-field flashes. Cells were sorted using Spike2 and classified into ON, OFF, ON-OFF and non-responsive types based on peak firing during full-field, light on and off, stimuli. When all groups were combined, RGCs from diabetic rats (n=92) had a significantly lower peak firing rate compared to control cells (n=61). OFF cells from diabetic retinae (n=45) had reduced peak spike frequency in response to light off (p=0.0003) and increased latency to peak (p=0.0003) compared to control OFF cells (n=20). Although ON RGCs from diabetic retinae showed a similar trend, there were no significant changes in peak spike rate or latency (peak spike rate, p= 0.1137; latency, =0.1471). There were no significant differences between ON-OFF RGCs of control or diabetic retinae. The results suggest changes in RGC function occur early in diabetes and further investigation is required to determine if cells with higher firing rates are lost, or if RGCs are less sensitive due to changes in membrane or receptive field properties.

NEURONAL REPRESENTATION OF VOCALISATION PITCH IN MARMOSET AUDITORY CORTEX.

Zhu S1,2, Allitt B1,2, Samuel A1, Rosa MG1,2, and Rajan R1.
1. Department of Physiology, Monash University, Melbourne, Australia. 2.ARC Centre of Integrative Brain Function, Australia.

Information about speaker gender, emotion, and education, etc. is often contained in non-verbal communication signals such as pitch. The pitch of vocalisations is a key feature used for subject recognition by humans and vocal non-human primates. The cortical representation of the pitch of low-frequency periodic sounds, as found in music, has been well studied in primate cortex and it has been proposed that there is a specialised area devoted pitch processing. However, little
is known about what mechanism is used for cortical processing of the high-frequency pitch of complex vocalisations, the form of pitch critical for recognition of conspecifics and use in auditory streaming in sound-degraded environments. Using cortical electrophysiology in anaesthetised marmosets, we investigated how neurons in the primary auditory cortex (A1), rostral auditory field (R) and auditory caudal belt (CB) of the highly-vocal marmoset respond to changes in the pitch of natural vocalisations. We found that neurons in different regions have different response pattern to vocalization sound, and these auditory neurons have differentiated firing rate to pitch changes, with a small population of neurons showing pitch-invariant responses. We found two major mechanisms that were utilised to give pitch responses, the frequency response area (FRA) based mechanism and the spectral temporal receptive field (STRF) based mechanism. Our results suggested that vocalisation pitch response is not merely frequency response given by neurons’ FRA. In fact, for most cases, integration of binaural inputs captured using STRF which considered both excitation and inhibition underlies vocalisation pitch responses.

Christine Barry – Flinders University

PEPTIDERGIC NERVE FIBRES IN THE FEMALE URETHRA: MORPHOLOGICAL AND NEUROCHEMICAL CHARACTERISTICS IN MICE OF REPRODUCTIVE AGE

Barry CM1,2, Ji E1, Sharma H1,2, Yap Y1,2, Vilimas P1,2, Kyloh M1,2, Spencer N1,2 and Haberberger RV1,2.

1Anatomy and Histology, College of Medicine and Public Health, Flinders University
2Human Physiology, College of Medicine and Public Health, Flinders University
Centre for Neuroscience, Flinders University

Peptidergic nerve fibres, including projections from sensory neurons, provide important contributions to urethral function. To understand the organisation of urethral innervation in female mice we used anterograde axonal tracing from lumbosacral dorsal root ganglia in vivo and multiple labelling immunohistochemistry of urethral sections from young nulliparous and older multiparous mice. Nerve fibres immunoreactive for vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP), substance P (SP) and neuropeptide tyrosine (NPY) were identified in the adventitia, muscularis and lamina propria of proximal and distal segments of the urethra. Many were closely associated with blood vessels, glands and epithelial cells identified by immunoreactivity for PGP9.5. Abundant fibres were traced from L5 to S1 DRG to all regions of the urethra. The epithelium received fibres immunoreactive for CGRP and/or SP. Many VIP-immunoreactive nerve fibres surrounding glands were immunoreactive for vesicular acetylcholine transporter (VAChT). A proportion of fibres immunoreactive for NPY were tyrosine hydroxylase (TH) immunoreactive. In the proximal urethra, epithelial innervation density was similar in multiparous and nulliparous mice (14.7 ± 5.5 vs. 9.9 ± 3.6 fibres per field), but density increased in the distal urethra of multiparous mice (22.3 ± 9.9 vs. 11.7 ± 2.8 fibres per field, ANOVA p < 0.05, n = 4 per group). We present the first identification of spinal afferent endings in the urethra. Peptidergic fibres, including multiple populations of sensory fibres, provide rich innervation of the female mouse urethra. The morphology of fibres in the epithelium and other regions suggests multiple nerve-cell interactions impacting on urethral function.

Jennie Cederholm – UNSW Sydney

SUSTAINED SOUND ELEVATION ACTIVATES LOCALISED PURINERGIC ADAPTATION IN THE COCHLEA

Cederholm JME1, Ryan AF2, Housley GD1.

1. Translational Neuroscience Facility and Department of Physiology, School of Medical Sciences, UNSW Sydney, Sydney, New South Wales 2052, Australia. 2. Departments of Surgery and Neurosciences, University of California San Diego, and Veterans Administration Medical Center, La Jolla, California 92039-0666, USA

Sustained sound causes cochlear tissue to release ATP, which activates ATP-gated ion channels assembled from P2X2 receptor subunits. In wild-type mice, moderate (85 dB SPL) noise normally induces a reversible threshold shift, measured by auditory brainstem response (ABR), which recovers over ~ 24 hours. In contrast, P2rx2 null mice (P2rx2-/-) lacking P2X2 receptors maintain their hearing sensitivity despite the noise. However, at higher sound levels P2rx2-/- mice are particularly vulnerable to permanent hearing loss (Housley et al. PNAS, 2013). In the current study we found that there was a significant difference in the distortion product otoacoustic emissions (DPOAE) between wild-type and P2rx2-/- mice during noise exposure. Moreover, we know from a previous study that ipsilateral noise can suppress the DPOAE in the same ear (Froud et al. Nat Comm, 2015) via the efferent olivocochlear innervation to the cochlear outer hair cells. This cross-over between
Australasian Neuroscience Society Annual Scientific Meeting 2017
International Convention Centre, Sydney, December 3rd – 6th 2017

Efferent adaptation and purinergic adaptation was evident with sustained noise presentation in the first ten minutes of observation. Supported by NHMRC APP1089838.

Juliette Cheyne – University of Auckland

**IN VIVO IMAGING AND WHOLE CELL RECORDING OF SPONTANEOUS ACTIVITY IN THE DEVELOPING MOUSE AUDITORY CORTEX**

*Cheyne JE*\(^1,2\), *Thorne PR*\(^1\), *Montgomery JM*\(^2\)

1. Auditory Neurobiology Research Group, University of Auckland, Auckland, New Zealand.
2. Synaptic Function Research Group, University of Auckland, Auckland, New Zealand.

Autism Spectrum Disorders (ASD) are a set of developmental disorders defined by impaired learning, sensory disorders, communication difficulties, social deficits and stereotyped behaviours. The social and communication difficulties in ASD are thought to be due to the distorted processing of sounds, which in turn impairs language abilities. Because ASD symptoms appear during infancy, it is crucial to examine how brain development is altered, as this could underlie behavioural deficits. We hypothesise that the auditory cortex circuitry develops incorrectly, which results in abnormal neuronal connectivity and impaired ability to process sound. During development, sensory organs and their associated cortical regions are spontaneously active, before the onset of sensory perception. We and others have shown that this activity is crucial for the correct wiring of developing networks in the brain. Here we will utilized state-of-the-art in vivo cellular recording techniques to examine how spontaneous activity is altered in the auditory cortex during development in an ASD mouse model. This research has revealed alterations in spontaneous activity during development in ASD and determined the underlying factors leading to these alterations, which may provide new ways to improve communication and social difficulties in ASD in the future.

Shaun Cloherty – Monash University

**MOTION PERCEPTION IN THE COMMON MARMOSET**

*Cloherty SL*\(^1,2\), *Yates JL*\(^1\), *DeAngelis GC*\(^2\), *Mitchell JF*\(^3\)

1. Biomedicine Discovery Institute, Department of Physiology, Monash University, Melbourne, Australia. 2. Brain and Cognitive Sciences, University of Rochester, Rochester, New York.

Studies of motion perception have advanced our understanding of neural population coding and perceptual decision making. The dominant model for this work has been the macaque monkey. However, the common marmoset is a New World primate that offers new opportunities for the study of population coding and decision making. The marmoset brain shares clear homologies with the macaque. However, unlike the macaque, in the marmoset most brain areas involved in motion processing (e.g., the middle temporal area, MT) lie on the surface of the brain and are well suited to large scale population recordings using electrode arrays or imaging.

Here we describe a behavioral paradigm for studying motion perception in the marmoset. We trained two marmosets to perform a motion estimation task: reporting the perceived direction of motion of a noisy random dot-pattern. The direction of each dot was drawn randomly from a uniform distribution with a mean direction assigned randomly from a set of possible directions. The difficulty of the task was manipulated from trial to trial by changing the width of the distribution. Marmosets reported the direction of motion by making an eye movement to a point on a target ring surrounding the stimulus.

Both marmosets learned this task over 6 months of training. The angular error of reports by both marmosets increased with task difficulty and showed a typical trade-off between speed and accuracy. Combined with large-scale recording techniques, this behavioral paradigm offers new avenues for studying the neural code underlying motion perception and decision making.

Calvin Eiber - Save Sight Institute, University Of Sydney

**SINGLE UNIT AND BETA-BAND LOCAL FIELD POTENTIAL RESPONSES TO VISUAL STIMULUS IN PRIMATE LATERAL GENICULATE NUCLEUS (LGN)**

*Eiber CD*\(^1,2,3\), *Pietersen AN*\(^1,2,3\), *Zeater N*\(^1,2,3\), *Townsend R*\(^1,4\), *Solomon SG*\(^3,5\), *Martin PR*\(^1,2,3\)
ELUCIDATING THE FUNCTIONAL STATE OF THE COCHLEAR AMPLIFIER IN HUMANS BASED ON THE TEMPORAL ARRANGEMENT OF THE STIMULUS TONES

Gummer AW, Krokenberger M, Dalhoff E, Zelle, D.
Sectional of Physiological Acoustics and Communication, Faculty of Medicine, University of Tübingen, Tübingen, Germany.

Distortion-product otoacoustic emissions (DPOAEs) emerge as a by-product of the nonlinear amplification in the cochlea when presenting two tones of frequencies $f_1$ and $f_2$. DPOAEs comprise two main components – a nonlinear-distortion and a coherent-reflection component. The aim of the study was to design and test an algorithm for using the nonlinear-distortion component to elucidate the functional state of nonlinear amplification in the human cochlea. These components can be separated in the time domain using short pulses (SP) as stimulus. The study utilized two acquisition paradigms with different primary-tone arrangements, denoted as SP-$f_1$ and SP-$f_2$. For SP-$f_1$, the $f_2$ tone was presented for 25 ms, whereas the $f_2$ tone was switched on 5 ms after $f_1$ onset for frequency-dependent durations between 3 and 11 ms to elicit the DPOAE. SP-$f_2$ interchanged the temporal arrangement and durations of the primary tones. DPOAEs were recorded at eight frequencies ($f_2 = 1–8$ kHz; $f_2/f_1 = 1.2$) and five primary-tone levels $L_2 = 30–70$ dB SPL in 56 normal-hearing ears from 33 subjects. On average, the $f_1$ elicitor of DPOAEs (the SP-$f_1$ algorithm) produced a larger nonlinear-distortion component with smaller delay; the differences were 1.54 dB (inter-quartile range, IQR, of 3.58 dB; $p < 0.001$) and 2.84 cycles (IQR = 2.95 cycles; $p < 0.001$), respectively. The differences were larger at lower stimulus intensities (one-sided Wilcoxon rank sum test; $p < 0.001$). In conclusion, this dependence on temporal arrangement of the primary tones provides a more comprehensive method to investigate cochlear nonlinearity.

WHEN MALE AND FEMALE EYES DIFFER: SEXUALLY DIMORPHIC RETINAL RESPONSES IN A MOTH

Henze MJ$^1$, Lind O$^3$, Alan Ha$^2$, Mappes J$^4$, Rojas B$^4$, Kelber A$^1$

1. Lund Vision Group, Department of Biology, Lund University, Sweden. 2. Queensland Brain Institute, University of Queensland, Queensland, Australia. 3. Cognitive Science, Department of Philosophy, Lund University, Sweden. 4. Centre of Excellence in Biological Interactions, University of Jyväskylä, Finland

Wood tiger moths (Arctia plantaginis) display a contrasting pattern of black stripes against bright patches on their wings. In Scandinavia, these patches are white or yellow on the hindwings of males, and vary from orange to red in females. To investigate the potential role of vision in mate detection and mate choice, we recorded electroretinograms from the compound eyes of the moths over the course of the day. Our results show that both sexes possess the insect standard...
reertoire of UV-, blue- and green photoreceptors, with no obvious sex-difference in spectral tuning. The maximal sensitivity of the blue receptor type is red-shifted by more than 20 nm compared to an equal spacing of sensitivities across the spectrum. However, model calculations suggest that this shift has no significant effect on colour discrimination. While the eyes of males and females are optimized for the same range of light intensities, an equal increase in intensity causes a bigger increase in response strength in males. Maximal responses to flashes of white light in males were more than twice as large as in females. This difference, which was highly significant and consistent over the course of the day, fits well to the moths’ behaviour. Females mostly sit in the vegetation and emit pheromones, whereas males fly to find a mate. Pheromones help males to locate females from afar, but they have to detect them visually in dense foliage once they are close. For flight control as well as for finding females, males need sensitive eyes.

Zoei Isherwood – UNSW Sydney

MODULATION OF NATURAL SCENE STATISTICS IN SPACE AND TIME – ITS EFFECT ON THE BOLD RESPONSE IN HUMAN VISUAL CORTEX

Zoei Isherwood1, Colin Clifford1, Mark Schira1,2, Branka Spehar1.

1. UNSW Sydney, Sydney, Australia. 2. University of Wollongong, Wollongong, Australia. 3. Neuroscience Research Australia, Sydney, Australia.

Purpose: Natural scenes are characterised by a specific distribution of spatial and temporal frequencies and associated luminance intensities, known as the 1/fα amplitude spectrum (α = 1.2). While this property is present in both spatial and temporal domains, little work has investigated the tuning of the visual system to this property in the temporal domain. Methods: Psychophysics was used to measure visual sensitivity and fMRI was used to measure BOLD responses in early visual areas (V1-V4) toward a wide range of spatial (α = 0.25, 1.25, 2.25) and temporal (α = 0.25, 0.75, 1.25, 1.75, 2.25) slope values. Results: Psychophysics – a significant effect was found for both spatial slope and temporal slope, whereby sensitivity peaked for stimuli with natural 1/fα spectra in both space (1.25) and time (1.25). The interaction between spatial and temporal slope was also significant, whereby peak sensitivity for shallow spatial slopes (0.25) peaked for shallow temporal slopes (0.25 – 0.75), for intermediate spatial slopes (1.25) sensitivity peaked for intermediate temporal slopes (1.25), and for steep spatial slopes (2.25) sensitivity peaked for steep temporal slopes (1.75 – 2.25). fMRI – following the psychophysics experiment, the same significant effects and interactions were found with the exception that peak responses for intermediate spatial slopes (1.25) were for stimuli with a temporal slope of 0.75, which is within the natural range. Conclusion: Our results show high correspondence between behavioural and cortical responses, providing evidence that the visual system is tuned to natural 1/fα distributions of frequency in both space and time.

Sammy Chi Sam Lee - Save Sight Institute - University of Sydney

SUBPOPULATIONS OF WIDE-FIELD GANGLION CELLS EXPRESS THE TRANSCRIPTION FACTOR FOXP2 IN MARMOSET RETINA

Lee SCS1,2, Martin PR1,2, Rosa, MGP3,4, Grünert U1,2

1. Save Sight Institute and Department of Clinical Ophthalmology, The University of Sydney, Sydney, NSW, Australia. 2. Australian Research Council Centre of Excellence for Integrative Brain Function, Sydney Node, The University of Sydney, NSW, Australia. 3. Department of Physiology, Monash University, VIC 3800, Australia. 4. Australian Research Council Centre of Excellence for Integrative Brain Function, Monash Node, Monash University, VIC 3800, Australia.

Purpose: The transcription factor FoxP2 is specific for the parvocellular layers of marmoset lateral geniculate nucleus (Iwai et al., Cerebral Cortex, 2012) and FoxP2 has been suggested to be expressed by parvocellular projecting retinal (midget) ganglion cells. The purpose of this study was to identify the retinal ganglion cells expressing FoxP2. Methods: Two retinas from were obtained from two marmosets (Callithrix jacchus). The retinas were fixed in 4% paraformaldehyde for 1 hour and then incubated with rabbit antibodies against Forkhead box protein P2 (FoxP2). Ganglion cell nuclei labelled with FoxP2 were targeted for intracellular injection with the lipophilic dye, Dil. Ganglion cells were imaged with a Zeiss confocal microscope and classified according to dendritic field size, stratification and branch density. Results: A total of 64 ganglion cells was injected at eccentricities from 0.4 mm to 11 mm. All cells were classified as wide-field ganglion cells. Most of the FoxP2 labeled cells were multi-tufted ganglion cells (34/64; 53%). Other ganglion cell types included recursive cells and thorny cells. Midget ganglion cells were never found to be labeled with FoxP2. Conclusion: Multi-tufted cells are a largely uncharacterized type of ganglion cell which, like recursive bistratified cells, are suggested to correspond to direction.
selective ganglion cells. Thus, FoxP2 may be a means to characterize direction selective ganglion cells in primate retina.

Alexander Pietersen – The University of Sydney

SPLIT IDENTIFY OF BLUE-OFF AND SUPPRESSED-BY-CONTRAST CELLS IN LATERAL GENICULATE NUCLEUS OF ANAESTHETISED MARMOSETS.

Pietersen A N J1,2,3, Eiber C D1,2,3, Zeater N1,2,3, Solomon S G3,4, and Martin P R1,2,3.
1. ARC Centre of Excellence for Integrative Brain Function, The University of Sydney. 2. Save Sight Institute, The University of Sydney. 3. School of Medical Sciences, The University of Sydney. 4. Experimental Psychology, University College London.

Purpose: We previously reported that neurons in the intercalated (koniocellular, K) layers of the lateral geniculate nucleus (LGN) show brain state-related variability in spike rate, in absence of visual stimulation (Cheong SK et. al., P.N.A.S. 2011). Suppressed-by-contrast (SBC) cells are characterised by high baseline spike rate which is transiently suppressed by stimulus presentation (Solomon et al., J Nphys 2010). ‘Blue-OFF’ cells are characterised by low spike rate and respond to short wavelength (S) cone decrement and medium-long (ML) wavelength increment stimuli. We here show that either signature can appear if the same cell is recorded during different brain states. Methods: Extracellular spike activity of K cells in the LGN provisionally classified as ‘blue-OFF’ (n=19) or SBC (n=9) were recorded in Sufentanil-anaesthetised marmosets (Callitrichix jacchus). Visual stimuli (achromatic and cone-isolating, uniform fields and gratings) were presented against a uniform grey background near 50 Cd / m^2. Results: Cone opponent signature (S-, ML+) was found in 89% of cells (25/28). In 93% (26/28) when baseline spike rate is low, S cone decrements cause excitation (n=13 cells) or no change in spike rate (n=13 cells) whereas ML+ are ineffective and ML- stimuli suppressive. When baseline spike rate is high the ML+ and ML- stimuli become suppressive, and therefore the blue-OFF cells acquire an SBC response profile. Conclusion: We propose that blue-OFF and SBC cells are a single cell type in which response signature depends on the baseline spike rate.

Markus Rothermel - RWTH Aachen University

IMPACT OF BASAL FOREBRAIN STIMULATION ON OLFACTORY BULB OUTPUT IN AWAKE MICE

The olfactory bulb (OB) receives neuromodulatory input from diverse areas whose role in shaping early olfactory processing, especially in the awake behaving animal, remain unclear. A specific activation of cholinergic diagonal band of Broca (HDB) terminals within the OB was shown to add an excitatory bias to OB output activity in anesthetized animals. However, wakefulness strongly modulates both, HDB as well as OB network activity posing the question of how HDB influences OB output in the awake animal. In order to answer this question we used a retrograde tracing approach to ensure GCaMP6 expression solely in OB output neurons and visualized their activity using imaging approaches. Widefield MT cell odor responses in awake animals consist of complex spatiotemporal sequences of short term excitation and longer lasting inhibition. Electrical HDB stimulation revealed an excitatory effect visible as a decrease of the inhibitory component of these sequences. This effect was still visible immediately after odor presentation as well as during odor presentation in the following trial, pointing to a relatively long lasting effect. Preliminary data acquired using 2-Photon imaging displayed mixed effects following HDB stimulation. Together, in agreement with data from anesthetized animals, these experiments reveal enhanced odor evoked responses also in the awake condition but also point to more complex responses. Future experiments will investigate HDB stimulation effects also in response to inhalation of odorless air alone and make use of behavioral tests to investigate the influence of modulating HDB activity on odor-discrimination learning.

Tamara Watson – Western Sydney University

MODULATION OF STIMULUS PROCESSING IN HUMAN PRIMARY VISUAL CORTEX AROUND THE TIME OF SACCADIC EYE MOVEMENTS

Watson TL1, Mannion DJ2, Clifford CWG2
1. School of Social Sciences and Psychology, Western Sydney University.
2. School of Psychology, UNSW Sydney.

Just before a saccadic eye movement, behavioural sensitivity is reduced for stimuli presented at fixation but increased for stimuli presented at the saccade target. To investigate the neural mechanisms underlying these effects we measured the Blood Oxygen Level Dependent (BOLD) response of human primary visual cortex (V1) to brief stimuli presented immediately prior to or immediately post a large horizontal saccade. Two stimuli were presented simultaneously, one close
to the initial fixation location and the other close to the saccade target. The BOLD response to each stimulus was compared to the sum of the response to that stimulus presented when no eye movement was made and the response to a saccade executed without the stimuli. When the stimuli were presented prior to the saccade, the response to the stimulus presented close to the saccade target was significantly increased, consistent with previous reports of increased behavioural sensitivity at the saccade target. However, there was also a significantly increased response in the part of V1 representing the retinotopic location that the pre-saccadic stimulus would have occupied after saccade execution had it remained onscreen. For stimuli presented immediately after a saccade, response was increased at the location on the screen from which the eyes had just departed. This pattern of results highlights the complexity of the dynamic neural processing occurring around the time of a saccade.

Molis Yunzab - National Vision Research Institute

**CONTRAST-DEPENDENT PHASE-SENSITIVITY IN MOUSE PRIMARY VISUAL CORTEX: AN INTRACELLULAR STUDY**

Yunzab M1,2, Choi V1, Meffin H1,2, Cloherty SL1, Scholl B3, Priebe NJ3, Ibbotson MR1,2

1. National Vision Research Institute, Australian College of Optometry, 374 Cardigan Street, Carlton, VIC 3053, Australia. 2. ARC Centre of Excellence for Integrative Brain Function, Department of Optometry and Vision Sciences, University of Melbourne, Parkville, VIC 3010, Australia. 3. University of Texas Austin, Centre for Perceptual Systems, 2415 Speedway, Austin, TX 78712, USA.

Neurons in the mammalian primary visual cortex (V1) are typically classified as either simple or complex on the basis of their receptive field structures. Simple cells have spatially offset ON and OFF subfields whereas complex cells have overlapping subfields. This receptive field difference can be quantified by measuring the phase sensitivity to sinusoidal gratings. Simple cell responses depend on the spatial phase of the stimulus while complex cell responses are phase invariant. We have previously demonstrated, using extracellular recording that the degree to which complex cell responses are invariant to spatial phase depends on the contrast of the drifting gratings. This contrast dependence could arise either from differences in the synaptic inputs of neurons or from differences in the intrinsic properties of neurons. To determine which of these mechanisms account for the contrast dependence of spatial phase invariance we made intracellular recordings of membrane potential from neurons in V1 of anaesthetised mice. We find that there is no change in the biophysical spike threshold of neurons, whereas synaptic drive is contrast dependent. Our result reveals that contrast-dependent changes in spatial phase sensitivity arise from network activity rather than a neuron’s intrinsic properties.

Ashleigh Chandra – University of Sydney

**IDENTIFICATION OF CALBINDIN POSITIVE CELLS IN THE HUMAN RETINA.**

Chandra AJ1,2, Lee S C1,2, Masri RA1,2, Grünert U1,2


**Purpose:** Retinal ganglion cells send visual signals from the eye to the brain. In the human retina over 20 morphological types of retinal ganglion cell have been distinguished. Here we studied calbindin positive cells in the ganglion cell layer of the human retina. **Methods:** Post mortem human eyes (n=2) were obtained from the Lions NSW Eye Bank with ethical approval. Retinas were fixed in 2% paraformaldehyde and sectioned vertically with a Vibratome, or processed as whole mounts. Sections were labelled with antibodies against calbindin and the ganglion cell marker RBPMS, antibodies against choline acetyl transferase (ChAT, a marker for starburst amacrine cells) and antibodies against melanopsin. Whole mounts were processed with antibodies against calbindin. Calbindin positive cells were intracellularly injected with the lipophilic dye, DiI. Preparations were imaged with a Zeiss confocal microscope. **Results:** In the ganglion cell layer, all of the ChAT positive cells were also labelled for calbindin, indicating that calbindin labels the displaced starburst amacrine cells. In addition, a small population (50/762 cells) of the calbindin positive cells in the ganglion cell layer were positive for RBPMS and thus are ganglion cells. Double labelling showed that the calbindin positive ganglion cells include melanopsin positive cells. Consistently, DiI injected calbindin immunoreactive cells had the morphology of starburst amacrine and melanopsin expressing ganglion cells. **Conclusions:** Ganglion cells and displaced amacrine cells in human retina are labelled with antibodies against the calcium binding protein calbindin. Calbindin is also a marker for melanopsin expressing ganglion cells in the human retina.
Tristan Chaplin – Monash University

**EFFECTS OF SPIKE COUNT CORRELATIONS ON POPULATION DECODING OF MOTION EMBEDDED IN NOISE IN THE MIDDLE TEMPORAL AREA**

*Chaplin TA*1,2, Allitt BJ1,2, Hagan MA1,2, Price NS1,2, Rajan R1,2, Rosa MGP1,2, & Lui LL1,2

1. Biomedicine Discovery Institute, Neuroscience Program and Department of Physiology, Monash University. 2. ARC Centre of Excellence for Integrated Brain Function, Monash University

The study of single neurons in the middle temporal area (MT) in response to motion embedded in noise has provided some of the most valuable insights into how the activity of neurons leads to perception. Yet clearly perception arises from the activity of many neurons, and whilst it was thought that correlations between neurons reduce the amount of information in a neuronal population, it is now recognised that certain correlation structures can actually enhance population coding.

To investigate population activity in this paradigm, we recorded 17 neuronal populations (consisting of 3-35 single or multi-units) in MT in 5 anesthetised marmosets with linear electrode arrays. We presented random dot motion stimuli and varied the strength of the motion signal by manipulating the coherence, and decoded the direction of motion at each level of coherence.

We tested whether a decoder that learnt the correlations performed better than a "correlation-blind" decoder, and whether the removal of correlations improved or impaired performance. We found that knowing the correlations generally improved decoding (p=0.048) as did the removal of correlations (p=0.02). We then examined the time-course of decoding and found that decoding was faster at higher coherences (p=0.031), in line with behavioural studies. Knowing the correlations resulted in faster decoding (p=0.038), but the removal of correlations did not improve the speed of decoding (p=0.487).

In summary, these results have implications for how information is encoded by populations of MT neurons, as well as how they may be decoded by downstream areas in decision making tasks.

Mark Gradwell – University of Newcastle

**DOING DIFFERENT THINGS THE SAME WAY: PARVALBUMIN+, CHOLINE ACETYLTRANSFERASE+, AND CALRETININ+ ISLET CELL CONNECTIVITY WITHIN THE DORSAL HORN OF THE SPINAL CORD**

*M Gradwell1, KM Smith1, LA Popp1, RJ Callister1, DI Hughes2, and BA Graham1*

1. School of Biomedical Sciences and Pharmacy, University of Newcastle, NSW; and Hunter Medical Research Institute (HMRI), NSW. 2. Veterinary and Life Sciences, University of Glasgow, Glasgow, UK.

Appropriate sensory perception occurs seamlessly despite the complex neural pathway from peripheral input to central perception. The dorsal horn of the spinal cord is an essential part of this pathway and inhibitory interneurons play a crucial role in maintaining contextually relevant sensory processing. Recent work has shown that disinhibition of the spinal cord allows tactile information to access nociceptive circuits (Takazawa & MacDermott 2010, Ishikawa et al, 2000), whereas activation of inhibitory interneurons can ‘rescue’ mice from neuropathic pain states (Petitjean H et al, 2015). Despite their important role, the connectivity of inhibitory interneuron sub-populations remains largely unknown. We have used ChR2 targeted against three inhibitory populations (parvalbumin, choline acetyltransferase, and calretinin islets) as a genetic entry point to manipulate both postsynaptic and presynaptic inhibition in mouse spinal cord slices. Our findings indicate that each inhibitory interneuron population strongly regulates local circuit excitability via direct postsynaptic inhibition onto a majority of local interneurons (79%, 64%, and 80%), including interconnectivity (61%, 58%, and 63%). We show that these post-synaptic connections rely on the inhibitory neurotransmitters GABA and glycine. Each population also forms axo-axonic contacts onto the same primary afferent terminal type from which they receive input, forming a feedback pathway. This presynaptic inhibition is GABA dependent and can be completely abolished by bath application of bicuculline. Taken together these findings suggest a common connectivity dogma exists within the dorsal horn for inhibitory interneurons, providing extensive post-synaptic but specific presynaptic contacts to effectively gate incoming sensory information.
Rania Masri – University of Sydney

**DISTRIBUTION OF THREE TYPES OF CONE BIPOLAR NEURONS IN THE INNER NUCLEAR LAYER OF THE HUMAN RETINA.**

*Masri RA1,2, Purushothuman S1, Lee SCS1,2, Martin PR1,2, Grünert U1,2*


**Purpose:** Neurons in the retina are organized into parallel pathways that convey signals serving distinct submodalities of vision. This study aimed to map retinal interneurons involved in pathways serving high acuity vision (flat midget bipolar, FMB cells) and motion perception (diffuse bipolar types DB3a and DB3b) in humans. **Methods:** Post mortem human eyes (n=6) were obtained from the Lions NSW Eye Bank with ethical approval. Retinal pieces of defined eccentricity were embedded in Agarose and sectioned vertically using a Vibratome. The sections were double or triple labeled using a variety of immunohistochemical markers: cone photoreceptors were labeled with antibodies against cone arrestin, FMB cells were labeled using antibodies against recoverin, and bipolar cell types DB3a and DB3b were labeled with antibodies against calbindin and CD15 respectively. Sections were imaged using a confocal microscope and cell populations were counted across the length of the section. **Results and conclusions:** We found that the density of FMB cells is much higher than that of DB3a and DB3b cells. At 1mm eccentricity, the average density of FMB cells is 30,000 cells/mm$^2$ whereas the density of DB3a and DB3b cells is 5,000 and 7,000 cells/mm$^2$, respectively. Cone photoreceptors and FMB cells show matched density (implying one-to-one connectivity) at least to 8 mm eccentricity, where the FMB density averages 7,700 cells/mm$^2$ and the cone density averages 7,600 cells/mm$^2$. Our results indicate a differential distribution of neurons involved in high acuity and motion vision pathways in human retina.

Victor Perez-Fernandez – Western Sydney University

**PATHWAYS LEADING TO DOPAMINE RELEASE IN THE MAMMALIAN RETINA**

*Pérez-Fernández V, Morley JW, Cameron MA.*

*Department of Anatomy and Cell Biology, School of Medicine, Western Sydney University, Australia.*

Adaptation of the retina to distinct light conditions relies considerably on modulation of retinal pathways by dopamine (DA) released in response to light by a sub-set of neurons: dopaminergic amacrine cells (DACs). Cones, rods and intrinsically photosensitive retinal ganglion cells (ipRGCs) have been shown to provide an input to DACs, but thus far, only rods and cones have been shown to cause DA release. We measured light-induced DA release in an *ex vivo* preparation of the mouse eye in animals with impaired photoreceptor function. Irradiance-response curves were performed for wild-type, Gnat2$^{A517G}$ (lacking functional cones) and *rd/rd* mice (lacking both rods and cones). A significant increase (p-value<0.001) in DA was observed in response to high irradiances (>1000 lux) in wild-type and Gnat2$^{A517G}$ but not in *rd/rd*. Interestingly, while all evidence suggests rods are driving the majority of this input, the threshold for DA release is 6 log units above rod threshold. Gap junction blockage by meclofenamic acid caused a significant reduction, but not total loss of light-induced DA in both wild-type and Gnat2$^{A517G}$, meaning a rod-driven gap-junction-independent pathway is involved. In *wild-types,* GABA antagonists significantly increased DA release in darkness suggesting a constitutive block of DACs by GABAergic amacrine. In agreement with the literature, glutamate receptor blockers L-AP4 and CNQX completely blocked light-driven DA release. Moreover, the NMDA receptor blocker APS abolished the majority of DA release. We postulate that rods and ipRGCs work in synergy through NMDA/AMPA coincidence detection to provide a reliable signal for light-adaptation in the retina.
A PROTECTIVE ROLE FOR COMPLEMENT C3aR ACTIVATION IN SOD1G93A MICE.

Conroy JN1, Noakes PG1,2, Woodruff TM1, Lee JD1,3

1. School of Biomedical Sciences, the University of Queensland, QLD. 2. Queensland Brain Institute, the University of Queensland, QLD. 3. University of Queensland Centre for Clinical Research, the University of Queensland, QLD.

The complement system is an integral component of innate immunity and has been implicated in the pathogenesis of motor neuron disease (MND). Complement is activated in human MND and in mouse models, and its activation generates the bioactive fragment C3a, which can modulate the inflammatory response by binding to its receptor, C3aR. However, the contribution of C3aR to disease progression in MND is still unknown. The current study aimed to determine the function of C3aR in the disease progression of MND using SOD1G93A mice lacking the C3aR (SOD1G93A x C3aR−/−). Behavioral tests were conducted to observe any differences in motor symptoms, along with body weight and survival. Neuroinflammation was also measured in these animals by determining the degree of gliosis and cytokine expression using quantitative PCR at defined disease stages. SOD1G93A x C3aR−/− mice showed significantly reduced survival and body weight relative to SOD1G93A mice. SOD1G93A x C3aR−/− mice also displayed greater motor dysfunction, at an earlier age, compared to SOD1G93A mice. In line with this worsened disease progression, there was increased gliosis gene expression at later stages of disease, while inflammatory cytokine gene expression was increased at earlier disease stages in SOD1G93A x C3aR−/− mice, relative to SOD1G93A. These results indicate that the C3a generated during complement activation in MND, has anti-inflammatory activities in the SOD1G93A mouse. Hence modulating complement activation in MND should ideally be targeted towards downstream C5a inhibition, in order to avoid blocking any endogenous protective effects of upstream factors such as C3a.

ELEVATED SURFACE EXPRESSION OF C5AR1 ON PERIPHERAL BLOOD MONOCYTES FROM MOTOR NEURON DISEASE PATIENTS

McGill R1, Mantovani S2, Lee JD1,3, Steyn FJ1-4, Ngo ST1-6, Ruitenber MJ1, Henderson RD4,5, McCombe PA 3,4, Woodruff TM1,2.

1. School of Biomedical Sciences, University of Queensland, QLD. 2. Wesley Medical Research, The Wesley Hospital, QLD. 3. University of Queensland Centre for Clinical Research, University of Queensland, QLD. 4. Department of Neurology, Royal Brisbane & Women’s Hospital, QLD. 5. Queensland Brain Institute, University of Queensland, QLD. 6. Australian Institute for Bioengineering and Nanotechnology, University of Queensland, QLD.

The complement system is a key component of the innate immune response, and has been implicated in the pathogenesis of motor neuron disease (MND). Complement activation produces C5a, which binds to its receptor, C5aR1 to induce potent inflammatory responses. Emerging research suggests that peripheral immune cell activation and central nervous system (CNS) infiltration, perpetuates chronic neuroinflammation in MND. Our prior work has demonstrated increased circulating blood C5a in MND patients. Therefore, the current study aimed to investigate surface and intracellular C5aR1 expression on various peripheral blood leukocyte populations from MND patients and healthy control subjects. Flow cytometry was used to measure C5aR1 expression on CD16+ granulocytes, monocyte subset populations and CD4+ T cells. We identified significantly increased C5aR1 surface expression on HLA-DR+ CD14+ CD16- classical monocytes from MND patients compared to control participants. No change of surface or intracellular C5aR1 expression was observed on other leukocyte populations. These results demonstrate that there is a selective upregulation of C5aR1 on the pro-inflammatory M1-like classical monocytes in MND patients. These cells may be engaging with C5a to drive their recruitment to, and inflammatory responses within, the diseased CNS. Therapeutic inhibition of C5aR1 may therefore be an
option to reduce neuroinflammation and slow disease progression in MND.

Victoria McLeod (Florey Institute Of Neuroscience And Mental Health)

**ANDROGEN RECEPTOR TRANSCRIPTIONAL TARGETS IN MUSCLE AND MOTOR NEURONS**

McLeod VM, Lau CL, Boon WC, Turner BJ
1. Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, Australia.

The androgen receptor (AR) is a major transcriptional regulator of many genes throughout the body. Of growing interest is its diverse and complex roles throughout the central nervous system. AR is expressed in motor neurons of the spinal cord where it has been shown that androgens can have both direct and indirect trophic effects supporting both survival and regeneration after damage. AR is also expressed in myonuclei and satellite cells of adult muscle tissue where it plays a major role in myogenic commitment and anabolic processes. Trophic factors, such as BDNF have also been shown to be retrogradely transported to motor neurons form their innervation targets in the muscle. While previous studies elude to the neuroprotective effects of androgens and AR signaling the mechanisms and identity of transcriptional targets often remains obscure. Here we have identified and confirmed direct AR target genes in human embryonic stem cell (hESC) derived mature motor neurons and mouse C2C12 myoblasts and differentiated myotubes. Cells expressing endogenous AR or overexpressing native human AR were treated for 24h with the AR agonist, dihydrotestosterone, alone or in combination with the antagonist flutamide and RNA was extracted for qPCR analysis of gene targets, including VEGF, BDNF, IGF-1, NTF4, NRN1, CALCA & TGFβ1. Future studies will confirm AR regulation of neurotrophic genes in AR knockout and overexpression models.

Gayathri Perera (The University of Sydney)

**INCREASED LRRK2 KINASE ACTIVITY FOLLOWING ACTIVATION OF TOLL-LIKE RECEPTORS**

Perera G, Halliday G.M, Dzamko N
1. Faculty of Medicine, Central Clinical School, University of Sydney

Mutations in leucine-rich repeat kinase 2 (LRRK2) that affect the enzymes GTPase and kinase activities are linked to familial Parkinson’s disease (PD), as well as an increased risk of sporadic PD. However, how LRRK2 predisposes to PD is unknown, largely because the biological function(s) of LRRK2 are unclear. LRRK2 is highly expressed in monocytes and macrophages, and has been implicated in the regulation of inflammatory pathways. Using a recently described LRRK2 autophosphorylation site (Ser1292) as a measure of LRRK2 kinase activity, we have discovered that LRRK2 activity is increased in both human peripheral blood mononuclear cells, and primary human macrophages, treated with agonists of the toll-like receptor (TLR) inflammatory signaling pathway. To determine if LRRK2 kinase activity is required for the production of inflammatory cytokines following TLR activation, we treated primary human macrophages with the TLR2 agonist pam3CSK4, in the presence or absence of two selective LRRK2 kinase inhibitors, and measured levels of inflammatory cytokines by ELISA. Secreted levels of IL6, IL12, CCL5, TNFa, MIP1a and MIP1b were significantly reduced (p<0.05) in the LRRK2 inhibitor treated groups. As the reduced cytokines were largely regulated by the NfkB transcription factor, we also measured NfkB transcriptional activity and indeed, this was also significantly reduced in LRRK2 inhibitor-treated, TLR2-stimulated macrophages. These results strongly suggest a role for LRRK2 in regulating inflammatory signaling downstream of TLR activation. As inflammation is associated with PD, these results may be important for understanding LRRK2 pathogenicity.

Sharn Perry (Wicking Dementia Research And Education Centre,University Of Tasmania)

**DMRT3-DERIVED NEURONS MODULATE THE ALTERNATION-SYNCHRONY LOCOMOTOR SWITCH**

Perry S*1,2, Larhammar M*1, Nagaraja C1, Hilscher MM1, Tafreshiha A1, Potter E1, Edwards SJ1, Caixeta FV1 and Kullander K*1
1. Department of Neuroscience, Uppsala University, Uppsala, Sweden. 2. Wicking Dementia Research and Education Centre, University of Tasmania, Hobart, Tasmania, Australia.

*Authors contributed equally to this work
*Correspondence: klas.kullander@neuro.uu.se
Neuronal networks within the spinal cord coordinate rhythmic movements such as locomotion. The transcription factor DMRT3 is involved in the differentiation of the d16 class of spinal cord interneurons. A non-sense mutation in the horse Dmrt3 gene has major effects on gaiting ability, whereas mice lacking the Dmrt3 gene display impaired CPG activity and locomotion. Although the Dmrt3 gene is required for normal spinal neuronal network formation and function, a role for Dmrt3-derived neurons has not been demonstrated. Here we show that inhibitory Dmrt3 interneurons in mice receive extensive synaptic inputs from several sources, display accommodating properties, and are rhythmically active during fictive locomotion when they fire at frequencies independent to the ventral root output. Conditional removal of VIAAT dependent inhibitory neurotransmission from the Dmrt3 population resulted in an uncoordinated CPG output in vitro, severely impaired limb coordination in vivo, and increased limb synchrony at high running speeds. The present study provides novel insights on the role of Dmrt3 neurons in locomotor coordination and suggests that inhibition arising from Dmrt3 interneurons acts to balance excitatory inputs, with subsequent impact on locomotor coordination.

**AXONAL SPIKE BURSTING IN THE INFERIOR OLIVE IS OSCILLATORY STATE-DEPENDENT**

Tang AD and Uusisaari MY

Neuronal Rhythms in Movement Unit, Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan.

The inferior olive (IO) is thought to relay instructive signals required for motor learning to the cerebellum. Information from the IO is transmitted as bursts whereby single somatic action potentials are followed by short, high frequency axonal spikes ("spikelets"). The number of spikelets in each IO spike burst is known to modulate cerebellar output. Despite their importance, the factors which determine the number of spikelets are unclear. Furthermore, whether spikelet number modulation is similar in both oscillating and non-oscillating IO neurons is unknown. We investigated whether the intrinsic passive and active membrane properties of IO neurons determines the number of spikelets in both oscillating and non-oscillating neurons. Somatic whole-cell current-clamp recordings were made from adult mouse brainstem slices and spike analysis was restricted to cells displaying spontaneous somatic action potentials. Preliminary results suggest that the number of spikelets in oscillating neurons is correlated to the high-threshold calcium-related after depolarisation (r=0.83, p<0.01) but not to the phase of the oscillation (r=0.06, p=0.77), or threshold voltage (r=-0.64, p=0.74) at which the somatic action potential is initiated. Non-oscillating IO neurons displayed spikelets which were correlated with threshold voltage (r=-0.81, p<0.01) and the high-threshold calcium-related after depolarisation (r=0.33, p=0.02). Our results suggest that information transfer from the IO in the form of axonal spikelets is in part dependent on the oscillatory state of the IO neuron. These preliminary findings help to elucidate the function of IO oscillations and axonal spike bursting in motor learning.

Brittney Black (Centre For Brain Research, University Of Auckland)

**GLUTAMATE RECEPTOR STUDIES IN THE HUMAN GLOBUS PALLIDUS**

Black BL, Waldvogel HJ, Faull RLM

Centre for Brain Research, University of Auckland, Auckland, New Zealand

The basal ganglia are a group of interconnected nuclei in the center of the brain, associated with controlling mood and movement, and converge on the internal segment of the globus pallidus (GP). Dysfunction of the basal ganglia nuclei and their pathways cause a variety of movement disorders including Huntington’s and Parkinson’s disease, two devastating disorders that are characterized by opposing motor symptoms. The objective of this study is to characterize the neuronal populations in the human globus pallidus, with a focus on the subpopulations expressing glutamatergic receptors and to compare between normal and disease states. Using immunohistochemistry, the GP of normal human brains are microscopically examined and imaged. The 70µm slices are immunostained for glutamatergic receptors (GluN1), GABA\textsubscript{A} receptors, vesicular transporters (VGLUT2, VGAT), and calcium binding proteins (Parvalbumin, Calretinin). We have discovered a new configuration of excitatory and inhibitory receptor systems on cells in the GP. The results indicate that there are separate input patterns converging on two different cells types in the GP, where it has been previously accepted as converging on only one, suggesting that there are at least two unique cell types within the human GP. Some of which contain mainly inhibitory receptors and others which contain mainly excitatory receptors. If this is true, it will
change the way we think about how the basal ganglia operates: two different pathways. Thus having major implications for understanding the processing of mood and movement in the human brain and how we may develop more effective treatments for neurological diseases.

Zora Chui Kuen Chan (The University Of Hong Kong)

EXTRACELLULAR MATRIX PROTEIN-INDUCED ASSEMBLY OF PODOSOME-LIKE STRUCTURES FOR ANEURAL ACETYLCHOLINE RECEPTOR CLUSTER REMODELING

Chan ZC, Wong YS and Lee CW
School of Biomedical Sciences, LKS Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong.

The presence of high acetylcholine receptor (AChR) density is a prominent feature of neuromuscular junctions (NMJs). Previous studies have shown that integrin-beta-1 is localized at the cortex domain of podosome-like structures at aneural AChR clusters induced by extracellular matrix (ECM) proteins, suggesting the involvement of integrin-mediated signaling pathway in the formation and/or remodeling of aneural AChR clusters. However, the causal relationship among different ECM proteins, podosome-like structures, and aneural AChR cluster remodeling is not fully understood. In this study, we first showed that the formation of aneural AChR clusters is spatially induced in muscle cells cultured on micro-patterns of various extracellular matrix (ECM) proteins by utilizing multiphoton-crosslinking fabrication technique. Additionally, several typical core and cortex markers of podosome-like structures are detected in the perforated regions of the aneural AChR clusters induced by those ECM proteins. Next, we used total internal reflection fluorescence (TIRF) microscopy and fluorescence recovery after photobleaching (FRAP) to further understand the functional involvement of podosome-like structures at developing NMJs. Live cell time-lapse imaging of microtubule plus-end protein EB1 demonstrated that microtubules are targeted to and captured at the cortex domains of perforated aneural AChR clusters, suggesting that the podosome-like structures may direct the local surface delivery of new AChR molecules to the aneural AChR clusters via microtubule-based vesicle transport. Further work aimed to investigate the molecular mechanisms involved in ECM protein-induced assembly of podosome-like structures through integrin-mediated signaling pathways to facilitate the formation and/or remodeling of aneural AChR clusters at developing NMJs.

Amal Galal (Unsw Sydney)

THE EFFECT OF RUBROSPINAL TRACT (RST) AND VENTROLATERAL FUNICULUS (VLF) LESIONS ON RODENT OVERGROUND LOCOMOTION

Galal AS\(^1\), Brown JS\(^1\), Morris R\(^1\)

1. Translational Neuroscience Facility (TNF), School of Medical Sciences (SoMS), UNSW Sydney, Australia.

Creating accurate cervical injury models that replicate human forelimb behaviour in the rodent is an imperative objective in the search for effective therapies to spinal cord injury. Rodents exhibit distinct motor components during the stepping motion comprising ‘lift and release’, ‘carry’, ‘advance’ and ‘arpeggio’ (Whishaw et al., 2010). The carry and advance motions constitute the swing phase of the step, whilst the arpeggio is defined as the sequential lateral to medial placement of the digits. In order to understand the effect of rubrospinal tract (RST) lesions in the context of locomotion, female Long Evans rats were trained to walk down a straight alley prior to a sequential lateral to medial placement of the digits. In order to advance the stepping motion comprising ‘lift and release’, ‘carry’ and ‘advance’, the ‘arpeggio’ was defined as the sequential lateral to medial placement of the digits. In order to understand the effect of rubrospinal tract (RST) lesions in the context of locomotion, female Long Evans rats were trained to walk down a straight alley prior to a unilateral cervical RST transection. Frame-by-frame video analysis showed that lesions limited to the RST specifically interfered with the ability to perform the arpeggio (Morris et al., 2011). However, some of the transections produced larger lesions that encompassed a portion of the ventrolateral funiculus (VLF). Such lesions resulted in dysfunction of the carry and advance components of rodent locomotion. Comparison of qualitative scores (mean ± SEM) for carry, advance and arpeggio were carried out before and after these two lesion types. Rats with larger RST+VLF lesions were significantly impaired in carry (*p=0.0406, p<0.05), advance (*p=0.0408, p<0.05) and arpeggio (**p=0.005, p<0.01). Small RST lesions however, resulted in significant impairments in the arpeggio alone (*p=0.0339). We are now investigating whether the VLF is solely responsible for the carry and advance components of locomotion and does not contribute to the arpeggio movement.

Samantha Levin (University of Queensland)

ABLACTION OF FREE FATTY ACID RECEPTOR 2 (FFAR2) SIGNALING ACCELERATES EARLY DISEASE PROGRESSION IN THE SOD1G93A MOUSE MODEL OF MOTOR NEURON DISEASE
The gut microbiome and short chain fatty acids (SCFAs) have been shown to modulate immune and inflammatory responses in both the CNS and peripheral tissues. Specifically, SCFA signaling through its receptor, FFAR2, has been shown to modulate innate immune responses, as well as microglia morphology and activation. Chronic neuroinflammation plays a pathological role in motor neuron disease (MND), and both MND patients and mouse models show microbiome dysbiosis. This suggests that FFAR2 signaling could play a potential role in the progression of this disease. This study aimed to determine the function of FFAR2 signaling in the disease progression of MND by comparing SOD1<sup>G93A</sup> mice to SOD1<sup>G93A</sup> mice lacking FFAR2 (SOD1<sup>G93A</sup> x FFAR2<sup>−/−</sup>). Neuromotor assessments and qPCR analysis identified increased inflammation in the lumbar spinal cord and a corresponding increase in motor deficits in the SOD1<sup>G93A</sup> x FFAR2<sup>−/−</sup> mice when compared to SOD1<sup>G93A</sup> mice, at mid symptomatic disease stage (130days). Interestingly, the survival of the SOD1<sup>G93A</sup> x FFAR2<sup>−/−</sup> mice was not significantly different from the SOD1<sup>G93A</sup> mice, and at disease end stage, SOD1<sup>G93A</sup> x FFAR2<sup>−/−</sup> mice had reduced spinal cord inflammation compared to SOD1<sup>G93A</sup> mice. Overall, these results indicate that FFAR2 may play a protective role in the early stages of disease progression in the SOD1<sup>G93A</sup> mouse model of MND, but switches to a pathological impact at later stages of the disease. This data therefore suggests that therapeutic targeting of FFAR2, or manipulation of dietary SCFAs in MND, must be carefully approached to target the appropriate disease stage.

Esmeralda Parić (University of New South Wales)

**VOLATILE VS INJECTABLE ANAESTHETICS: CONSIDERATIONS FOR ELECTROPHYSIOLOGICAL STUDIES IN THE RAT.**

Parić E<sup>1</sup>, Wild BM<sup>1</sup>, Arnold R<sup>2</sup>, Morris R<sup>1</sup>
1. Translational Neuroscience Facility (TNF), School of Medical Sciences (SoMS), UNSW Sydney, Australia. 2. Exercise Physiology Department, School of Medical Sciences (SoMS), UNSW Sydney, Australia.

Nerve excitability testing (NET) is a reproducible and reliable means of testing axonal ion channel function and membrane potential in clinical settings. The use of this technique in rodent models of disease provides an attractive translational methodology. Unlike in humans, anaesthetics are required in animal research, therefore, systematically examining the effects of different anaesthetics is important for the implementation of this technique. Isoflurane and ketamine/xylazine are widely used in experimental settings, however, their effects on NET are unclear. NET was performed on the rat ulnar nerve after administration of either isoflurane or ketamine/xylazine using a cross over design (n=12). Comparing the ulnar NET results obtained under these two anaesthetic agents revealed multiple significant differences in various parameters. There was a significant difference between the two anaesthetic agents in the threshold electrotonus parameters TEd(90-100ms), p=0.016 and TEd(overshoot), p=0.005. Additionally, significant differences were revealed in the subexcitability parameter (p<0.001) of the recovery cycle. These results demonstrate that different anaesthetics have different effects on motor excitability properties. However, the contribution of xylazine in the injectable agent i.e., the muscle relaxant counterpart of ketamine, to NET remains to be clarified. To isolate the effects of xylazine on NET, a follow-up study is currently being conducted using isoflurane with xylazine. Holistically, this study has the potential to reveal the effects of anaesthetic agents and muscle relaxants on axonal ion channel function and membrane potential. This study also has the potential to dictate the optimal agent to use for improved translatability of nerve excitability testing.

Jane Wu (University of Auckland)

**THE SUBTHALAMIC NUCLEUS SHOWS PROFOUND GENE EXPRESSION CHANGES IN PARKINSON’S DISEASE**

Xi H. Wu<sup>1,2,3</sup>, Suzanne J. Reid<sup>3,4</sup>, Russell G. Snell<sup>3,4</sup>, Michael Dragunow<sup>2,3</sup>, Maurice A. Curtis<sup>1,3</sup>, Richard L.M Faull<sup>1,3</sup>, Henry J Waldvogel<sup>1,3</sup>
1. Department of Anatomy and Medical Imaging, 2. Department of Pharmacology, 3. Centre for Brain Research, University of Queensland, St. Lucia, Australia.
Parkinson’s disease (PD) is a progressive neurodegenerative movement disorder characterised by extensive death of dopaminergic neurons in the substantia nigra. The global decrease in dopamine levels within the basal ganglia leads to the emergence of distinctive pathophysiology in multiple signalling midbrain regions. The subthalamic nucleus (STN) is one of the critical basal ganglia nuclei affected, which displays elevated activation in PD and contributes to the cardinal hypokinetic motor symptoms. In order to better understand the role of the STN in PD, we have investigated the human STN transcriptome in seven PD and nine neurologically normal post-mortem brains. Differential gene expression analysis conducted between PD and normal brains yielded 157 statistically significant differentially expressed genes, 126 up-regulated and 31 down-regulated, in the PD STN. Collectively, the up-regulated genes showed enrichment in multiple cellular processes including neuroinflammation, immune response, regulation of cell death and blood vessel homeostasis. Conversely, the down-regulated genes showed profound dysregulation of dopamine biosynthetic and metabolism processes. A smaller subset of identified genes was then validated using Nanostring nCounter expression analysis, confirming expression differences in 21 of 26 assessed genes. Our results show the occurrence of a distinct pattern of gene expression changes within the human STN following degeneration of dopaminergic nigrostriatal neurons in PD. Although the STN constitutes one of the brain regions that shows minimal neurodegeneration in PD, our differential gene expression data suggest that this structure is fundamentally altered in PD.

Nadia Carinaria (University of Bristol)

AN ACTION BASED MAP OF C3 CEREBELLAR MICROZONES

Cerminara NL1, Garwicz M2, Marple-Horvat DE3, Apps R1
1. School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, UK. 2. Division of Neuroscience, Department of Experimental Medical Science, Lund University, Sweden. 3. Centre for Biomedicine, Manchester Metropolitan University, Manchester, UK.

A fundamental principle of cerebellar functional organization is its division into a series of longitudinally oriented modules. Each module is defined by its climbing fibre input from a specific subdivision of the contralateral inferior olive, which targets one or more longitudinal zones of Purkinje cells within the cerebellar cortex. In turn, Purkinje cells within each zone project to specific regions of the cerebellar or vestibular nuclei. Within several zones, smaller units known as ‘microzones’ have been identified electrophysiologically in anaesthetized animals. Within a given microzone, all Purkinje cells have climbing fibre-mediated input with similar receptive fields. Microzones and their associated input–output connections are thought to represent the basic operational units of the cerebellum. However, little is known about their function in awake, behaving animals. The aim of the present study has been to study microzones in the forelimb part of the C3 zone (lobule V) ‘in action’ by recording from single cerebellar neurons located in different microzones during performance of a visually guided forelimb reach/retrieval task in cats. Findings to date suggest that that individual classes of microzones monitor different aspects of a skilled movement, probably reflecting their distinct functional responsibilities in motor control. This suggests that rather than conforming solely to sensory maps of the body surface, cerebellar topography can be viewed as an action based map.

Victoria Mcleod (Florey Institute of Neuroscience and Mental Health)

EFFECT OF ANDROGEN RECEPTOR ANTAGONISM ON DISEASE PROGRESSION IN THE SOD1G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

McLeod VM1, Lau CL1, Rupasinghe TW2, Boon WC1, Turner BJ1
1. Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, Australia. 2. Metabolomics Australia, School of BioSciences, University of Melbourne, Parkville, Victoria, Australia

In amyotrophic lateral sclerosis (ALS) a gender bias towards higher male incidence exists suggesting underlying hormonal differences may be influencing disease progression. Activation of androgen receptor (AR) provides trophic support to motor neurons (MNs) and a mutation in the AR gene gives rise to selective lower MN vulnerability in spinal bulbar and muscular atrophy (SBMA). Therefore, we sought to block AR signaling in the SOD1G93A mouse model of ALS using the antagonist, flutamide, predicting a hastening of disease progression. Flutamide (5mg/day) or placebo were administered to mice from presymptomatic P60 onwards via a slow-release subcutaneous implant. Circulating testosterone and drug levels were confirmed by liquid
progressive motor deficits in male flutamide treated mice compared to placebo, however, chronic AR blockade was unable to slow late disease progression in this mouse model. Pathology on symptomatic P120 tissue showed no change in MN and glial cell counts, although indicators of muscle atrophy, myogenin and TGFβ1 transcript levels, were significantly elevated in the muscle tissue of flutamide treated male mice.

In conclusion, systemic blockade of AR signalling from presymptomatic age was unable to modulate motor deficits, neurodegeneration or disease outcome in SOD1G93A mice, despite aggravating peripheral muscle atrophy. Recent evidence implicating prenatal testosterone exposure as a risk factor in ALS, in addition to conflicting outcomes on the protective effects of estradiol, highlights the complexity of the steroid hormone systems which may be involved in driving sex-specific differences in ALS.

Annika van Hummel (UNSW)

PROGRESSIVE MOTOR DEFICITS IN AN AGED MUTANT TDP-43 TRANSGENIC MOUSE MODEL OF ALS

A. van Hummel1,2, J. van der Hoven2, S. Ippati2, M. Morsch1, G. Chan2, Chung R1, L. M. Ittner2,4, Y. D. Ke1,2
1. Motor Neuron Disease Unit, Department of Anatomy, School of Medical Sciences, Faculty of Medicine, University of New South Wales, NSW, Australia. 2. Dementia Research Unit, Department of Anatomy, School of Medical Sciences, Faculty of Medicine, University of New South Wales, NSW, Australia. 3. Motor Neuron Disease Research Group, Faculty of Medicine and Health Sciences, Macquarie University Sydney, NSW, Australia. 4. Neuroscience Research Australia Sydney, Australia.

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, fatal neurological disease that results from the gradual death of motor neurons. Mutations in several genes including TARDBP, which encodes TDP-43, have been identified in familial ALS. In ALS and frontotemporal dementia (FTD), the nuclear factor TDP-43 undergoes relocation and deposition into the cytoplasm of neurons. Pathogenic mutations in the TARDBP gene in familial ALS as well as non-mutant human TDP-43 have been utilized to model FTD/ALS in cell culture and animals, including mice. We have recently reported a novel A315T mutant TDP-43 transgenic mouse with inducible neuronal over-expression (Ke YD et al., Acta Neuropathol 2015). In the present follow-up study of aged mice, we focused on the progression of motor deficits, and degeneration of musculature and upper and lower motor neurons. We found that constitutive expression of human TDP-43A315T resulted in progressive deterioration of gait and a progressive decline in a range of motor tests, including rotarod, vertical pole test and hanging wire test, accompanied by muscle atrophy. Magnetic resonance imaging (MRI) and histology revealed cortical atrophy, including motor areas, in TDP-43A315T mice. In contrast to cortical motor areas and despite the pronounced motor deficits and TDP-43 pathology, stereological analysis did not show cell loss in spinal cords or any overt changes in neuromuscular junctions (NMJ). Taken together, aged TDP-43A315T mice present with selective vulnerability of upper motor neurons to TDP-43 pathology leading to progressive motor and gait deficits, recapitulating aspects of ALS.

Mathew Chiam (Florey Institute of Neuroscience and Mental Health)

ATP13A2 DEFICIENCY LEADS TO ENDO-LYSOSOMAL DYSFUNCTION AND ALPHA SYNUCLEIN ACCUMULATION IN TRANSGENIC MICE EXPRESSING PATHOGENIC ALA53THR HUMAN A-SYN

Chiam Mathew1, Liu JP2, He L2, Horne MK1, Turner BJ1
1. Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Australia. 2. Murdoch Childrens Research Institute, Parkville, Australia

Parkinson’s disease (PD) is characterized by cytoplasmic accumulation and aggregation of α-synuclein which may reflect impairment of autophagy and lysosomal-mediated protein degradation pathways. Recently, familial PD genes such as ATP13A2 have been suggested to converge on modulation of autophagy pathways and therefore α-synuclein metabolism. In this current study, we have crossbred A53T M83 mice on an ATP13A2 null background (A53T M83-ATP13A2null mice) in efforts to investigate the impact of ATP13A2 genetic deficiency on α-synuclein accumulation, autophagy and neurodegeneration in a mouse model of PD. Brain regions including cortex, cerebellum and olfactory bulb were analysed for α-synuclein accumulation, autophagic and endocytic marker levels using Western blotting. Our current immunoblot analyses have shown the accumulation of SDS-soluble α-synuclein accumulation in the cortex of 3-month-old crossed mice but not in other analysed brain regions. Here we report that there were some alterations in autophagy pathways, whereby we saw an altered
levels of matured autophagosomes in both the olfactory bulb and cortex. Also, our preliminary data have shown the possible role of ATP13A2 in mitochondrial dynamics whereby the mitochondrial fission process was affected in the cerebellum of 3-month-old crossed mice. Despite the changes in protein degradation pathways, the locomotor activities of 3-month-old mice were still comparable to A53T M83 mice. In summary, our results may highlight the role of ATP13A2 in maintaining α-synuclein homeostasis and regulation of endo-lysosomal pathways.

Hossai Gul (Macquarie University)

**DISCOVERY OF PROTEINS AND PATHWAYS CONTRIBUTING TO TDP-43-MEDIATED NEURODEGENERATION IN A NOVEL TRANSGENIC MOUSE MODEL OF DISEASE.**

Gul H\(^1\), Mehta P\(^1\), Hedl T\(^1\), Le S\(^1\), Wright A\(^1\), Berning B\(^1\), Riddell W\(^1\), Lee A\(^1\), Krisp C\(^2\), Molloy MP\(^2\), Walker AK\(^1\).

1. Centre for MND Research, Department of Biomedical Sciences, Macquarie University, NSW, Australia. 2. Australian Proteome Analysis Facility, Macquarie University, NSW, Australia.

Motor neuron disease (MND)/amyotrophic lateral sclerosis (ALS) is an incurable and fatal neurodegenerative disease caused by progressive loss of motor neurons controlling movement. The main pathology exhibited by 97% of ALS cases is the aggregation of the RNA/DNA-binding protein TDP-43 within the cytoplasm of affected neurons. Although ALS pathology is characterised by TDP-43 accumulation, it is not understood how this dysfunction causes disease. Using a new transgenic mouse model that exhibits TDP-43 pathology as well as a disease phenotype very similar to human ALS, we performed a large-scale proteomic study to discover the protein changes involved in TDP-43-mediated pathogenesis. Advanced quantitative mass spectrometry (SWATH-MS) identified 200 proteins with statistically significant increased or decreased levels (>1.5-fold) within the brain and/or spinal cord of TDP-43 transgenic mice at early, mid and late stages of disease development, compared to matched non-transgenic littermate controls. Immunoblotting and immunofluorescence using specific antibodies against a subset of the highest-prioritised identified targets confirmed that numerous proteins and pathways showed altered levels and subcellular location throughout disease onset and progression. Bioinformatic analysis using several analysis tools showed a dynamic pathology with non-linear changes within the pathways and cellular processes during disease progression. Protein network analyses revealed up-stream regulators that provide valuable insight into the mechanistic drivers of pathogenesis and offer additional hypotheses for causation of disease, which will be further studied using mechanistic cell culture and human tissue experiments. These findings add to the understanding of TDP-43-driven neurodegeneration and reveal new protein candidates that can be targeted for therapy.

Emily McCann (Centre for MND Research, Macquarie University)

**ANAlYSIS OF GENETIC VARIATION AND IMMUNOPATHOLGY OF CHCHD10 IN AUSTRALIAN FAMILIAL AND SPORADIC AMYOTROPHIC LATERAL SCLEROSIS**

McCann EP\(^1\), Fifita JA\(^1\), Yang S\(^1\), Williams KL\(^1\), Twine N1,2, Bauer D2, Rowe DB1,, Nicholson GA1,3,4,5, Blair IP1.

1. Faculty of Medicine and Health Sciences, Department of Biomedical Sciences, Centre for MND Research, Macquarie University, Sydney, New South Wales, Australia. 2. Commonwealth Scientific and Industrial Research Organization, Health & Biosecurity Flagship, Sydney, Australia. 3. Northcott Neuroscience Laboratory, ANZAC Research Institute, Sydney, New South Wales, Australia. 4. Sydney Medical School, University of Sydney, Sydney, New South Wales, Australia. 5. Molecular Medicine Laboratory, Concord Hospital, Concord, New South Wales, Australia.

Amyotrophic lateral sclerosis (ALS) is a debilitating late onset neurodegenerative disease, causing the elimination of both upper and lower motor neurons, resulting in progressive muscle weakness, wasting, spasticity and eventually paralysis. Death from associated respiratory failure generally occurs within just two to five years from symptom onset. Approximately 20% of patients exhibit co-morbid frontotemporal dementia (FTD), while roughly 10% of all ALS patients have a family history (FALS). To date, the only proven cause of ALS is gene mutations. Recently, variants in CHCHD10 have been identified as a cause of, or associated with, pure ALS, FTD and ALS-FTD in families and sporadic patients of European ancestry. We sought to uncover the prevalence of CHCHD10 mutations among 81 Australian FALS patients negative for known ALS gene mutations, 643 sporadic ALS and 143 FTD patients. We also examined whether any common polymorphisms showed association with disease. Thus far, no pathogenic or associated variants have been identified among FALS patients, while sporadic and FTD...
patient analysis is underway. Further, we will conduct immunopathological analysis of ALS patient spinal cord tissue, to determine whether the hallmark protein aggregates found in ALS patient motor neurons contain the CHCHD10 protein. Our preliminary findings in FALS suggests that CHCHD10 mutations are not a common cause of ALS in Australian patients of predominately European ancestry, and our follow up analyses will help confirm whether this is likely due to population structure or whether CHCHD10 is more relevant to FTD dominant phenotypes opposed to pure ALS.

Benjamin Trist (University of Sydney)

LESSONS LEARNT FROM SOD1 DYSFUNCTION IN PARKINSON’S DISEASE AND FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

Benjamin G Trist¹, Dominic J Hare², Kay L Double¹

Affiliations:
2. Discipline of Biomedical Science and Brain and Mind Centre, Sydney Medical School, The University of Sydney, Sydney, NSW 2006, Australia.
1. Elemental Bio-imaging Facility, University of Technology Sydney, Broadway, New South Wales 2007, Australia and The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria 3052, Australia.

Clinically, primary disability in idiopathic Parkinson’s disease (PD) and amyotrophic lateral sclerosis (ALS) results from denervation of different neuronal populations; dopaminergic neurons in the substantia nigra pars compacta (SNc), and upper and lower motor neurons within the motor cortices, brainstem and spinal cord respectively. Despite this distinction, clinical co-occurrence of ALS and parkinsonism is common in some populations (Papua, Guam and Kii), and post-mortem studies demonstrate up to 17% of ALS patients present with clinical parkinsonism and mild-moderate SNc denervation. These data suggest common disease features are present in both disorders which have been under appreciated to date, and which may represent shared pathways to neuronal death. We recently described abnormal accumulation of SOD1 protein in degenerating regions of the idiopathic PD brain, bearing remarkable similarities to SOD1 proteinopathy in both the brain and spinal cord of cases of familial (f)ALS exhibiting mutations to the sod1 gene. We hypothesise that separate but converging pathways of dysfunctional SOD1 occur in specific populations of vulnerable neurons in these disorders, leading to distinct topographic patterns of neuron death and characteristic disease phenotypes. These data further suggest that treatments targeting SOD1 toxicity may possess efficacy across multiple disorders, evidenced by the recent success of one such therapy, CuII(atsm), in multiple murine models of both PD and fALS.

Ye Zhao (Neura, Unsw, Bmc)

LRRK2 IS DECREASED IN THE BRAIN OF PATIENTS WITH LRRK2 MUTATIONS AND IS ASSOCIATED WITH DYSFUNCTION OF THE RETROMER COMPLEX

Zhao Y¹,²,³, Perera G¹,³, Takahashi-Fujigasaki J⁴, Mash D.C⁵, Vonsattel J. G⁶, Hasegawa K⁷, Nichols R. J⁸, Holton J. L⁹, Murayama S⁴, Dzamko N¹,²,³, Halliday G.M¹,²,³

3. Brain and Mind Centre, Sydney Medical School, University of Sydney, Camperdown, 2050, Australia.
1. School of Medical Sciences, University of NSW, Kensington, 2033, Australia.
2. Neuroscience Research Australia, Randwick, 2031, Australia.
3. Department of Neuropathology, Brain Bank for Aging Research, Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Tokyo, 173-0015, Japan.
4. University of Miami Brain Endowment Bank, University of Miami Miller School of Medicine, Miami, Florida, 33136, USA.
5. New York Brain Bank, Taub Institute for Research on Alzheimer’s Disease and the Aging Brain, Columbia University, New York, 10032, USA.
6. Department of Neurology, Sagamihara National Hospital, Kangawa, 252-0315, Japan.
7. Parkinson’s Institute and Clinical Center, Sunnyvale, California, 94085, USA.
8. Queen Square Brain Bank, UCL Institute of Neurology, University College London, London, WC1N 1PJ, UK.

Mutations in leucine-rich repeat kinase 2 (LRRK2) are the most frequent genetic cause of Parkinson’s disease (PD)
and one of the most promising therapeutic targets. Retromer dysfunction is pathogenically linked to PD, and an emerging role for LRRK2 in retromer function has been suggested by several recent studies. However, the levels of LRRK2 and retromer proteins in the brains of PD patients with LRRK2 mutations is unknown. To test if retromer function is altered in PD, brain samples from cases with the G2019S (N=12) or I2020T (N=5) LRRK2 mutations were used for protein extraction and Western blotting and matched to unaffected controls and idiopathic PD patients. Protein levels of LRRK2, along with important components of the retromer complex system, including vacuolar protein sorting associated protein 35 (VPS35) and cation-independent mannose-6-phosphate receptor (MPR300), were measured in brain extracts. The cases with LRRK2 mutations showed significantly reduced LRRK2 levels compared to matched controls and idiopathic PD cases. Intriguingly, there was a solubility shift in VPS35, with a reduction in the TBS-soluble fraction and an associated increase in the SDS-soluble VPS35. MPR300 was reduced in cases with LRRK2 mutations, and this reduction was correlated with the levels of LRRK2 (p-value < 0.01). This study provides new data on LRRK2 expression in LRRK2-associated PD, which supports a core pathophysiological relationship between LRRK2 and retromer dysfunction.

Dzung Do-ha (University of Wollongong)

**IMPROVED DIFFERENTIATION AND NEURONAL MATURATION OF INDUCED PLURIPOTENT STEM CELLS INTO MOTOR NEURONS USING BRAINPHYS AND SMALL MOLECULE INHIBITORS**

Do-Ha D, Stevens CH, Cabral-da-Silva MC, Bax M, Yang S, Blair I, Engel M, Buskila Y, Ooi L
1. Illawarra Health and Medical Research Institute, School of Biological Sciences, Faculty of Science Medicine and Health, University of Wollongong, Wollongong, NSW, Australia
2. Centre for MND Research, Faculty of Medicine and Health Sciences, Macquarie University
3. School of Medicine, Western Sydney University, Penrith, NSW, Australia
4. The MARCS Institute, Western Sydney University, Penrith, NSW, Australia

The use of induced pluripotent stem cells (iPSCs) has revolutionised research into neurodegenerative diseases, including motor neuron disease. However, the challenge remains to generate, in a short time period, mature neuronal cultures that closely resemble neurons in vivo in terms of their protein expression profile and electrophysiological properties. In this study we investigated the effects of altering basal medium and growth factor composition to differentiate functional and mature motor neurons. Using immunocytochemistry and whole cell patch clamping we confirmed the expression of motor neuron markers and measured neuronal membrane properties, voltage-dependent K+ and Na+ currents and firing properties of iPSC-derived motor neurons. After 4 weeks of maturation, neurons cultured in Neurobasal medium exhibited a resting membrane potential (RMP) of -10 mV, while neurons cultured in BrainPhys, which closely resembles physiological ion concentrations, exhibited a significantly lower RMP of -35 mV (p < 0.0001). The use of small molecule inhibitors (SMIs) in combination with BrainPhys yielded neurons with a RMP of -45 mV within 3 weeks of maturation. Only 50% of patched neurons were active using BrainPhys without SMI, while 100% of patched neurons were electrophysiologically active when maturing with SMIs. Spike trains, which were only observed in SMI treated neurons, were present in 76% of neurons. Concurrently Na+ currents in SMI-treated neurons peaked at 2.4 nA, whilst neurons cultured in BrainPhys without SMIs only reached 0.75 nA. Together this data suggests the use of BrainPhys with SMI is useful for efficient generation of homogenous, functionally mature motor neurons in vitro.

**Autonomic/Neuroendocrine systems**

Femke Buisman-Pijlman (University of Adelaide)

**CONSISTENT SALIVARY OXYTOCIN AWAKENING RESPONSE IN LATE ADOLESCENCE IN A CONTROLLED SETTING.**

1. Robinson Research Institute, Adelaide Medical School, University of Adelaide, Adelaide, AUSTRALIA.
2. Discipline of Pharmacology, Adelaide Medical School, University of Adelaide, Adelaide, AUSTRALIA.
3. Centre for Sleep Research, University of South Australia, Adelaide, AUSTRALIA
4. School of Psychology, Flinders University, Adelaide, AUSTRALIA
Many studies investigate the impact of intranasal oxytocin, but we know little about the precise temporal features of the endogenous oxytocin circadian rhythm. Forsling et al. (1998), first reported a diurnal rhythm in young adult males, but little has been done since. Research has highlighted the impact of a large range of environmental factors on circulating oxytocin. We therefore set to record oxytocin in a standardised setting to investigate the stability of the diurnal rhythm of oxytocin while controlling for potential influences such as sleep, stress and methodological inconsistencies.

The aim of the present study was to characterise the diurnal pattern in adolescents in a highly-controlled sleep laboratory environment, over three consecutive days. Oxytocin concentrations from saliva samples collected at 0, 15, 30, and 45 minutes, and 8 and 12 hours, post-awakening (PA) in 12 healthy adolescents aged 15 to 17 years (mean = 16.25) were assessed using ELISA.

We present the first evidence for an oxytocin awakening response, as salivary oxytocin dropped dramatically in the first 15 minutes PA. This effect was highly consistent across participants and days and was not affected by sex. However, older subjects had significantly higher awakening OXT concentrations. Importantly, these data were collected in a highly controlled laboratory environment, and participants spent 3 days and nights both prior to and following the study period. Thus, these data are unlikely to be significantly influenced by environmental factors including variation in daily activities, sleep habits, exposure to a novel environment, or anticipatory stress.

Hsiao-Jou Cortina Chen (The University of Queensland)

CSA RECEPTOR KNOCKOUT MICE SHOW ALTERED STRESS RESPONSES AND LOCOMOTOR ACTIVITY FOLLOWING ACUTE AND SUB-ACUTE RESTRAINT STRESS

Chen HJ1, Spiers JG1, 2, Lavidis NA1, Woodruff TM1, Lee JD1, 2
1. School of Biomedical Sciences, The University of Queensland, St. Lucia, 4072, Australia. 2. University of Queensland Centre for Clinical Research, The University of Queensland, Herston, Australia. 3. MRC Toxicology Unit, Hodgkin Building, University of Leicester, Leicester, LE1 9HN, UK.

Immune dyshomeostasis plays a major role in the pathophysiology of stress-related disorders and previous studies have implicated a complex modulatory relationship between stress and different facets of immunity. The complement system represents one of the major effector mechanisms of the innate immune system with signalling by the activation product C5a via the C5a1 receptor (C5aR1) being one key component of the complement cascade. However, C5a-C5aR1 signalling in stress responsiveness has not been well characterised. In the present study, we aimed to determine the role of C5aR1 signalling on hormonal and behavioural responses following acute and sub-acute restraint stress using C5aR1 deficient mice (C5ar1-/-). Male C57BL/6J or C5ar1-/- mice (n=7-10) were exposed to 30 minute (acute), or 7 days of 2 hour (sub-acute) restraint stress after which the plasma corticosterone concentrations and locomotor activity were determined. The results demonstrated higher circulating corticosterone concentrations at 1 and 1.5-hour post-acute stress in the C5ar1-/- mice when compared to wild-type mice while no difference in basal corticosterone levels was observed. However, following 7 days of repeated stress, there was a decrease in the stress-induced corticosterone response in C5ar1-/- mice. This was observed alongside a restoration in the stress-induced reductions in locomotor activity in the C5ar1-/- mice monitored by the TSE PhenoMaster system from days 2 to 7 of sub-acute restraint stress when compared to wild-type mice. These findings suggest that C5a-C5aR1 signalling may play an important role in corticosterone responses following short and prolonged stress exposure which further modulates the behavioural responses to stress.

Shanti Diwakarla (Florey Institute of Neuroscience and Mental Health)

DISTRIBUTION AND LOCALISATION OF GFP UNDER TPH1 CONTROL AND ITS RELATION TO 5-HT IN THE MOUSE GIT

Diwakarla S1, Callaghan B2, Hunne B2, Wykosky J1, Huang J3, Syder D2, Furness JB1, 2
1. Florey Institute of Neuroscience and Mental Health, Parkville, Victoria 3010, Australia. 2. Department of Anatomy & Neuroscience, University of Melbourne, Parkville, Victoria 3010, Australia. 3. Takeda Pharmaceuticals, California, USA
Although enteroendocrine cells (EECs) are known to secrete multiple regulatory molecules, the lack of a transcriptional profile of individual types has limited our understanding of their precise functions and roles in gastrointestinal pathologies. To overcome this challenge, a bacTRAP transgenic mouse line where the L10a ribosomal subunit is tagged with GFP and placed under the control of the tph-1 promoter was generated. Tryptophan hydroxylase (TPH) catalyzes the rate limiting step in the biosynthesis of serotonin. This study immunohistochemically characterised the bacTRAP transgenic mouse line to determine the specificity of the reporter for EECs. The numbers of cells immunopositive for GFP and 5-HT, as well as their morphologies and distribution patterns were determined. Cells were counted at 63x from each GIT region of 3 bacTRAP mice. The morphology and staining pattern of cells differed along the GIT. Numerous EECs with long processes were observed in the distal colon, but long processes on 5-HT cells were not discernible in duodenal samples. The majority of tph-1 EECs contained 5-HT. However, in the stomach there were two distinct groups of 5-HT immunoreactive cells, with cells at the base of the glands staining positive for tph-1 and 5-HT, and those further towards the lumen staining positive for 5-HT only. Although GFP-Tph-1 staining has made it possible to distinguish the morphology of 5-HT cells more clearly, further characterisation is required to establish if another Tph enzyme is responsible for 5-HT synthesis in cells that do not express GFP.

Phillip Jobling (University of Newcastle)

DEVELOPMENT OF A MOUSE MODEL TO STUDY THE NERVE CANCER CONNECTION IN BREAST TUMOURS.

Mišićić M1, Hondermarck H2, Roselli S2, Faulkner S3, Jobling P4.
1. School of Biomedical Sciences and Pharmacy, University of Newcastle, Australia

Nerves have been shown to infiltrate the tumour microenvironment in a number of solid tumours [1]. Recently in a study of women with breast cancer we found that axon infiltration into the tumour was correlated with poor prognosis and lymph node involvement [2]. The major aim of this study was to investigate the possibility that the mouse mammary tumour virus model (MMTV)-polyoma middle T-antigen (PyMT) (MMTV-PyMT) was suitable to investigate the nerve cancer connection. First, we investigated mammary gland innervation in five normal Swiss mice (sacrificed under anaesthesia with 10% isoflurane in air). Tyrosine hydroxylase immunoreactivity (TH-IR) accounted for 82% of the mammary gland innervation whereas substance P–IR or neuronal nitric oxide synthase –IR accounted for 11% and 7% of the nerves detected, respectively. Axons were found surrounding the vasculature and also within ducts. Vesicular acetylcholine transporter was only sparsely present in the normal mammary gland. The extent of innervation was then assessed in mammary tumours isolated from MMTV-PyMT mice (n=8), 6-8 weeks after tumour initiation. In two of six mice tested for TH-IR, axons immunoreactive for TH infiltrated the tumour microenvironment. A further two mice had axons immunoreactive to PGP9.5 within the tumour microenvironment. This study demonstrates that mouse mammary tumours can attract axons and suggests that this mouse model may represent a way of understanding the nerve cancer connection.


Joon Kim (University Of Otago)

REGULATION OF STRESS NEURON ACTIVITY DYNAMICS IN VIVO

Kim JS, Iremonger KJ
Centre for Neuroendocrinology and Department of Physiology, University of Otago, Dunedin, NZ

Corticotropin-releasing hormone (CRH) neurons are the final output cells that drive the neuroendocrine stress response, resulting in corticosteroid (CORT) release. It is widely accepted CORT acts back on the brain to inhibit and thereby prevent excessive CRH neuron activity. However the activity dynamics of CRH neurons in vivo remain unknown and therefore current models for the stress circuitry have never been directly tested. Using fibre photometry to record the natural activity of the CRH neuron population in freely behaving mice, we report that CRH neuron excitability can be rapidly modulated, revealing distinct patterns of activity at rest, during stress, and after stress. CRH neurons are tonically active independent of changes in circulating CORT, with low stochastic patterns of activity. In response to an acute white noise stress, CRH neuron activity is rapidly synchronised with peak activity observed within 5 seconds of stress onset. This increase in activity continues post stress, in oscillating burst-like dynamics, lasting >20min. We then aimed to investigate the
CHARACTERISATION OF ENTERIC NEURAL CIRCUITRY AND MOTILITY IN THE NEUROLIGIN-3 KNOCKOUT MOUSE MODEL OF AUTISM

Leembruggen AJL1, Balasuriya GK2, Seger GO1, Bornstein JC1, Nithianantharajah J3, Hill-Yardin EL1,2.

1. Department of Physiology, University of Melbourne, Parkville, Melbourne, Victoria, Australia
2. School of Health and Biomedical Sciences, RMIT University, Bundoora, Victoria, Australia
3. Florey Institute of Neuroscience and Mental Health, Parkville, Melbourne, Victoria, Australia

Mutations in the synaptic adhesion molecule, neurexin-3 (NL3), are associated with autism spectrum disorder, in which gut dysfunction is common. NL3 knockout (KO) mice have altered brain function, but whether the enteric nervous system is also altered is unknown. To determine whether NL3 deletion impacts enteric neural circuitry, we performed immunofluorescent staining for neuronal markers (pan-neuronal marker Hu; neuronal nitric oxide synthase (nNOS); calretinin (CR)) in the myenteric plexus of jejunum and colon of NL3 KO and wildtype (WT) mice. Video-imaging was used to assess colonic migrating motor complexes (CMMCs) ex vivo. WT and NL3 KO mice showed significant variation in neuron number (Hu-immunoreactive (IR)) in the proximal jejunum, and proximal, mid and distal colon (p<0.0001, n=5). However, no significant differences were observed between WT and NL3 KO mice in any region, contrasting with another mouse model of NL3 dysfunction expressing the NL3R451C point mutation, which showed increased neurons per ganglion in the jejunum (p=0.03, n=5). While there was a trend for decreased CR-IR neurons in the mid colon of NL3 KO mice (p=0.07, n=5), CR-IR and nNOS-IR neuron numbers were similar between NL3 KO and WT mice. Further, both NL3 KO and WT mice showed similar motility under control conditions (8±1 and 7±2 CMMCs per 15 min, respectively, n=4). These findings suggest complete loss of NL3 does not significantly change proportions of motor neurons or interneurons, or motility in the enteric nervous system. Further work is needed to assess structural and functional changes at the level of the synapse.

Jiamei Lian (University Of Wollongong)

GLUCO-LIPID METABOLIC DISORDERS INDUCED BY ANTIPSYCHOTIC DRUG IN RATS: MECHANISMS FOR TIME-DEPENDANT CHANGES THROUGH MODULATION OF HEPATIC SREBP/CHREBP PATHWAY

Lian J1,2, Huang X1,2, Deng C1,2.

1. School of Medicine, University of Wollongong, Wollongong, NSW, Australia. 2. Illawarra Health and Medical Research Institute, Wollongong, NSW, Australia.

The second-generation antipsychotic drug olanzapine is widely used to treat schizophrenia and other mental disorders. However, it is associated with adverse obesity and other metabolic disorders. Histamine H1 receptor (H1R) plays an important role in olanzapine-induced weight gain, while muscarinic M3 receptor (M3R) involves in the gluco-metabolic side-effects of olanzapine. The upregulation of sterol regulatory element-binding proteins (SREBP-1 and SREBP-2) are associated with olanzapine-induced abnormal lipid and cholesterol synthesis. The carbohydrate response element binding protein (ChREBP) contributes to improve insulin sensitivity in the liver. Therefore, this study investigated the effect of olanzapine treatment on gluco-lipid homeostasis and their potential mechanisms. To address these issues, female Sprague Dawley rats (201-225g) were treated with olanzapine (2 mg/kg, t.i.d.) for 2, 3, 4, 5, 7 and 9 weeks. Plasma glucose, insulin and lipid levels were measured. Hepatic protein levels of SREBP-1, SREBP-2, ChREBP, H1R and M3R were examined by Western Blots. The result shows that olanzapine significantly increased body weight in all treatment durations. Furthermore, olanzapine elevated glucose level in 2, 3, 4 weeks’ treatment, and insulin levels in 4,5 and 9 weeks’ treatment. The upregulation of cholesterol was observed after 2,5,7,9 olanzapine treatment. Olanzapine treatment upregulated levels of H1R, M3R, SREBP-1, SREBP-2 and ChREBP protein level in the liver in a time-dependent manner. Therefore, this study provides evidence underlying the time-dependent effects.

Anita Leembruggen (University of Melbourne)
of olanzapine on weight gain, glucose and lipid metabolic disturbances associated with the activation of H1R, M3R, SREBP, ChREBP pathway in the liver.

Alice McGovern (University of Melbourne)

MOLECULAR DIVERSITY OF AIRWAY VAGAL SENSORY NEURONS REVEALED BY SINGLE CELL RNA-SEQ

McGovern AE\(^1\), Keller J\(^1\), Tian L\(^2\), Ritchie ME\(^2\), Mazzone SB\(^3\)

4. Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Victoria, AUSTRALIA. 2. Department of Molecular Medicine, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, AUSTRALIA.

The respiratory system is densely innervated by heterogeneous populations of sensory neurons that monitor airway physiological state. When activated, these neurons send information to the brainstem and higher brain leading to the production of reflex and complex behavioural responses, critical for the ongoing physiological control of respiratory function and pulmonary defense. However, very little is known about the specific molecular characteristics of these heterogeneous cell types. In C57/B6 mice, airway-specific vagal sensory neurons were identified by retrograde tracing with 2% DiI (n=5). Following 10 days of recovery, vagal ganglia (jugular and nodose) were removed, enzymatically dissociated and 72 DiI+ neurons were randomly collected into individual micropipettes and prepared for high-resolution single cell mRNA-seq. Unbiased SC3 clustering analysis revealed 5 novel major classes of sensory neurons that were characterised by the highest expression of marker genes Sprr1a, Lypd1, Trappc3l, Lypd6 or Rasgrf1. When the expression level (high versus low) of P2rx2 (to differentiate nodose from jugular neurons) and Trpv1 (to identify nociceptors) were included as known pre-existing population markers, 967 differentially expressed genes were identified, 26 of which represented unique markers of the 4 possible P2rx2/ Trpv1 populations, including: Chrnb4 and Lypd6b (in P2rx2+/ Trpv1+ neurons); Adora2a and Prdm12 (in P2rx2+/ Trpv1- neurons); Serpinb8 (in P2rx2-/ Trpv1+ neurons); and Barh2 (in P2rx2-/ Trpv1- neurons). These data highlight novel approaches to understanding the molecular diversity of vagal afferent neurons, which ultimately may provide new insights into airway sensory neurobiology.

Youichirou Ootsuka (Flinders University)

THE MEDULLARY SEROTONERGIC SYSTEM MEDIATES TACHYCARDIA AND HYPERPNEA RESPONSE TO PSYCHOLOGICAL STRESS

Ikoma Y\(^1\), Kusumoto I\(^1\), Yamanaka A\(^1\), Ootsuka Y\(^1,2\), Kuwaki T\(^1\)

1. Department of Physiology, Graduate School of Medical & Dental Sciences, Kagoshima University, Kagoshima, Japan. 2. Centre for Neuroscience, College of Medicine and Public Health, Flinders University of South Australia, Australia. 3. Department of Neuroscience II, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan

When animals are confronted by an unpleasant and aversive situation, they often show physiological responses including tachycardia, hyperthermia and tachypnea. The medullary raphé has been shown to mediate the stress-induced tachycardia and hyperthermia responses. It contains several groups of serotonergic neurons and is involved in controlling cardiovascular, respiratory and thermoregulatory functions. It is for this reason that the medullary serotonin system has been suggested to be involved in controlling the stress-induced physiological responses. We investigated in this study. We used transgenic mice that expressed light-driven outward proton pumps (Archaeorhodopsin T) selectively in the central serotonergic neurons with a tetracycline-controlled transcription activation system and a tryptophan hydroxylase 2 promoters. Under anaesthesia, we implanted, a telemetry probe (ETA-F10, DSI) to measure body temperature and heart rate as well as a piezo sensor for respiratory measurements. We transmitted green light (523nm, 15mW) in the medullary raphé via a pre-implanted optical fibre (200um core diameter) to selectively inhibit serotonergic neurons in the medullary raphé. We performed the following two stress tests: a sudden 2cm drop of the experimental cage and the introduction of an intruder confined in a small cage. The intruder stress increased heart rate by 28.6±4.1% (n=15, P<0.001), respiratory by 47.2±18.5% (n=9, P<0.05) and body temperature by 0.7±0.1% (n=15, P<0.001). Optical inhibition of the medullary serotonergic neurons significantly attenuated the tachycardia and hyperpnoea, but not the hyperthermia. Our results indicate that serotonergic neurons in the medullary raphé contribute to controlling cardiovascular and respiratory function during aversive situations.
DISTINCT POPULATIONS OF ARC NPY NEURONS CONTROL DIFFERENT ASPECTS OF ENERGY HOMEOSTASIS

Yue Qi, Ronaldo Enriquez, Lei Zhang & Herbert Herzog
Department of Eating Disorders, Neuroscience Division, Garvan Institute of Medical Research, Sydney, Australia

Two specific population of arcuate nucleus (Arc) neurons have been identified as the most critical players responsible for the control of energy homeostasis, namely the orexigenic acting neuropeptide Y (NPY)/agouti-related peptide (AgRP) neurons and the anorexogenic acting proopiomelanocortin/cocaine amphetamine regulated transcript neurons. While many aspects of energy homeostasis control seem to be assigned to these two neuronal populations, discrepancies have emerged that suggest a greater heterogeneity in Arc neuronal populations with additional or diverse actions assigned to subpopulations of them. Specifically a group of Arc NPY neurons exist that do not co-express AgRP. Since most past and current research investigating hypothalamic NPY function employ AgRP driven Cre-lines, functional contributions of other NPY neuronal population in the Arc have either been missed or overlooked. To investigate the properties of different NPY neuronal subpopulations we have generated a new mouse model specifically only lacking NPY in AgRP neurons. These mice show a reduced response to fasting induced food intake and are generally smaller in size. Also specifically only activating these neurons using DREADD technology reveals a diminished food intake response indicating that AgRP alone is not sufficient to drive feeding and maintain a balanced energy homeostasis. Importantly, lack of NPY only in this population of neurons resulted in a significant decrease in whole body bone mineral content and density, the complete opposite as seen in global NPY knockout mice. Together these results demonstrate that different population of NPY neurons exist in the Arc and that these different NPY neurons fulfil specific functions.

SUBPOPULATIONS OF SACRAL PREGANGLIONIC NEURONS EXPRESS ACTIVATED TRANSCRIPTION FACTOR – 3 AFTER PELVIC NERVE INJURY

Wong AW1, Osborne PB1, Keast JR1.

1. Department of Anatomy and Neuroscience, School of Biomedical Sciences, The University of Melbourne, VIC.

Sacral preganglionic neurons (SPNs) are a diverse group of autonomic neurons critical for micturition, defeecation and sexual function. Their long axons extend from sacral spinal cord to the pelvic ganglia, making them particularly vulnerable to injury. Previously we showed that SPNs survived after pelvic nerve transaction and a subpopulation expressed activated transcription factor-3 (ATF3), a transcription factor highly related to regeneration. Here we aimed to examine if this selective upregulation of ATF3 expression in SPNs was confined to certain molecular subclasses that have specific functions. Unilateral transection of pelvic nerves was performed on male Sprague-Dawley rats (7-9 weeks). One week after axotomy, spinal cords were removed after intracardial perfusion with fixative, and immunolabelled for ATF3, heat shock protein-25 (Hsp25), choline acetyltransferase (ChAT), somatostatin (SOM), neuropekin-1 receptor (NK1R), and calbindin (CAL). We found that subpopulations of SPNs expressed SOM (30%), CAL (24%) and NK1R (45%)(n=6). Expression of ATF3 was induced in many injured SPNs and its expression was found in each SPN subpopulation (51-53% of NK1R and SOM SPNs; 21% of CAL+ SPNs; n=6). We also found that, in contrast to more rostral preganglionic neurons, Hsp25 was not expressed in naïve rat SPNs but significantly upregulated in these neurons after injury; after axotomy a majority expressed ATF-3. Together, these studies reveal the complexity of sacral preganglionic neurons and their responses to injury. The simultaneous upregulation of Hsp25 and ATF3 in a subpopulation of sacral preganglionic neurons may indicate a distinct level of regenerative capacity after injury.

PROLACTIN ACUTELY INFLUENCES RUNNING WHEEL ACTIVITY BUT NOT AMBULATORY ACTIVITY IN FEMALE MICE

Kirsten Carter (University of Otago)
Carter KM, Grattan DR, Ladyman SR.
Department of Anatomy and Centre for Neuroendocrinology, University of Otago, Dunedin, NZ

Prolactin has been implicated in a broad range of functions in the brain. In particular, prolactin plays a role in energy homeostasis as prolactin can stimulate food intake and increase body weight. We have recently observed a link between prolactin and running wheel activity; male mice with prolactin-receptors deleted from RIP-cre cells (beta cells and subset of neurons) have increased running wheel activity\(^1\), suggesting that prolactin may suppress activity and thus facilitate a positive energy balance. The aim of this study was to further investigate the effect of prolactin on physical activity to determine if 1) prolactin can acutely influence running wheel behaviour and 2) if this effect is specific to running wheel activity or generalized for other forms of physical activity. Female C57B6/J mice received an i.p. injection of prolactin (5mg/kg) or saline then running wheel activity was measured for 12 hours. Prolactin treatment significantly decreased dark phase running wheel distance compared to vehicle treatment, especially with in the first 4 hours. In a second cohort of mice, prolactin or saline was similarly administered and locomotor activity (distance traveled) was measured during an open field test, then one week later this was repeated using an elevated plus maze test. Prolactin and saline treated mice traveled similar distances during both tests suggesting no effect of prolactin on ambulatory activity. Overall, these data describe a novel suppressive role for prolactin specifically on running wheel activity, as opposed to general activity, and also suggest potential action of prolactin on reward-inducing behaviour. (248 words)


Khalid Elsaafien (The Florey Institute of Neuroscience and Mental Health)

**CCL2-CCR2 SIGNALLING IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS ELICITS SYMPATHETIC-MEDIATED BLOOD PRESSURE ELEVATIONS THROUGH MONOCYTE AND LYMPHOCYTE RECRUITMENT**

Elsaafien K, Korim WS, May CN, Yao ST
The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Victoria 3010, Australia.

The plasma levels of the chemokine C-C motif ligand 2 (CCL2) are elevated in hypertension. Additionally, in experimental models of hypertension CCL2 levels increase by 3-fold in the paraventricular nucleus of the hypothalamus (PVN). However, the underlying neural and molecular mechanisms remain unknown. We believe that CCL2 in the PVN contributes to sympathetic-mediated increases in blood pressure. Here, we show that in the PVN of rats receptors for CCL2, termed CCR2, are predominantly expressed in the membrane of astrocytes that make close appositions to PVN neurons projecting to the rostral ventrolateral medulla (RVLM)-where cardiovascular sympathetic premotor neurons are found. Activation of CCR2 receptors in the PVN elicited biphasic responses in mean blood pressure (MAP), and renal sympathetic nerve activity (RSNA), an early sympatho-inhibition was followed by a long-lasting increase in RSNA and MAP. These responses were abolished by prior injection of the selective CCR2 antagonist (RS-102895). The first phase of the response was dependent on putative purinergic transmission between astrocytes and neurons, whereas monocyte and lymphocyte infiltration into the PVN was responsible for sympatho-excitation. These findings suggest that combined neural and pro-inflammatory mechanisms in the PVN mediate sympathetic and blood pressure responses to CCL2 upregulation in hypertension.

Caitlin Finney (University of New South Wales)

**LABORATORY DIETS HIGH IN SOY LEAD TO SEX-SPECIFIC CHANGES IN BODY WEIGHT AND ESTROGEN RECEPTOR GENE EXPRESSION IN RATS**

Finney C\(^1\), Jenner AM\(^2\), Westbrook RF\(^1\), Clemens KL\(^1\)
1. School of Psychology, University of New South Wales. 2. Bioanalytical Mass Spectrometry Facility, University of New South Wales.

Standard laboratory rodent diets used in Australia often contain high amounts of soy-derived xenoestrogens
known as isoflavones. These isoflavones are direct, high-affinity agonists at brain estrogen receptors (ER) α and β. The consequences of dietary isoflavones on estrogen signaling in the brain are unknown. We compared the effects of two commonly used Australian standard rodent diets, Gordon’s Premium Rat and Mouse Pellets (G-Std) and Specialty Feed’s Irradiated Rat and Mouse Diet (SF-Std), with Specialty Feed’s phytoestrogen-free diet (SF-AIN93G) on body weight and estrogen receptor expression in the striatum of adult male and female rats. Gas Chromatography-Tandem Mass Spectrometry (GC/MS/MS) analysis of each diet confirmed isoflavone content was highest in SF-Std and ordered SF-Std > G-Std > SF-AIN93G. Male, but not female, rats fed the SF-AIN93G diet gained significantly more weight than those fed the other diets. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) analysis revealed that expression of the ERβ gene in the striatum was less in both male and female rats receiving SF-AIN93G diet than those fed the other diets. However, there were no between-group differences in ERα gene expression. These data show that dietary isoflavone content affects estrogen receptor expression in the rat brain, which may have implications for rodent models of neurobiological diseases that are influenced by estrogen, including schizophrenia and addiction.

Julia Gouws (University of Otago)

NORADRENERGIC REGULATION OF THE CRH NEURONAL NETWORK

Gouws JM, Iremonger KJ
Centre for Neuroendocrinology, Department of Physiology, University of Otago, Dunedin, New Zealand.

The stress response is crucial for maintenance of homeostasis. Stress is controlled by corticotropin-releasing hormone (CRH) neurons within the paraventricular nucleus (PVN) of the hypothalamus. During stress, noradrenaline (NA) is released into the PVN causing activation of CRH neurons. However, the effects of NA on the patterns of CRH neuron activity are unknown. This study aimed to investigate this, using genetically encoded calcium indicators. In vitro calcium imaging was performed on brain slices containing the PVN from CRH-GCaMP6f mice. We found that NA (10 mM) induced a robust increase in CRH neuron excitability, with most CRH neurons switching into a bursting pattern of activity. NA induced excitation was blocked with the adrenergic α1 receptor antagonist prazosin (10 mM). In control, NA activated, on average, 7.3 ± 1.0 CRH neurons per slice, however, in slices treated with prazosin, NA only activated 0.9 ± 0.3 CRH neurons per slice (NA, n = 8 slices; Prazosin, n = 14 slices; unpaired t-test, p < 0.05 ). The excitatory effects of NA on CRH neurons persisted if ionotropic glutamate receptors were blocked with CNQX (10 mM) and AP5 (40 mM) (# neurons active per slice: 11.3 ± 1.0, n = 10 slices) or ionotropic GABA receptors were blocked with picrotoxin (50 mM) (# neurons active per slice: 10.3 ± 1.1, n = 7 slices). This data demonstrates that CRH neurons adopt a bursting pattern of activity when excited by NA, and that this is mediated through direct activation of α1 adrenergic receptors.

Anthony Setiadi (The Florey Institute of Neuroscience and Mental Health)

PENTOXIFYLLINE ATTENUATES HYPERTENSION BY DECREASING SYMPATHETIC ACTIVATION IN RENOVASCULAR HYPERTENSIVE RATS

Setiadi A1, Korim WS1, May CN1, Yao ST1.
5. The Florey Institute of Neuroscience and Mental Health, University of Melbourne, VIC, Australia.

Hypertension is known to involve a chronic upregulation of pro-inflammatory mediators both within the periphery and the brain. Circulating levels of pro-inflammatory cytokines such as tumour necrosis factor-a (TNF-a) are upregulated in hypertensive patients and animals. Pentoxifylline, amongst other pharmacological actions, can decrease the production of TNF-a. In this study, we hypothesised that pentoxifylline can decrease blood pressure in a two kidney, one-clip rat model of hypertension. We administered pentoxifylline orally (30 mg/kg/day in drinking water) to renovascular hypertensive rats implanted with radiotelemetry transmitters over 8 consecutive weeks. Blood pressure and heart rate were continuously monitored throughout. We found that pentoxifylline treatment significantly attenuated the increase in blood pressure when compared with vehicle treated controls. Furthermore, c-Fos immunoreactivity, a marker of neuronal activation, was significantly decreased within the paraventricular nucleus of the hypothalamus (PVN) in these animals. In addition, spectral analysis of systolic blood pressure variability showed decreased in the low frequency band of rats treated with pentoxifylline compared with vehicle controls. Altogether, these results demonstrate that pentoxifylline can decrease blood pressure and these actions maybe mediated, in part, by decreasing PVN
GHRELIN'S ROLE IN STRESS RESPONSE TO LIPOPOLYSACCHARIDE IN MALE WISTAR RATS

Ilvana Ziko (RMIT University)

Ghrelin is a major metabolic hormone involved in growth, obesity and stress. Recent research has demonstrated ghrelin’s role in regulation of the hypothalamic-pituitary-adrenal (HPA) axis responses to stress and immune challenge. Ghrelin exists in two main biologically active isoforms – acylated ghrelin (AG), which is the result of ghrelin-O-acyl-transferase (GOAT)-mediated acylation and des-acylated ghrelin (DAG), which is the unacylated form. Previous studies have shown that AG and DAG have opposing effects on stress and anxiety with AG having mainly a suppressive effect and DAG contributing to an enhanced stress response. Despite evidence for a pituitary-specific effect of at least AG, ghrelin’s involvement in regulating the extra-hypothalamic components of the HPA axis have not yet been investigated. Here we aimed to assess how AG and DAG modulate HPA axis responses to immune challenge with lipopolysaccharide (LPS) in vivo and 2) assess how AG and DAG influence the pituitary and adrenal responses to stimulation in vitro. Our findings show that neither AG nor DAG affect pituitary ACTH responses to a stress-mimicking stimulus (CRH) in male rats, although DAG can increase adrenal CORT release (with a slightly altered time course). Ghrelin also does not influence neuronal activation in the paraventricular nucleus of the HPA axis in response to LPS. On the other hand, both DAG and AG suppress LPS-induced cytokine release into circulation. The findings with respect to the HPA axis suggest DAG and AG do not mediate cytokine suppression via corticosterone release.

Pascal Carrive (Unsw Sydney)

DISTRIBUTION OF OREXIN RECEPTORS IN RAT SYMPATHETIC PREGANGLIONIC NEURONS AND C1 NEURONS OF THE ROSTRAL VENTRAL MEDULLA

Duong F, Kumar NN and Carrive P
School of Medical Sciences, The University of New South Wales, Sydney, New South Wales 2035, Australia

Orexin, aka hypocretin, contributes to the regulation of cardiovascular function during wakefulness and motivated behaviors. Consistent with this, terminals of orexin neurons are found in regions involved in cardiovascular regulation such as the vasopressor area of the rostral ventrolateral medulla (RVLM) where C1 catecholaminergic neurons are found and the lateral horn of the thoracic cord where sympathetic preganglionic neurons (SPN) are located. These are the last two central neurons controlling the sympathetic outflow to cardiovascular effectors. Here we used in situ hybridization with digoxigenin-labelled riboprobes directed against orexin receptors Ox1R and Ox2R mRNA in combination with choline acetyl transferase and tyrosine hydroxylase immunofluorescence to detect the expression of orexin receptors in rat SPN and RVLM C1 neurons, respectively. In the rostral ventral medulla, strong OxR expression was found medially (RVMM), especially in neurons of the gigantocellular and lateral paragigantocellular nuclei. OxR expression laterally in the RVLM was comparatively weaker. Approximately 19%±4% of C1 RVMM and 17%±3% of C1 RVLM neurons expressed Ox1R, while 31%±11% C1 RVMM and 21%±10% of C1 RVLM expressed Ox2R. In the thoracic cord, OxR expression was found in all layers and was particularly strong in somatic motoneurons. Approximately 37%±9% of SPN neurons were Ox1R compared to 43%±3% for Ox2R. Thus our results so far indicate that both receptors can be expressed in RVLM C1 neurons and SPN. To summarize, orexin can act directly on the last two central neurons controlling the sympathetic outflow to cardiovascular effectors and both receptors can potentially mediate this effect.

Philip Ryan (The Florey Institute of Neuroscience and Mental Health)

OXYTOCIN RECEPTOR-EXPRESSING NEURONS IN THE PARABRACHIAL NUCLEUS SUPPRESS NON-CALORIC FLUID INTAKE

Ryan PJ1, Ross Si1, Campos CA1, Derkach VA1, Palmiter RD1
1. Howard Hughes Medical Institute, University of Washington, Seattle, WA, USA. 2. The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, VIC, Australia.
Brain regions that regulate fluid intake are not well characterised, yet are essential for understanding fluid homeostasis. We identify oxytocin receptor-expressing neurons in the parabrachial nucleus (OxtrPBN) as key regulators of fluid intake. Chemogenetic activation of OxtrPBN neurons in mice robustly suppressed non-caloric fluid intake and decreased food intake during dehydration, but not after fasting. Activation of OxtrPBN neurons did not alter salt intake following salt depletion, but inactivation increased saline intake after dehydration or hypertonic saline. Under physiological conditions, OxtrPBN neurons were activated by fluid satiation and hypertonic saline. OxtrPBN neurons were directly innervated by oxytocin neurons in the paraventricular hypothalamus (OxtPVH), which mildly attenuated fluid intake. Activation of neurons in the nucleus of the solitary tract (NTS) substantially suppressed fluid intake and activated OxtrPBN neurons. Our study suggests that OxtrPBN neurons decrease saline and/or water intake to decrease or prevent hypervolaemia and/or hypernatraemia.

Danielle Burgess (The University of Queensland)

PERICONCEPTIONAL ALCOHOL PROGRAMS AN INCREASE IN OFFSPRING CORTICOSTERONE CONCENTRATIONS AND HIPPOCAMPAL REGULATION OF STRESS RESPONSIVENESS IN RAT OFFSPRING.

Burgess DJ1, Cuffe JSM2 and Moritz KM1,3
1. School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, 4072. 2. School of Medical Sciences, Griffith University, Australia, QLD, 4222. 3. Child Health Research Centre, The University of Queensland, Brisbane, 4072.

Ethanol (EtOH) consumption during pregnancy alters offspring hypothalamus-pituitary-adrenal axis (HPA) activity and regulation. While most Australian woman will cease alcohol consumption upon pregnancy detection, little is known about the impacts of alcohol exposure before this time. This study aims to investigate periconceptional ethanol (PC:EtOH) exposure on HPA axis function and regulation in aged rat offspring. Female Sprague-Dawley rats were treated with PC:EtOH (12/5% v/v EtOH, liquid diet) from 4 days before conception (E-4), until embryonic day 4 (E4). Dams littered down naturally. At 6 and 18 months of age, plasma was collected for measurement of corticosterone. At 18 months of age, a subset of animals was culled and adrenal gland, hypothalamus and hippocampus collected for gene expression analysis. PcEtOH exposure reduced plasma corticosterone in female offspring at both 6 and 18 months of age (p<0.05). Furthermore, while key adrenal steroidogenesis genes (Mc2r, StAr, Cyp11a1, Cyp11b2 and 11bhsd2) and hypothalamic genes (Nr3c1, Hsp90, Crh or Crh-r1) were not affected by PC:EtOH in either sex, Nr3c1 and Hsp90 were increased in the hippocampus of female offspring exposed to PcEtOH. This study demonstrated that ethanol exposure around the time of conception reduces basal corticosterone. These hormonal adaptations are likely related to the expression of key hippocampal regulators of HPA function. As the HPA axis and it’s appropriate regulation is essential for the function of many important biological systems, these results suggest PC:EtOH may have long term impacts on physiological function, especially in females.

Natasha Kumar (UNSW Sydney)

DISTRIBUTION OF GALANIN AND GALANIN RECEPTOR 1 IN THE BRAINSTEM OF THE MOUSE

Dereli AS1, McMullan S2, Kumar NN1.
1. Department of Pharmacology, University of New South Wales, NSW, Australia.
2. Department of Biomedical Sciences, Macquarie University, NSW, Australia.

The inhibitory neuropeptide transmitter galanin is involved in multiple autonomic behaviours including control of breathing. Microinjection of galanin into the ventral respiratory column (VRC), the brain region that generates the rhythmic breathing pattern, inhibits breathing in the rat. Galaninergic projections from the retrotrapezoid nucleus (RTN) chemosensory neurons to the VRC have been demonstrated in the rat. The mechanism by which galanin inhibits ventilation and the receptor substrates involved needs to be elucidated to understand its role in the CNS control of breathing. We aimed to examine the distribution of galanin receptor subtype 1 (GalIR1) mRNA and map all galaninergic populations including those that project to the VRC, in the mouse brainstem. Brainstem tissue sections from adult male C57BL/6 mice (n=4) were processed for galanin receptor 1 in situ
hybridisation in combination with immunohistochemistry for tyrosine hydroxylase. The results show that there is moderate expression of GalR1 in facial nucleus, lateral reticular nucleus, RTN and rVRG. Preprogalanin mRNA is expressed in NTS, RTN, inferior olive, spinal trigeminal nucleus and locus coeruleus. Following injection of the retrograde tracer, CTB 555, into the VRC in adult mice, we identified specific neuronal populations that project to VRC including NTS, locus coeruleus, parabrachial nucleus, Kolliker fuse nucleus, cuneate nucleus, SP5 and inferior olive however these were not galaninergic. In conclusion, we have identified all projections to the VRC in the mouse. The expression of galanin in RTN and GalR1 in VRC in the mouse provides for a circuit for homeostatic regulation of respiration by galanin.

Rahat Ul Ain Summan Toor (Macquarie University)

ACTIVITY IN THE POST-INSPIRATORY COMPLEX (PICO) IS NOT NECESSARY FOR THE GENERATION OF POST-INSPIRATORY VAGAL OR SYMPATHETIC NERVE ACTIVITIES IN THE RAT

Toor R, McMullan S, Hildreth CM, Phillips JR, Sun QJ
Faculty of Medicine & Health Science, Macquarie University, NSW 2109 Australia

A network of excitatory cholinergic interneurons immediately dorsomedial to the compact formation of the nucleus ambiguus, referred to as the post-inspiratory complex (PiCo), is reported to oscillate independently when uncoupled from the central respiratory rhythm generator and to generate the post-inspiratory (post-I) phase of the respiratory cycle in mice (Anderson et al. (2016). Nature 536, 76-80). Here we test the hypothesis that PiCo activity underlies post-I activity in vagal and renal sympathetic nerve activities in urethane-anaesthetised rats (n=9). Bilateral PiCo microinjection of the GABA receptor agonist isoguvacine significantly reduced, but never abolished, the amplitudes of post-I vagal (3.9 ± 0.5 to 1.4 ±0.3µV p < 0.0001) and renal sympathetic nerve activities (4.2 ± 0.7 to 2.1 ± 0.4µV p < 0.01) without affecting baseline activities, supporting the hypothesis that activities in PiCo contributes to vagal and renal post-I discharge. However, we found that post-I activity could be restored by brief hypoxia immediately after PiCo inhibition, with post-I activity reaching levels that were not significantly different to baseline, suggesting that neurons in the PiCo region contribute to but are not necessary for the generation of post-I drive. In contrast, isoguvacine microinjections in to the PiCo abolished fictive swallowing reflexes evoked by electrical stimulation of the superior laryngeal nerve, indicating that the neurons in Pico are crucial for swallowing. We therefore conclude that activity in the PiCo contributes to the transmission of post-I drive under normal circumstances but is not responsible for its generation, and may instead contribute to the swallowing reflex.
**INHIBITION OF THE VENTRAL TEGMENTAL AREA INCREASES SYMPATHETIC DISCHARGE TO BROWN ADIPOSE TISSUE**

Brizuela M\(^1\), Swoap SJ\(^2\), Blessing WW\(^1\), Ootsuka Y\(^1\).
1. Centre for Neuroscience, Department of Human Physiology, School of Medicine, Flinders University, South Australia, Australia.
2. Department of Biology, Williams College, Williamstown, MA, USA

The lateral habenula (LHb) plays an important role in the behavioral response to adverse environmental situations. This response to negative situations is mediated via the inhibitory control of the LHb over the dopaminergic neurons in the Ventral Tegmental Area (VTA). Physiological responses to adverse situations include emotional hyperthermia induced by brown adipose tissue thermogenesis (BAT). Our laboratory has elucidated the role of the LHb in physiological thermoregulatory changes, demonstrating that the LHb can mediate emotional hyperthermia by eliciting BAT thermogenesis and cutaneous vasoconstriction and that the inhibition of the medullary raphe reverses this response. Given that there are no directed projections from the LHb to the medullary raphe the specific brain pathways involved in this habenula-mediated autonomic response remains unknown. It is plausible that the VTA dopamine system represents such link. To address this issue, in the present study we evaluated the potential role of the VTA in BAT thermogenesis. Bilateral injections were made in the VTA of anesthetized male Sprague-Dawley rats with the GABAA receptor agonist muscimol (1nmol in 100nl). After VTA injection there was a significant increase in BAT sympathetic nerve discharge by 16±3 % (n=6, p<0.05) and BAT temperature by 1.34±0.18 ºC (n=6, p<0.01). Further injection of muscimol into the medullary raphe reversed these changes. The results suggest that the VTA-dopamine system might be part of the circuitry linking the LHb with the medullary raphe in emotional hyperthermia.

**FUNCTIONAL CHARACTERISATION OF VISCEROSENSORY INPUT TO THE NUCLEUS ACCUMBENS.**

McDougall SJ, Heller RC, Horton ALC, Dissanayake CA, Thek KR, Lee-Kardashyan L & Lawrence AJ.
Florey Institute of Neuroscience and Mental Health, University of Melbourne.

The nucleus of the solitary tract (NTS) is the first central region to receive direct sensory vagal afferent input. Viscerosensory signals drive autonomic reflexes, neuroendocrine function and modulate behaviors. A group of NTS neurons project forward to the nucleus accumbens (NAC), a key region in the mesocorticolimbic dopamine system, which is central for reward and motivation. Yet the function of this NTS-NAC pathway remains unknown. A combination of neuroanatomical tracing and slice electrophysiology was used to place NTS-NAC projection neurons within the viscerosensory network. NTS-NAC projection neurons are predominantly located in the medial and caudal portions of the NTS and 54.1±6.7% are TH-positive, representing the A2 NTS cell group. In horizontal brainstem slices, solitary tract (ST) stimulation evoked EPSCs in NTS-NAC projection neurons (n=14 cells across n=7 mice). The majority (79%) received low-jitter, zero-failure EPSCs characteristic of monosynaptic ST afferent input that identifies them as second order. Previous reports suggest a role for the NAC in satiety. We used neuroanatomical tracing and stimulated vagal afferents with CCK (20ug/kg i.p.) in vivo to investigate the response in these NTS-NAC neurons. Mice were processed for detection of c-Fos and Ctb. Surprisingly, and in opposition to our hypothesis, there was no difference in the number of activated NTS-NAC (c-Fos & Ctb) within the NTS between CCK and saline treated groups. These findings indicate high-fidelity afferent information from vagal sensory afferents reaches the NAC but this information apparently does not include satiety-like signals.

**THE VERY LARGE SEPTAL HIPPOCAMPUS IN THE ELEPHANT SHREW MAY PLAY A ROLE IN SPATIAL LEARNING**

Watson C, Binks C, France F
University of Western Australia, Perth, Australia

The eastern rock elephant shrew (Elephantulus myurus) escapes from attacks from hawks by its running speed and its
The tuberal hypothalamus regulates cardiovascular and locomotor responses that are essential for rodent survival, and orexin neurons, which are located in this region, make a significant contribution to these responses. Disinhibition of the tuberal hypothalamus through microinjection of bicuculline produces marked cardiovascular and behavioural effects. However direct activation via glutamate microinjection only evokes small changes. At present little is known about how direct chemogenetic excitation of the tuberal hypothalamus effects these responses. Using a chemogenetic approach expressing the excitatory hM3Dq designer receptor exclusively activated by a designer drug (DREADD) in tuberal hypothalamus neurons, we show that clozapine-N-oxide (3 mg/kg, i.P.) successfully activates these neurons, including orexin neurons. Also, we used telemetry to show that this activation caused significant increases in activity, heart rate and blood pressure (by 5.77 arbitrary units, 84.26 BPM and 14.03 mmHg respectively). Activating all neurons with a hSyn promoter and a subpopulation (of likely glutamatergic) CaMKIIα+ neurons with a CaMKIIα promoter had mostly similar effects, as did activating more medial or more lateral hypothalamic regions. We then challenged these responses with the dual orexin receptor antagonist Almorexant. We found that Almorexant (100 mg/kg I.P.) significantly reduced the activity, pressor and tachycardic responses to chemogenetic excitation (by 67%, 51% and 54% respectively). Therefore
we show for the first time that direct chemogenetic activation of tuberal hypothalamus neurons is a highly effective method through which to study the cardiovascular and locomotor responses regulated by the tuberal hypothalamus and confirm that orexin makes a major contribution to these responses.

Carine Lampert (UFRGS)

**SHORT POST-WEANING SOCIAL ISOLATION INDUCES LONG-TERM CHANGES IN DOPAMINERGIC SYSTEM AND INCREASES SWEET FOOD SEEKING IN FEMALE RATS**

Lampert C\(^1\), Arcego DM\(^1\), Couto-Pereira NdS\(^1\), Vieira AdS\(^1\), Garcia EdS\(^1\), Vendite DA\(^1\), Calcagnotto ME\(^1\), Dalmaz C\(^1\).

\(^1\) Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.

Food addiction is a new insight into the eating disorders, which resembles drug addiction and is a contributing factor to overeating and obesity. Stressful events at earlier ages, such as social isolation (SI), may alter the reward system development and increase the risk of addictive behaviors later in life. Therefore, the aim of this study was to analyze the effects of a short post-weaning social isolation combined with a chronic high sugar diet (HSD) on sweet food seeking behavior and dopaminergic parameters in adulthood. We used female Wistar rats that were socially isolated from postnatal days (PD) 21 to 35 and received chronic HSD until PD 60. From PD 65, the habituation protocol of sweet food seeking task (using Froot Loop\(^a\)) was performed, during 5 days under food restriction, and the test session was conducted on the 6\(^{th}\) day in a fed state. Dopaminergic parameters in the nucleus accumbens (Nac) were analyzed by Western Blot. We found that animals SI after weaning increased sweet food seeking \((p=0.004)\) as well as the amount of Froot Loop\(^a\) consumed \((p=0.025)\) in the test session. In the same way, SI animals showed a reduced basal immunocountent of D2R \((p=0.024)\) in the Nac. This study highlights that a short post-weaning social isolation is able to induce long-term changes in the Nac dopaminergic system and increase sweet food seeking. These results emphasize the importance of stressful experiences during a short period of development on reward circuit programming and susceptibility to food addiction later in life.

Caitlin Mitchell (University of Newcastle)

**CHANNELRHODOPSIN-ASSISTED CIRCUIT MAPPING OF NUCLEUS ACCUMBENS CONNECTIVITY TO THE LATERAL HYPOTHALAMUS**

Mitchell CS\(^1\), Yeoh JW\(^1\), Adams CD\(^1\), Graham BA\(^1\) and Dayas CV\(^2\)

\(^1\) School of Biomedical Sciences and Pharmacy and the Centre for Translational Neuroscience and Mental Health Research, University of Newcastle and the Hunter Medical Research Institute, Newcastle, NSW, Australia.

The nucleus accumbens shell (NACsh) has a well characterised role in motivated behaviour and transmission of reward-sensitive information. The orexin system, located in the lateral hypothalamus (LH), is also a critical region involved in reward-seeking and a possible target to ameliorate reduced motivated behaviour. There is evidence for inhibitory connections from the NACsh to the LH which, when activated, suppress reward-seeking behaviour but these connections do not appear to target orexin cells. Thus, the characteristics of NACshàLH circuits that control orexin cell activity is undetermined. To resolve this, we applied an electrophysiology and optogenetics approach. We injected an adenovirus-associated viral vector (AAVS-hSyn-YFP) carrying Channelrhodopsin-2 (ChR2) into the NACsh of orexin-GFP mice. Animals were allowed a 4-week incubation period. Acute hypothalamic brain slices were prepared for targeted recordings of orexin neurons. Examination of slices revealed ChR2-positive terminals within the LH. Photostimulation of the LH reliably evoked synaptic currents in identified orexin neurons, confirming direct (monosynaptic) functional projections from NACsh to LH orexin neurons (2 cells from 7 mice). These projections were classified as GABAergic based on insensitivity to glutamate antagonist CNQX and blockage by GABA antagonist picrotoxin. Photostimulation also identified indirect (polysynaptic) functional projections from the NACsh to orexin neurons (13 cells from 7 mice). Application of glutamate receptor antagonist CNQX showed that 70% of these responses were excitatory. The remaining 30% of responses were inhibitory. Overall, this study shows direct and indirect connectivity from the NACsh to LH. Further studies should assess how modifying this pathway could affect reward-seeking behaviour.

Jin Yang Ng (The University of Auckland)

**THE EFFECTS OF SITE-SPECIFIC GLYCATION OF A\(_2\)B(1-42) ON AGGREGATION KINETICS AND DIFFERENTIATED SH-SY5Y CELL SURVIVAL**

Ng JY\(^1,2\), Kaur H\(^1,3\), Collier T\(^4\), Allison J\(^4\) Brimble MA\(^1,3\), Birch NP\(^1,2\)

\(^1\) Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.
Aggregation of amyloid-β (Aβ) peptides into fibrils is a signature of Alzheimer’s disease. Aβ is a substrate for non-enzymatic glycation and reports suggest that glycated Aβ (Aβ-AGE, Advanced Glycation End-Products) is common in Alzheimer’s disease patients and is more pathogenic than unglycated Aβ. In this study, we have examined the effects of site-specific glycation of Aβ1-42 on aggregation into fibrils and the cytotoxicity of these peptides on retinoic acid-differentiated SH-SYSY cells. We have used a combination of organic and solid phase peptide synthesis to substitute the naturally occurring lysines at position 16 and 28 of Aβ1-42 with a commonly occurring AGE, N-(carboxymethyl)lysine (CEL), to produce three analogues; Aβ-CEL16, Aβ-CEL28 and Aβ-CEL16&28. All peptides were incubated under conditions known to produce fibrillar forms of Aβ and fibrillation were visualised and quantified using transmission electron microscopy. Aβ-CEL16 and Aβ-CEL16&28 displayed significantly reduced amounts of fibrils at 24 h compared to unglycated Aβ1-42. Analysis up to 120 h suggested delays in fibril formation were more pronounced for Aβ-CEL16 compared to Aβ-CEL28 while Aβ-CEL16&28 displayed the least fibrils. Testing of both unglycated and glycated Aβ1-42 peptides in differentiated SH-SYSY cells showed all peptides decreased metabolic activity (MTT reduction). Together these studies demonstrate that glycated Aβ1-42 peptides are cytotoxic to cells and show site-specific effects of glycation on aggregation. Thioflavin T assays revealed that Aβ-CEL16&28 was most prone to a fragmentation mechanism. In silico modelling of pentameric fibrils revealed Aβ-CEL16&28 was the least stable, giving the lowest free energy change upon binding.

Claire Foldi (Monash University)

ANHEDONIA, REWARD-BASED FEEDING AND HYPERACTIVITY: INSIGHTS FROM THE ACTIVITY-BASED ANOREXIA (ABA) RAT MODEL.

Foldi CJ, Milton LK, Oldfield BJ.
Department of Physiology, Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC, 3800 Australia.

Individuals suffering anorexia nervosa (AN) become anhedonic, most profoundly in relation to food intake. We have previously shown in the activity-based anorexia (ABA) rodent model of AN that increasing neuronal activity in the mesolimbic reward circuit prevents weight loss by increasing food intake. We hypothesise that enhanced feeding during reward circuit activation is underscored by an increase in the hedonic value of food.

Female rats (n=28; 6 weeks old) underwent bilateral stereotaxic injections of canine adenovirus-2-Cre (CAV-2-Cre) into the nucleus accumbens (NAC) and activating DREADDs (AAV-hSyn-DIO-hM3D(Gq)-mCherry) into the ventral tegmental area (VTA). DREADDs reorient in the presence of retrogradely-transported Cre and clozapine-n-oxide (CNO) causes DREADD-expressing neurons to fire with temporal and anatomical specificity. As defined in ABA, rats were given free access to running wheels and time-limited (90 min) access to food, with daily i.p. injections of 0.3 mg/kg CNO at the onset of the feeding period. The impact on anhedonia, measured using the two-bottle saccharin preference test, was assessed before, during and after access to running wheels. Anhedonia developed within 3 days of running wheel access (F1,83, 30.17=78.29, p<0.0001) and endured for 10 days after access was removed (t=4.76, p=0.005). Anhedonia was not associated with food restriction (F3,30=0.30, p=0.83) or food intake (r2=0.006, p=0.45). The impact of DREADD activation of the NAc-VTA pathway revealed complex interactions between reward associated with running, feeding and sweet taste. These results will inform the neurobiological underpinnings of AN, and provide insight into the mechanisms of reward circuitry relevant to feeding and weight loss.

David Reser (Monash Rural Health)

CYTOARCHITECTONIC PARCELLATION OF THE MARMOSET CLAUSTRUM

Pham , Atapour N, Watkins , Worthy , and Reser DH
1. 2. Department of Physiology, Monash University, Clayton, VIC, AU. . Biomedicine Discovery Institute, Monash University, Clayton, VIC, AU.

There has been a surge of interest in the structure and function of the mammalian claustrum in recent years, due in part to widely publicized theories surrounding a potential role for the claustrum in initiation or maintenance of consciousness. Most experimental work in the claustrum has been in rodent species, although recent studies from our lab and others have indicated that substantive differences in claustro-cortical connectivity exist between rodents and primates. In this study, we examined the cyto- and myelo- architecture of the claustrum of a small primate, the common
function. Nissl, NeuN, parvalbumin, calbindin, and myelin-stained sections from 4 adult marmosets were studied using light microscopy and serial reconstruction to identify potential internal compartments. Our findings suggest that there is an internal organization of the marmoset claustrum, including at least 2 major subdivisions, which correspond to the endopiriform and insular claustrum nuclei reported in other species. Continuing efforts are underway to relate these findings to the pattern of cortical connectivity revealed by tracer studies from multiple laboratories internationally.

John Van Horn (Usc Mark and Mary Stevens Neuroimaging and Informatics Institute)

ANALYSIS OF WHITE MATTER CONNECTIVITY AFFIRMS THE CLAUSTRUM AS A MEMBER OF THE RICH CLUB NETWORK OF THE HUMAN BRAIN.

Van Horn JD, Bhattrai A, Irimia A, and Torgerson C
Laboratory of Neuroimaging, Mark & Mary Stevens Neuroimaging and Informatics Institute, Keck School of Medicine of USC, University of Southern California, Los Angeles, CA USA

The claustrum has broad connectivity to most (sub)cortical regions (1, 2), yet its function is poorly understood. Here, we investigate the relationship between the claustrum and the ‘rich-club’ (RC) network of the human brain. Structural magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) volumes were acquired from N=100 healthy adults (53% female; ages: 18.6 ± 6.1yrs; μ±σ: 32.7 ± 11.6yrs). T1-MPRAGE volumes were acquired (TR=20ms, TE=88ms, flip angle=90°, voxel size=1mm3) and 68-direction DTI data were also obtained (TR=9.4s, TE=88ms, flip angle=90°, voxel size=8mm3). Image processing (FSL), tissue classification (FreeSurfer), tractography (TrackVis) and connectivity analyses were performed as detailed elsewhere (2-4). The claustrum was identified and segmented using in-house methods (2). Network analysis was implemented using the Brain Connectivity Toolbox (5, 6). RC membership of brain structures was based on previous reports (3). The claustrum was strongly connected to all regions comprising the RC. Specifically, the number of WM bundles (μ±σ) per cm3 between the claustrum and RC members was: 8.4±2.3 (paracent. gyr. and sulc.), 6.2±2.1 (sup. par. gyr.), 2.5±0.9 (mid. fr. gyr.), 3.0±0.9 (sup. fr. gyr.), 7.2±2.6 (precuneus), 4.5±1.1 (precent. gyr.), 3.7±1.4 (inf. precent. sulc.) and 4.1±2.1 (sup. precent. sulc.). It was also found that the removal of the claustrum from the network model led to statistically significant changes in the nodal betweenness centrality of RC nodes; e.g. left (t=2.01, df=98, p<0.04) and right (t=1.97, p<0.04) precent. gyr.; the left (t=2.17, p<0.03) and right (t=2.10, p<0.03) mid. fr. gyr. This study strongly supports the claustrum as a part of the human brain’s RC. Future research will investigate the role of the claustrum in cognitive operations which involve the RC network.

Sarah Ch’ng (Florey Institute Of Neuroscience And Mental Health)

CHARACTERISATION OF THE RXFP3 SYSTEM IN THE MOUSE BED NUCLEUS OF THE STRIA TERMINALIS

Ch’ng SC, Smith CM, Brown RM, Gundlach AL, Lawrence AJ
Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, VIC.

The BNST RXFP3 system is implicated in stress-induced alcohol seeking, but the neurochemical phenotype and connectivity of BNST RXFP3-expressing (RXFP3+) cells have yet to be elucidated. We interrogated the molecular signature and effenter connectome of BNST RXFP3+ neurons using RXFP3-Cre transgenic reporter mice. BNST RXFP3+ cells are circumscribed to the dorsal BNST (dBNST) and are a neurochemically heterogeneous population, comprising a mix of inhibitory and excitatory neurons. ~48% of BNST RXFP3+ neurons are GABAergic and ~25% co-express the calcium-binding protein, calbindin. A subset of BNST RXFP3+ cells (~41%) co-express CaMKIIα. In situ hybridisation using RNAscope® revealed that ~35% of BNST RXFP3+ cells express VGluT2 mRNA, suggesting that a subpopulation of these neurons are glutamatergic. We injected a Cre-dependent anterograde virus (AAV-FLEX-hSym-mGFP-SYP-mRuby) into the dBNST (coordinates from bregma: A/P −0.2mm, M/L ±1.0mm, D/V −4.0mm) and cholera toxin β (60-80nl) into the ventral tegmental area (VTA, coordinates from bregma: A/P −3.0mm, M/L ±0.4mm, D/V −4.5mm) to examine BNST RXFP3+ efferents throughout the neuraxis. dBNST RXFP3+ cells do not project directly to the VTA but may communicate with the VTA via either a ventral BNST and/or a medial preoptic area (MPO) relay. Other prominent targets of BNST RXFP3+ neurons include the medial amygdala, arcuate nucleus, rostral anterior hypothalamus, perifornical area, and tuber cinereum. These studies are the first to characterise the BNST RXFP3 system in mouse and lay the foundation for future functional studies.
Excitability and Synaptic transmission

Robert Callister (University of Newcastle)

**HUMAN FOETAL SENSORY NEURONS ACQUIRE INWARD CURRENTS DURING THE LATE FIRST TRIMESTER**

Tadors, Lim, Jobling, Brichita and Callister  
Biomedical Science & Pharmacy, University of Newcastle, Callaghan, NSW, 2308

Our ability to perceive sensations such as touch, warmth, cold, and pain begins in sensory neurons (SNs) dorsal root ganglia (DRG). Accurate discrimination of sensations in the adult embryonic development of SNs. However, studies on the early development of human SNs are lacking. Human foetal DRGs (11 to 15 weeks gestation; WG) were isolated and dissociated to obtain SNs, which were then plated onto culture wells. Whole-cell patch-clamp recordings were made 1 hour after plating (CsF-based internal solution) from visualised SNs at room temperature (22-24°C). Human foetal SNs could be separated into large and small populations from 11WG. At 11WG, SNs were unable to generate inward currents in response to membrane depolarization (n = 5/7; 71%), regardless of their size. The remaining SNs discharged small inward currents (pA). In contrast, all recorded SNs older than 11WG displayed inward currents during membrane depolarisation (n = 9/9), with amplitude 13 and 15WG (509 ± 100 vs. 727 ± 370 pA, n = 5 and 4). Profile of inward currents small SNs 13WGpeak current decay time SNs. These data suggest human foetal SNs acquire the ability to generate inward currents between 11 and 13WG.

Robert Harvey (University of The Sunshine Coast)

**GLYCINE RECEPTOR α4 SUBUNIT CONTROLS STARTLE AND ESCAPE RESPONSES**

Leacock S1, Syed P2, James VM3, Bode A2, Kawamaki K3, Keramidas A2, Suster M4, Lynch JW5,6 and Harvey RJ1,6  
1UCL School of Pharmacy, London, UK; 2Queensland Brain Institute, University of Queensland, Brisbane, Australia; 3National Institute of Genetics, Mishima, Japan; 4Uni Research AS, Bergen, Norway; 5School of Biomedical Sciences, Queensland, Brisbane, Australia; 6School of Health and Sport Sciences, University of the Sunshine Coast, Sippy Downs, Australia.

Inhibitory glycine receptors have major roles in startle disease (GlyR α1), autism spectrum disorder (GlyR α2), and inflammatory pain sensitization / rhythmic breathing (GlyR α3). However, the role of the GlyR α4 subunit has remained enigmatic, since GLRA4 is a pseudogene in humans, due to an in-frame stop codon in the M3-M4 intracellular loop. Despite this, a recent genetic study implicated GLRA4 in intellectual disability, behavioural problems and craniofacial anomalies. Analysing data from sequenced genomes, we found that GlyR α4 subunit genes are intact and functional all vertebrate species - with the exception of humans. Artificial restoration of the missing conserved arginine (R390) in the human GlyR α4 CDNA was not sufficient to restore function. Further bioinformatic and mutagenesis analysis revealed an additional damaging substitution at K59 that that ablates human GlyR α4 function, which is not present in other vertebrate GlyR α4 sequences. To gain insights into the biological role of GlyR α4 function in other species, we studied the duplicated genes glra4a and glra4b in zebrafish. While glra4b expression is restricted to the retina, we found that the zebrafish GlyR α4a subunit gene (glra4a) is strongly expressed in spinal cord and hindbrain. Using gene knockdown and a dominant-negative GlyR α4a mutant, we found that GlyR α4a contributes to touch-evoked escape behaviours in zebrafish. Thus, although GlyR α4 is extremely unlikely to be involved in human disease, this subtype may contribute to startle behaviours in other organisms.

Stewart Head (Western Sydney University)

**RESTING MEMBRANE POTENTIAL INPUT RESISTANCE AND LONG-TERM DEPRESSION IN ADULT MDX DYSTROPHIC MOUSE PURKINJE CELLS**

Head S1, Anderson JL2, Kueh S1, Morley JW1  
1. School of Medicine, Western Sydney University, Campbelltown Australia. 2. Westmead Childrens Hospital, Westmead, Australia.

While dystrophinopathies are mainly characterised by a progressive loss of skeletal muscle tissue with age, there is also a significant involvement of the CNS, with boys and mice displaying cognitive and behavioural deficits. We have previously shown in young mice that absence of dystrophin disrupts the cellular learning pathways, hetero- and homo-synaptic
Long Term Depression (LTD) in dystrophin-deficient mdx Purkinje cells. In this study, we examined the effect of age on electrophysiological properties of mdx Purkinje cells in cerebellum slices. There is a significant depolarisation of resting membrane potential (RMP) in adult mdx Purkinje cells (-61.6mV +/- 2.2 (n=13) c.f. age matched controls, -70.1 mV +/- 2.6 (n=9)). There was no significant difference in input resistance between mdx and wildtype indicating that membrane integrity and ionic conductances were unchanged (15.85 MΩ +/- 3.585 (n=9) c.f. 13.74 MΩ +/- 2.434 (n=13)). In contrast to the blunting in LTD we reported in young mdx, in adult wildtype and mdx mice LTD at the parallel fibre-Purkinje cell synapse is largely the same, (mdx early phase 59.5 +/- 2.5%, late phase 55.7 +/- 2.4% (n=6); wildtype early phase 71.4 +/- 4.1% late phase 63.7 +/- 3.5% (n=5)). We hypothesise the depolarised RMP in adult mdx is a consequence of a progressive disruption of Ca2+ homeostasis associated with the absence of dystrophin. An elevated [Ca2+]i would activate increased Na+ influx causing depolarisation and may also explain some of the subtle changes in LTD induction we report here in the adult mdx.

Timothy Lynagh (University of Copenhagen)

MOLECULAR BASIS FOR ALLOSTERIC INHIBITION OF ACID-SENSING ION CHANNEL 1A BY IBUPROfen

Lynagh T, Romero-Rojo JL, Lund C, Pless SA
Center for Biopharmaceuticals, Department of Drug Design and Pharmacology, University of Copenhagen, Denmark

Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen exert analgesic and neuroprotective effects on the nervous system. A growing body of evidence suggests that certain aspects of NSAID pharmacology are mediated by acid-sensing ion channels (ASICs), a small family of excitatory neurotransmitter receptors implicated in pain and neuroinflammation. Thus far, the mechanism and molecular basis of NSAID inhibition of ASICs have remained unknown, hindering the exploration of this line of therapy. Here, we examined the effects of ibuprofen on distinct aspects of ASIC1a function, explored the molecular determinants of inhibition and sought to establish informative structure-activity relationships, using electrophysiology, site-directed mutagenesis and voltage clamp fluorometry. Our results show that ibuprofen is an allosteric inhibitor of ASIC1a, binding to a crucial site in the agonist transduction pathway to cause conformational changes that oppose channel activation. Ibuprofen inhibits several ASIC subtypes, but certain ibuprofen derivatives show some selectivity for ASIC1a over ASIC2a and vice versa. These results thus illuminate the NSAID/ASIC interaction and pave the way for small-molecule drug design targeting pain and inflammation.

Roger Marek (Queensland Brain Institute)

PARVALBUMIN INTERNEURONS SHAPE HIPPOCAMPUS-DRIVEN PREFRONTAL ACTIVITY IN FEAR EXTINCTION

Marek R, Xu L, Sah P
The University of Queensland, Queensland Brain Institute, Brisbane, Australia

The amygdala, the main brain structure involved in encoding emotional memories, forms extensive connections with the hippocampus (HPC) and the medial prefrontal cortex (mPFC) to control extinction learning. In rodents, the prelimbic (PL) and infralimbic (IL) prefrontal cortices play distinct roles in fear learning and extinction, whereas the hippocampus is crucially involved in regulating the contextual information in which extinction occurs. By using a pharmacogenetic and optogenetic approach combining viral expression of either ChR2 (HPC) with chloride-sensitive ivermectin-receptors (IL) or Chronos (HPC) with ArcheardopisinT (IL), which allowed optical stimulation of hippocampal inputs and simultaneous silencing of neural activity of specific subtypes of interneurons in cre-dependent mouse lines, we investigated the neuronal prefrontal circuit underlaying fear extinction ex-vivo.

Ventral hippocampal projections were found to target both pyramidal cells and interneurons in the IL, with a predominant innervation of interneurons. Electrophysiological characterization of these interneurons revealed strongest innervation of fast-spiking interneurons (FS), which form local feed-forward inhibition of pyramidal cells, some of which project to the amygdala. Combined optical stimulation of HPC inputs and silencing of either parvalbumin (PV) or somatostatin (SOM)-positive interneurons in the IL revealed that the HPC targets PV+ interneurons (n=5) to shape activity of local projection neurons. Silencing SOM+ neurons (n=4) had no effect. These results show that HPC modulation of extinction occurs by modulating the activity of PV interneurons in the IL. Together, these findings improve our understanding of mPFC-driven neural circuitry that underlies the contextual extinction of learned fear.

Melissa Tadros (University of Newcastle)

YPOGLOSSAL MOTONEURONS
**Australasian Neuroscience Society Annual Scientific Meeting 2017**  
International Convention Centre, Sydney, December 3rd – 6th 2017

---

**Tadros, Smith Brichta Callister**  
*School of Biomedical Sciences & Pharmacy, Faculty of Health and Hunter Medical Research Institute, The University of Newcastle, Callaghan, NSW 2308, Australia*

Despite well documented decreases in tongue muscle strength, studies examining age-related changes in the electrophysiological properties of hypoglossal motoneurons (HMs) innervating the tongue are lacking. In this study, we used whole-cell patch-clamp recordings to compare the electrophysiological properties of HMs from young (~4 month) and aged (~30 month) mice. Transverse slices (300 mm thick) were obtained from the brainstem of male C57Bl/6 mice and whole-cell recordings were made from visualized HMs at 23°C using a KCH3SO4-based internal solution. Young and aged HMs discharged at higher frequencies (500 pA above rheobase; 61.7 ± 7.1 vs 38.8 ± 5.2 Hz, n = 10 and 6) and 0.089 ± 0.007 vs. 0.049 ± 0.005 Hz/pA, n = 10 and 6) compared to young HMs. HMs do not appear to change their term memories were not affected. However, a substantial knowledge gap exists in the world. As of 2016, only six drugs have been approved to treat the symptoms of AD, but none of them can slow or stop the disease. The major symptom of AD is a difficulty in forming and maintaining long-term memories, but their formation is not affected. However, a substantial knowledge gap exists in the molecular mechanism that allows Arc to regulate synaptic connections and affect memory formation, and this shortcoming in the literature has prevented Arc becoming a potential drug target for the treatment of AD. Current studies suggest that Arc may mediate synaptic development by regulating neuronal trafficking of AMPA receptor through interactions with neuronal endocytic machinery, such as dynamin and endophilin. Arc was also found to interact with presenilin to promote activity-dependent Amyloid-beta generation in an Alzheimer’s disease model. These data created a pressing need to solve the high-resolution structure of Arc and characterize Arc’s residue-specific binding sites for endophilin, dynamin, and presenilin. In our research project, we have focused on the structural study of

---

**Angelo Tedoldi (The University Of Queensland, Queensland Brain Institute)**

**EVIDENCE FOR NEWLY GENERATED INTERNEURONS IN THE BASOLATERAL AMYGDALA OF ADULT MICE.**

Jhaveri DJ1,4, Tedoldi A1, Hunt S1, Sullivan R1, Watts NR2, Power JM3, Bartlett PF4, Sah P1  
1. The University of Queensland, Queensland Brain Institute, Brisbane, QLD 4072, Australia. 2. School of Medical Sciences, University of New South Wales, Sydney NSW 2052, Australia. 3. Critical Care and Trauma Division, The George Institute for Global Health, Sydney, NSW, 2000, Australia. 4. Mater Research Institute - The University of Queensland, QLD 4102, Australia.

New neurons are continually generated from the resident populations of precursor cells in selective niches of the adult mammalian brain, but whether such cells are present in the adult amygdala is not known. Using the neurosphere assay, we demonstrate that a small number of precursor cells, the majority of which express Achaete-scute complex homolog 1 (Ascl1), are present in the basolateral amygdala (BLA) of the adult mouse. Using neuron-specific Thy1-YFP transgenic mice, we show that YFP+ cells in BLA-derived neurospheres have a neuronal morphology, co-express the neuronal marker βIII-tubulin, and generate action potentials, confirming their neuronal phenotype. In vivo, we demonstrate the presence of newly generated BrdU-labeled cells in the adult BLA, and show that a proportion of these cells co-express the immature neuronal marker doublecortin (DCX). Furthermore, we reveal that a significant proportion of GFP+ neurons (~23%) in the BLA are newly generated (BrdU+) in DCX-GFP mice, and using whole-cell recordings in acute slices we demonstrate that the GFP+ cells express electrophysiological properties that are characteristic of interneurons. Using retrovirus-GFP labelling as well as the Ascl1CreERT2 mouse line, we further confirm that the precursor cells within the BLA give rise to mature and functional interneurons that persist in the BLA for at least 8 weeks after their birth. These results demonstrate that neurogenic precursor cells are present in the adult BLA, and generate functional interneurons.

---

**Lei Wang (Hawaii Pacific University)**

**CHARACTERIZATION OF THE STRUCTURE OF ARC AND ITS INTERACTIONS WITH SYNAPTIC ENDOCYTIC PARTNERS**

Alzheimer’s disease (AD) is a devastating neurodegenerative disease, which affects approximately 30 million people in the world. As of 2016, only six drugs have been approved to treat the symptoms of AD, but none of them can slow or stop the disease. The major symptom of AD is a difficulty in forming long-term memories of newly acquired information, which requires proper development of synaptic connections between brain neurons at the cellular level. The activity-regulated cytoskeleton-associated protein (Arc) is a neuronal protein specifically linked to the formation of long-term memory: Arc-knockout mice showed substantial deficits in long-lasting memories, but their short-term memories were not affected. However, a substantial knowledge gap exists to explain the molecular mechanism that allows Arc to regulate synaptic connections and affect memory formation, and this shortcoming in the literature has prevented Arc becoming a potential drug target for the treatment of AD. Current studies suggest that Arc may mediate synaptic development by regulating neuronal trafficking of AMPA receptor through interactions with neuronal endocytic machinery, such as dynamin and endophilin. Arc was also found to interact with presenilin to promote activity-dependent Amyloid-beta generation in an Alzheimer’s disease model. These data created a pressing need to solve the high-resolution structure of Arc and characterize Arc’s residue-specific binding sites for endophilin, dynamin, and presenilin. In our research project, we have focused on the structural study of
Arc using solution-state NMR techniques, and we have also used the technique to characterize Arc interactions with dynamin and endophilin. We will present our latest research findings in the 37th ANS conference.

**Jesse Wark** (Children’s Medical Research Institute)

### IDENTIFYING BINDING PARTNERS OF RIM1 THROUGH PROTEOMIC ANALYSIS OF FUSION-PROTEIN PULLDOWNS

**Wark JR**, Engholm-Keller K, Fernando R, Graham ME

1. Synapse Proteomics, Children’s Medical Research Institute, Westmead, NSW, Australia
2. School of Biomedical Engineering, University of Sydney, Sydney, NSW, Australia.

Neurotransmitter release is regulated by scaffolding proteins at the active zone. RAB3A-interacting molecule (RIM1) is a large active zone protein (~180 kDa) with multiple protein interactions which facilitates synaptic vesicle docking and priming, as well as calcium channel clustering. We aim to identify binding partners of RIM1 using a pulldown system and proteomics. Initially, there was limited success in identifying known or novel binding partners using bacterially expressed GST (glutathione S-transferase)-RIM1 (full-length). The full-length protein had poor expression and resulted in many truncated by-products. This was likely due to cleavage of the fusion protein in the bacterial system. We moved to a HEK293T-produced GFP-RIM1 system, which produced whole, full length GFP-tagged RIM1, as confirmed by Western blot analysis. This fusion protein was immunoprecipitated using anti-GFP conjugated to magnetic beads and then used as bait in a pulldown with synaptosome lysate. Using proteomics, we confirmed that this system bound several known RIM1 binding partners, with significantly different binding compared to control. In future work, this full-length GFP-RIM1 system will be used to determine phospho-dependent binding partners, since RIM1 has recently been shown to have activity-dependent phosphorylation.

**Jing Xue** (Children’s Medical Research Institute)

### PHOSPHORYLATION OF DYNAMIN IXA ISOFORM REGULATES ENDOPHILIN INTERACTION FOR MULTIPLE CELLULAR FUNCTIONS

**Xue J**, Luo L, Hains PG, Quan A, Cousin MA, Robinson PJ

1. Cell Signalling Unit, Children’s Medical Research Institute, The University of Sydney, Locked Bag 23, Wentworthville 2145, NSW, Australia. 2. Institute for Molecular Bioscience (IMB), The University of Queensland, Brisbane, Queensland 4072, Australia. 3. Membrane Biology Group, Centre for Integrative Physiology, University of Edinburgh, George Square, Edinburgh EH8 9XD, United Kingdom.

Dynamin I (Dynl) is a phosphoprotein which mediates vesicle fission during synaptic vesicle endocytosis (SVE) in nerve terminals. Dyn I has a proline-rich domain (PRD) at its C-terminus which is the binding site for Src homology 3 (SH3) domain containing proteins. There are two main splice variants of dyn I PRD, long (dynixa) and short (dynlxb), varying at the C-terminus tail by 20 (xa) or 7 (xb) residues. We previously showed that dynlxb contains a conserved calcineurin (CaN) binding site at its tail to regulate the phosphorylation-dephosphorylation cycle in activity-dependent bulk endocytosis (ADBE) in neurons. Endophilin is known to bind dynl at Sites 2 + 3 (794RAPAVPPAR-793) present in both xa and xb PRD. We have discovered an additional binding site in the long tail of dynixa located at Sites 9 + 10 (831PSRPNRAPPVPP844) which is phosphorylation-dependent. We show that the spliced long tail of dynixa-PRD is essential for SVE, but not for ADBE in neurons. Both endophilin binding regions (Sites 2 + 3 and Sites 9 + 10) in dynixa are required for transferrin uptake in dynamin triple knock-out (TKO) cells, suggesting that both regions are essential for clathrin-mediated endocytosis (CME). Additionally, each of the binding sites differentially mediate the role of endophilin in neuronal cell development. Endophilin binding to the Sites 2 + 3 in dynixa regulates neurite outgrowth and branching, while binding to Sites 9 + 10 regulates dendritic spine morphogenesis. These observations show dynamin phosphorylation mediated-endophilin binding acts as a switch to change between cellular functions.

**Alba Bellot-Saez** (Western Sydney University)

### ASTROCYTIC MODULATION OF NEURONAL NETWORK OSCILLATIONS

**Bellot-Saez A**, Cohen G, Morley J, Buskila Y

1. Biomedical Engineering and Neuroscience group, The MARCS Institute, Western Sydney University, Penrith, NSW, Australia.
2. School of Medicine, Western Sydney University, Campbelltown, NSW, Australia.
Synchronous activity within neuronal networks gives rise to neural oscillations, which are thought to be involved in several physiological processes, such as bias of input selection, temporal linkage of neurons into assemblies and facilitation of synaptic plasticity. It has been postulated that at least ten distinct mechanisms are required to cover the large frequency range of cortical oscillations, however the mechanism that governs the transition between different oscillatory frequencies is still unknown. In this study, we have explored the potential involvement of astrocytic K+ clearance in the modulation of neural oscillations at the network and single-cell levels. Our results indicate that local rise in extracellular K+ concentration leads to alterations in oscillation frequencies and amplitude across a wide spectrum. Reduced astrocytic K+ spatial buffering capabilities through bath application of Barium (100 μM), as well as restricted astrocytic connectivity by the selective blockers GAP-26/27, resulted in increased cellular and network excitability, as indicated by elevated number of spikes, longer excitability durations and reduced number of Biocytin-stained astrocytes. In addition, altered K+ clearance shifted the neuronal resonance frequency towards higher frequencies and increased the power governing network oscillations in the range of beta and gamma frequencies. Our study suggests that neuromodulators of extracellular K+ likely influence the neuronal resonance frequency, which imposes alterations in network activity. Since astrocytes are essential for maintaining ion homeostasis in the CNS, we suggest that modulation of astrocytic K+ clearance mechanisms is a potential target to impact neural oscillations, and thereby mediating the transition between brain waves.

David Almeida Cardoso (Children's Medical Research Institute / University of Sydney)

DYNAMIN STRUCTURE AND FUNCTION HINGING ON RYNGOs

David A. Cardoso1, Mohammed K. Abdel-Hamid2, Adam McCluskey2, and Phillip J. Robinson1

1 Cell Signalling Unit, Children’s Medical Research Institute, The University of Sydney, Westmead, NSW, Australia
2 School of Environmental and Life Sciences, Faculty of Science, University of Newcastle, NSW, Australia

Dynamin I is a neuronal-enriched multi-domain GTPase enzyme capable of performing the final scission of invaginated plasma membrane prior to completion of synaptic vesicle endocytosis (SVE). Targeting dynamin in epilepsy mouse models can control seizure initiation and propagation. We have generated a series of small molecule dynamin modulators (Ryngos) which ‘lock’ dynamin into a ‘ring’ oligomeric state that structurally differs from the ‘helical’ state required for SVE. These compounds exhibit different activities on dynamin enzyme activity in vitro (Ryngo-1: mixed-mode / Ryngo-3: stimulation). Due to their chemical similarity, it can be surmised that these pharmacological agents share a common binding pocket. To establish their binding site, advanced computer modelling techniques were employed. The model predicted lead compounds; Ryngo-1-23 and Ryngo-3-32, independently localised to, and differentially interacted with Hinge 1, located between middle domain and bundle-signalling element of dynamin. A partial overlap of implicated residues between Ryngo-1-23 and Ryngo-3-32 suggests drug binding to different sub-regions of Hinge 1 may be capable of imparting different actions (stimulation/inhibition) on dynamin enzyme in vitro. To validate this model, mutagenesis of implicated Hinge 1 residues was carried out and resultant mutants characterised. Enzyme assays largely support the predictions i.e. single mutations specifically lost drug action. The data supports the proposed model of these compounds interacting with a flexible hinge within dynamin.

Xiumin Chen (QBI)

PHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF INHIBITORY POSTSYNAPTIC CURRENTS MEDIATED BY α5β1γ2, α5β2γ2 AND α5β3γ2 GABAA RECEPTORS

Xiumin Chen1, Angelo Keramidas1, Joseph W Lynch1*

1 Queensland Brain Institute, The University of Queensland, Brisbane, QLD 4072 Australia

α5-containing GABAARs are potential therapeutic targets for clinical conditions including age-related dementia, stroke, schizophrenia, Down syndrome, anaesthetic-induced amnesia, anxiety and pain. α5-containing GABAARs are expressed in layer 5 cortical neurons and hippocampal pyramidal neurons where they mediate both tonic currents and slow inhibitory postsynaptic currents (IPSCs). A range of drugs has been developed to specifically modulate these receptors. The main α5-containing GABAARs that are likely to exist in vivo are the α5β1γ2, α5β2γ2 and α5β3γ2 isoforms. We currently have few clues as to how these isoforms are distributed between synaptic and extrasynaptic compartments or their relative roles in controlling neuronal excitability. Accordingly, the aim of this study was to define the basic biophysical and pharmacological properties of IPSCs mediated by the three isoforms in a hippocampal neuron-HEK293 cell co-culture assay. The IPSC decay rates were slow (α5β1γ2L: 45 ms; α5β1γ2L: 80 ms; α5β3γ2L: 184 ms) and were largely dominated by the intrinsic channel deactivation rates. By comparing IPSC rise times, we inferred that α5β1γ2L
**THE REGULATORY ROLE OF OPIOID PEPTIDES ON FEEDBACK INHIBITION WITHIN THE AMYGDALA**

Gregoriou GG¹, Winters BL², Bagley EE¹

¹Discipline of Pharmacology, School of Medical Sciences, University of Sydney, Sydney, NSW, Australia. ²Pain Management Research Institute, Kolling School of Medical Research, University of Sydney, Sydney, NSW, Australia.

Neural circuits within the amygdala regulate the emotional learning and memory processes that motivate drug-seeking in abstinent opioid addicts. In particular, neurons within the basolateral amygdala nucleus (BLA), which innervate the learning and reward centres of the brain, are critically involved in cue-induced relapse to drug-seeking behaviours. GABAergic intercalated cells (ITCs), which form dense clusters that encapsulate the BLA, act as inhibitory gates; providing feedforward inhibition to the BLA's amygdala targets and feedback inhibition to the BLA itself. We have previously found that endogenous opioid peptides, which modulate BLA-mediated behaviours, are robust regulators of synaptic transmission in ITC clusters. However, the effects of opioid peptides at feedback ITC-BLA synapses remain unknown. Using whole-cell, patch-clamp electrophysiology in BLA PNs isolated from Sprague-Dawley rats, we show that opioid peptides can directly reduce BLA PN excitability. These data suggest that ITCs provide feedback inhibition onto BLA PNs and implicate a role for opioid peptides in regulating BLA-mediated behaviours.

Sumasri Guntupalli (Queensland Brain Institute)

**GLUA1 SUBUNIT UBIQUITINATION MEDIATES AMYLOID-B-INDUCED LOSS OF SURFACE A-AMINO-3-HYDROXY-5-METHYL-4-ISOXAZOLEPROPIONIC ACID (AMPA) RECEPTORS**

Guntupalli S¹, Jang SE¹, Zhu T¹, Huganir RL², Widagdo J¹, Anggono V¹.  
¹Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Queensland 4072, Australia. ²Department of Neuroscience and Kavli Neuroscience Discovery Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

The accumulation of soluble amyloid-β (Aβ) peptides produces profound neuronal changes in the brain during the pathogenesis of Alzheimer’s disease. Excessive levels of Aβ disrupt excitatory synaptic transmission by promoting the removal of synaptic AMPA receptors (AMPARs), dendritic spine loss, and synaptic depression. Recently, activity-dependent ubiquitination of the GluA1 subunit has been shown to regulate the intracellular sorting of AMPARs toward late endosomes for degradation. However, whether this ubiquitin signaling pathway mediates Aβ-induced loss of surface AMPARs is unknown. In this study, we demonstrate that acute exposure of cultured neurons to soluble Aβ oligomers induces AMPAR ubiquitination concomitant with the removal of AMPARs from the plasma membrane. Importantly, expression of the GluA1 ubiquitin-deficient mutants inhibited the adverse effects of Aβ on the surface expression of AMPARs in neurons. Furthermore, we revealed the cross-talk between GluA1 ubiquitination and phosphorylation, in particular phosphorylation at Ser-845, which is crucial for AMPAR recycling and is known to be dephosphorylated in the presence of Aβ. Our data showed that the GluA1 ubiquitin-deficient mutant enhances GluA1 phosphorylation on Ser-845. Conversely, the GluA1 S845D phosphomimetic mutant reduced binding with Nedd4-1 and hence the ubiquitination of AMPARs. Importantly, the GluA1 S845D mutant also prevented Aβ-induced removal of surface AMPARs. Taken together, these findings provide the first demonstration of the dynamic cross-modulation of GluA1 ubiquitination and phosphorylation, a process that is perturbed by Aβ, in regulating the membrane sorting decision that ultimately determines the number of AMPARs on the cell surface.
THE IMPACT OF ACUTE AND CHRONIC NEUROINFLAMMATION ON THE ELECTROPHYSIOLOGICAL PROPERTIES OF CHOLINERGIC NEURONS

Kekesi O 1,2, Gyengesi E 1, Muench G 1, Buskila Y 2
1: Dept. of Pharmacology, School of Medicine; Western Sydney University
2: Biomedical Engineering and Neuroscience Group, The MARCS Institute; Western Sydney University

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by significant impairment of cognitive function and memory. There are several hypotheses regarding the etiology of AD. Loss of cholinergic innervation in the hippocampus and neocortex contributes significantly to the cognitive symptoms associated with AD, and gave rise to the "cholinergic hypothesis" of AD. However, the source underlying the loss of cholinergic cells is still unknown. Recent findings suggest that neuroinflammation is an early process in the pathogenesis of AD. However, the impact of neuroinflammation on the physiological properties of cholinergic neurons is still unknown.

In this study, we have investigated the impact of acute and chronic neuroinflammation on the biophysiological properties of cholinergic neurons. Acute neuroinflammation was induced by peripheral administration of lipopolysaccharide (500 μg/kg) into ChAT(BAC)-eGFP mice at different ages (3-18 months), while age-matched control animals received saline. Chronic neuroinflammation was investigated in GFAP-IL6 mice (3-18 months), which overexpress the proinflammatory cytokine IL-6 in astrocytes. The electrophysiological properties of cholinergic neurons were measured in acute brain slices containing the septo-hippocampal pathway.

Our results indicate that while acute neuroinflammation had only minor influence on neuronal excitability, chronic neuroinflammation caused a significant decrease in neuronal excitability as seen by the increase of rheobase and spike amplitude. Moreover, aged, saline-injected animals showed a significant decrease of membrane excitability, comparing to young animals. These results are indicative of alterations in intrinsic excitability, which may contribute to the cholinergic loss during aging and thus promote the formation of AD.

RIBOSOME PROFILING REVEALS MEMBRANE DEPOLARISATION OF SH-SY5Y CELLS REMODELS THE GLOBAL TRANSLATOME

Kiltschewskij DJ 1,2, Cairns MJ 1,2,3
1. School of Biomedical Sciences and Pharmacy, Faculty of Health, The University of Newcastle, Callaghan, Australia.
2. Priority Research Centre for Brain and Mental Health Research, Hunter Medical Research Institute, New Lambton, Australia.
3. Schizophrenia Research Institute, Sydney, Australia.

Neuronal excitation is thought to rely on a cascade of transcriptional, translational and post-translational events to modify excitation-associated gene expression. Although changes in transcription and protein expression have been thoroughly investigated, excitation-associated translational activity has been difficult to characterize due to technological limitations. In the current study, we therefore used the recently developed ribosome profiling sequencing (Ribo-Seq) to investigate global changes in active translational activity in retinoic acid differentiated, membrane depolarized SH-SY5Y cells. We found that in excess of 1230 genes were subject to a significant (p < 0.05, q < 0.1), excitation-associated change in translation 1 hour after depolarization, which expanded to 2040 genes after 2 hours. When Ribo-Seq data were integrated with steady-state mRNA expression data (RNA-Seq), we observed a weak correlation (R² = 0.1105) between translational activity and mRNA abundance, whereby most genes were subject to modulation primarily at the translational level. This correlation was strengthened (R² = 0.3568) 2 hours post-depolarisation, suggesting translational changes at the later time-point were more accurately explained by modification of mRNA abundance. We therefore suspect that initial translational responses to membrane excitation are dictated by post-transcriptional regulation of mRNA translational competency, prior to increases in transcriptional activity.

PAIN INDUCED SYNAPTIC PLASTICITY IN THE AMYGDALA

Sarah Kissiwaa (University Of Sydney)
The spino-parabrachial-amygdala pathway is critical for the development of persistent pain. This pathway delivers nociceptive information to the laterocapsular amygdala (CeLC) and undergoes plasticity in conditions with ongoing injury. This project defined how a brief nociceptive stimulus changes the synaptic properties of the parabrachial (PB) inputs to the CeLC and whether these inputs are modulated by opioids. We used electrophysiology to define the synaptic properties of the parabrachial-CeLC synapse in acute brain slices taken from male Sprague-Dawley rats that have undergone a brief nociceptive stimulus (paw immersion in 44°C water bath for 2 minutes). We found that the brief nociceptive stimulus increases AMPA/NMDA ratios at PB-CeLC synapses. The increase in AMPA/NMDA ratio (marker for synaptic potentiation) was maximal 1 day after the nociceptive stimulus (nociceptive stimulus AMPA/NMDA ratio 10.58 ± 1.650, n=65 vs. control 4.535 ± 0.8973, n=32, Students t-test p=0.02) and persisted for 3 days. This data shows that a brief nociceptive stimulus produces a potentiation that can outlast the original stimulus. The potentiation could result in a previously innocuous stimulus producing an increased pain experience or alter endogenous analgesia. We used electrical and light-activated stimulation to determine if opioids regulate the PB-CeLC synapse. We found that while opioids modulate this synapse; the receptors involved differed with the stimulation. This difference may be due to the enhanced selectivity of optogenetics over electrical stimulation or changes caused by viral vectors. Nonetheless given this synapse’s involvement in pain, it could be part of the site of action for opioid analgesia.

Laura Leighton (Queensland Brain Institute)

A FUNCTIONAL ROLE FOR THE EPIGENETIC REGULATOR ING1 IN ACTIVITY-INDUCED GENE EXPRESSION IN PRIMARY CORTICAL NEURONS


Neuronal plasticity is dependent on activity-induced changes in gene expression and RNA metabolism. However, many of the epigenetic regulators and molecular interactions which drive these changes have not been investigated in neurons. We have found that the genome-wide deposition of inhibitor of growth family member 1 (ING1), which is a central epigenetic regulatory protein, is dynamically regulated in response to activity in primary cortical neurons. ING1 knockdown leads to decreased activity-responsive expression of genes involved in synaptic plasticity, including the regulatory subunit of calcineurin, Ppp3r1. In addition, ING1 binding at a site upstream of the transcription start site (TSS) of Ppp3r1 depends on the activity of neuronal Piwi-like proteins. Our results suggest that these pathways may be linked through their shared role in chromatin remodeling during DNA repair. These findings provide new insight into a novel mode of activity-induced genetic regulation in neurons.

Holly Melland (The Florey Institute of Neuroscience and Mental Health)

NEURODEVELOPMENTAL DISORDER-ASSOCIATED MUTATIONS IN SYNPOTAGMIN-1 DIFFERENTIALLY AFFECT SYNAPTIC VESICLE DYNAMICS

Melland H1, Jiang TJ1, Leech SL1, Raymond FL2, Baker K2, Gordon SL3.
1. The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Australia.
2. Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, Cambridge, UK.

Synaptotagmin-1 (syt-1) is an essential synaptic vesicle protein that acts as the Ca²⁺-sensor for fast, synchronous neurotransmitter release. Syt-1 has also been implicated in other aspects of synaptic physiology including the endocytic retrieval of synaptic vesicles. Recently, whole exome sequencing of a child with a novel neurodevelopmental disorder revealed the first known human mutation in syt-1 (I368T). We have now identified an additional 4 distinct mutations in syt-1 in a total of 9 individuals exhibiting a phenotypic spectrum of symptoms including cognitive impairment, motor delay and infantile hypotonia. These missense mutations are all clustered within the C2B domain of syt-1 and correctly localise to nerve terminals at rest, with the exception of one mutant (p<0.01, n=3-4). Of note, and supplementing our previous work showing altered exocytic and endocytic kinetics in the presence of syt-1I368T, we demonstrate here that syt-1 variants affect synaptic vesicle dynamics in a mutation-specific manner (p<0.05, n=5). Our findings reveal that a syt-1-associated neurodevelopmental disorder is caused by mutations that differentially affect the Ca²⁺-dependent function of syt-1. This highlights the importance of syt-1 functionality in human cognitive and motor development.
### DENDRITIC ENCODING IN LAYER 2/3 NEURONS DURING ACTIVE SOMATOSENSATION

Micallef AH\(^1\), Palmer LM\(^1\)

**Neural Networks Laboratory, The Florey Institute of Neuroscience & Mental Health, Melbourne, Victoria, Australia**

In the canonical cortical circuit information is anatomically segregated between two processing streams. The feed-forward pathway, driven by input from the sensory systems via thalamic nuclei, and a feed-back stream, integrating information across brain regions. Layer 2/3 neurons sit at the intersection of these two pathways and are thought to combine information between the streams. However it is unknown how this occurs in the context of an active behavioural task. To investigate this we performed 2-photon calcium imaging from layer 2/3 pyramidal neuron dendrites in the primary somatosensory cortex that were labelled with the genetic calcium indicator Gcamp6f. Calcium activity was recorded from 178 dendrites while animals performed a somatosensory Go/No-Go task. In hit trials, a subset (14.0%) of dendrites had increased activity during the response epoch, while a smaller subset (8.4%) were found to have increased calcium activity during the stimulus presentation. This illustrates that dendrites can respond differentially during distinct behavioural epochs, potentially contributing to the successful performance of a sensory Go/No-Go task.

### K3 TRANSGENIC AND ROTENONE TREATED MOUSE MODELS OF NEURODEGENERATIVE DISEASE SHOW HYPEREXCITABLE DISCHARGE PROPERTIES

Mo M\(^1\), Matthews M\(^1\), Johnstone D\(^2\), Mohammed Ali F\(^3\) and Camp A.J\(^1\)

**1Discipline of Biomedical Science, Sydney Medical School, University of Sydney; 2Discipline of Physiology, Sydney Medical School, University of Sydney. 3Discipline of Anatomy and Histology, Sydney Medical School, University of Sydney.**

**Objective:** Many neurodegenerative tauopathies including Progressive Supranuclear Palsy (PSP), lack validated animal models. In order to validate these models a description of their underlying neuronal properties is required. Here we compared electrophysiological properties of striatal neurons of wildtype mice with an existing transgenic model of Pick’s disease (K3 mice) and an occasional Parkinson’s model, rotenone injected mice.

**Methods:** The electrophysiological discharge properties of mouse striatal neurons (n= 38) were characterized in wildtype (n= 8), K3 transgenic (n= 8), and rotenone-treated mice (n= 8), all on the C57BL/6 background. Recordings were made from coronal slices (200 μm) distributed evenly across the striatum in whole-cell current-clamp mode at room temperature.

**Results:** The proportion of striatal neuron discharge profiles in wildtype mice was significantly altered when compared with the K3 transgenic and rotenone- treated groups (p= 0.006). In general this alteration was characterized by a shift towards burst firing discharge profiles in the K3 transgenic and rotenone treated mice. Further, both the K3 transgenic, and rotenone treated mice showed significantly higher subthreshold EPSP activity at rest (p= 0.03). The passive membrane properties including input impedance and capacitance of striatal neurons were not significantly different between the three mouse groups.

**Conclusion:** Neurons in the striatum of K3 transgenic mice display a hyper-excitable state that can be mimicked at least in part by injection of rotenone. This suggests that rotenone may model other frontotemporal lobar degenerative conditions like PSP, rather than Parkinson’s disease.

### MU-OPIOID RECEPTOR REGULATION OF GABAERGIC NEURONS IN THE AMYGDALA FOLLOWING FEAR LEARNING

Patel SD\(^1\), Medagoda DD\(^1\), Wells OA\(^1\), Bagley EE\(^1\)

**2Discipline of Pharmacology, School of Medical Sciences, University of Sydney, Sydney, NSW, Australia**

The Amygdala, brain region involved in the fear response, is made up of several nuclei including the intercalated cells (Im), a group GABAergic neurones. Im regulates fear by reducing the Amygdala’s output. With strong expression within the Im, the endogenous opiate system is known to have strong regulatory function of the Im and therefore, of the fear response. However, the functions of the opiate system in the fear response within the Im are yet to be fully defined. This study aims to outline the role of the mu-opioid receptor (MOR) in the Im and how this changes during fear learning. Using immunohistochemistry, we found after fear learning, in male Sprague-Dawley rats (4-8 weeks old), the MOR
AN N-TERMINAL MOTIF UNIQUE TO HUMAN TAU IS REQUIRED FOR DIFFERENTIAL PROTEIN-PROTEIN INTERACTIONS

Stefanoska K1, Volkerling AM1, Bertz J2, Poljak A2, Ittner LM1 & Ittner A1
1Dementia Research Unit, School of Medical Sciences, The University of New South Wales, Sydney, NSW 2052, Australia.
2Bioanalytical Mass Spectrometry Facility, The University of New South Wales, Sydney, NSW 2052, Australia.

Humans are particularly susceptible to tau-mediated neurodegenerative disorders as compared with other mammalian species. Both, physiological and pathological functions of tau are incompletely understood. Recent studies have suggested that the differential interactions of tau with other cellular proteins mediates tau functions. Here, we show that a 10-amino acid motif in the N-terminal region of human tau, which is not present in non-primate tau, mediates specific protein-protein interactions. Here, we used deletion mutagenesis in the longest human tau isoform and subsequent glutathione-S-transferase (GST) pull down approaches, in combination with mass spectrometry and isobaric tags for relative and absolute quantitation (iTRAQ) multiplex labelling. We found that amino acid residues 18 to 28 in human tau differentially mediates interactions with neuronal proteins. Specifically, the differential binding of vesicle associated machinery, synaptic transmission, signalling and actin-binding proteins, all involved this human-specific N-terminal tau motif. These results provide novel insight into tau interactions and suggest that human tau has evolved specific residues, which mediate its functions through the differential regulation of protein-protein interactions as compared to other non-primate, mammalian tau genes.

Prajwal Thakre (School of Biomedical Sciences)

CAPSAICIN ENHANCES EXCITATORY GLUTAMATERGIC SYNAPTIC TRANSMISSION TO NEONATAL MOUSE HYPOGLOSSAL MOTOR NEURONS

Thakre PP and Bellingham MC
School of Biomedical Sciences, The University of Queensland, Brisbane, Australia.

Capsaicin (CAP) is a common ingredient in chili peppers and is known to selectively activate transient receptor potential vanilloid type-1 (TRPV1) receptors. TRPV1 expressing neurons are present in several brain regions and activation of TRPV1 by CAP enhances neurotransmitter release. However, whether synaptic transmission to hypoglossal motor neurons (HMNs), which control tongue muscles, is modulated by CAP remains unexplored. We evaluated the effect of CAP in spontaneous excitatory synaptic transmission to mouse HMNs. Whole-cell patch-clamp recordings were made from HMNs in 300μm-thick transverse brainstem slices from 7-14 days-old C57BL/6 mice after sodium pentobarbitone anaesthesia. Spontaneous excitatory postsynaptic currents (sEPSCs) were recorded as inward currents at holding potential of -60mV using a CsCl-based internal solution and in presence of strychnine-HCl (20μM) to block inhibitory synaptic currents. A 10μM CAP solution was bath applied onto the brainstem slices. CAP caused a significant increase in sEPSC frequency (+117% from control, p=0.03, n=8 HMNs) as well as a significant increase in sEPSC amplitude (+24% from control, p=0.021, n=8 HMNs). Other sEPSC parameters (half-width and 10-90% rise-time) remained unchanged after CAP application. An interesting additional finding was a negative shift in baseline holding current (Ihold) after application of CAP. A significant increase in Ihold was noted (+90% from control, p=0.011, n=8). Further electrophysiology must be performed to see if increased MOR expression post-fear learning leads to enhanced morphine inhibition.

Kimberly Thek (Florey Institute of Neuroscience and Mental Health)

SELECTIVE OPTOGENETIC ACTIVATION OF VAGAL AFFERENTS REVEALS EXTENSIVE FEEDFORWARD INHIBITION WITHIN
THE SOLITARY TRACT NUCLEUS.

Thek KR, Allen AM & McDougall SJ. 
Florey Institute of Neuroscience and Mental Health and Dept. of Physiology, University of Melbourne.

The nucleus of the solitary tract (NTS) is the first central region to receive direct sensory vagal afferent input. Viscerosensory signals drive autonomic reflexes, neuroendocrine function and modulate behaviors. The vagal afferent/NTS synapse is a high probability release site utilizing glutamate as the fast neurotransmitter. The ‘rules’ for defining synaptic connections in electrophysiological assays have been well established for electrical stimulation with its superior temporal activation. Yet for optogenetics there are currently no well characterised rules to determine network connectivity without pharmacological interventions. To activate vagal afferents optogenetically we infected the nodose ganglion to induce ChR2 expression. In brainstem slices containing the NTS and vagal axons and terminals we compared electrical and optogenetic stimulation of vagal afferents via whole-cell electrophysiology. Monosynaptic inputs were identified by electrical stimulation of the solitary tract evoking low-jitter glutamatergic EPSCs. In the same neurons, EPSCs evoked with light stimulation of the solitary tract had longer latency, higher synaptic jitter, and lower amplitudes compared to electrical EPSCs. A surprising finding was that a large proportion of recorded NTS neurons exhibited light evoked IPSCs that were GABA mediated. Furthermore, these light evoked IPSCs were blocked by the AMPA receptor antagonist, NBQX. Thus vagal afferents drive NTS inhibitory interneurons that feedback onto other second order NTS neurons, a classic feedforward arrangement that has new implications for understanding how viscerosensory signals are integrated in the CNS.

Peng Zheng (University of Wollongong)

D2R-DISC1 COMPLEX FORMATION IMPAIRS PGSK3B AND NEURITE GROWTH IN PREFRONTAL CORTICAL NEURONS

Centre for Translational Neuroscience, School of Medicine, University of Wollongong, Wollongong, 2522, NSW, Australia.
Ilawarra Health and Medical Research Institute, Wollongong, 2522, NSW, Australia.

Background: D2 receptor (D2R) hyperactivity induces psychosis and neural connectivity impairment. The pGSK3β is regulated by D2R to control neurite growth. However, the mechanism is still largely unknown. This study aims to investigate the effect of over-activation of D2R affecting pGSK3β signalling and neurites in the prefrontal cortical neurons.

Methods: (1) Primary prefrontal cortical neurons were cultured from mice with or without DISC1 locus impairment (LI) at postnatal day 0; (2) After 7 days of culturing, cortical neurons were treated with D2R agonist (quinpirole) only or pretreated with partial agonist (aripiprazole) or antagonist (haloperidol); (3) Fluorescence resonance energy transfer (FRET) was applied to examine protein interaction; (4) Western blot was used to quantify protein expression.

Results: This study showed that (1) Over-activation of D2R reduced pGSK3β and impaired neurites growth of prefrontal cortical neurons; (2) Specific D2R antagonist prevented neurite impairment and reinstated GSK3β phosphorylation level; (3) D2R partial agonist also prevented the impairment induced by quinpirole; (4) When the D2R/DISC1 binding site was abolished, the preventative effect of D2R antagonist and partial agonist was not observed; (5) Specific D2R agonist significantly increased D2R/DISC1 complex formation.

Conclusions: The D2R-DISC1 complex formation induced by D2R over-activation decreased GSK3β phosphorylation and neurite growth of prefrontal cortical neurons. Antipsychotic drug haloperidol and aripiprazole prevent neurite lesion via reduction of D2R/DISC1 complex formation. Thus, a functional interplay between D2R and DISC1 might be a novel therapeutic target for treating cognitive decline.

Nathan Absalom (University of Sydney)

PHARMACOLOGY OF GABA RECEPTOR MUTATIONS THAT CAUSE SEVERE CHILDHOOD EPILEPSIES

Absalom NL, Liao V, Ahring P, McGregor I and Chebib M. 
Faculty of Pharmacy, University of Sydney, Camperdown NSW 2006 Lambert Initiative for Cannabinoid therapeutics, University of Sydney, Brain and Mind Centre, Camperdown NSW 2006.

Single point mutations in the γ-aminobutyric acid type A (GABA<sub>A</sub>) receptor have long been known to cause epilepsy. However, advances in DNA sequencing technolgies has enabled the identification of a number of de novo mutations.
within the α1, β3 and γ2 subunits of the receptor that lead to severe childhood epilepsies including Lennox-Gastaut and Dravet syndromes that are resistant to pharmacological treatment. These mutations lead to either a loss of function of the receptor, a reduction in surface expression of the receptor or both. We sought to determine whether common and experimental anticonvulsants that act at GABA_A receptors were able to restore the function of receptors containing these mutations in an in vitro system. Using molecular biological techniques, we created a concatenated α1B3γ2 receptor that enabled the expression of both heterozygous and homozygous mutations in Xenopus oocytes and measured the responses using two-electrode voltage clamp electrophysiology. For a mutation in the γ2 subunit (γ2R323Q), the mutation significantly shifted the EC50 of GABA 5-fold from the wild-type value. We performed concentration-response curves to GABA in the presence of anticonvulsants that act at GABA_A receptors, and demonstrate that neurosteroids, but not benzodiazepines, were able to restore the function of the receptor.

Annie Quan (Children’s Medical Research Institute)

IDENTIFICATION OF NEW SYNDAPIN-I BINDING PROTEINS IN NERVE TERMINALS

Annie Quan, Peter Hains, Jing Xue, Martin R. Larsen2, and Phillip J. Robinson

Cell Signalling Unit, Children’s Medical Research Institute, University of Sydney, Westmead, NSW, Australia. 2Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark

Syndapin-I is a synaptically enriched member of the F-BAR (FCH-BIN amphiphysin RVS) family of proteins. It consists of two functional domains; an N-terminal F-BAR, which can bind to and deform phospholipid membranes, and a C-terminal src homology 3 (SH3) flanked by a middle NPF-rich variable region. Syndapin-I is an important regulator of activity-dependent bulk endocytosis (ADBE) of synaptic vesicles (SV). It is an in vitro phospho-protein, and we have identified that syndapin-I is phosphorylated in rat brain nerve terminals and total brain. Quantitative mass spectrometry (MS) analyses on syndapin-I phosphorylation indicates that Ser-358 phosphorylation changes with KCl-dependent depolarization of the nerve terminals. Using the latest quantitative SWATH-MS technology, we have identified 917 proteins binding to the different syndapin-I functional domains and region. Thirteen (13) of these proteins coordinately function in the glycolytic and the Krebs cycle pathways, both important to energy metabolism and ATP production required for activity-dependent synaptic function. Protein domain and motif mapping of the MS data on the 13 glycolysis and Krebs cycle proteins, identified that fructose 6-bisphosphate aldolase A (ALDOA) strongly binds the syndapin-I NPF-rich variable region. Mutational analyses of the amino acids in the variable region using protein-binding assays show that Trp (W)-357 residue in an acidic patch flanking the Ser-358 phosphosite (350-GQTYATEWSDDE-361) is the direct ALDOA

Guy Barry (QIMR Berghofer Medical Research Institute)

UNCOVERING SYNAPTIC LINKS BETWEEN CANNABIS USE AND PSYCHIATRIC DISEASE

Guennewig B1,2,3, Bitar M1, O’Brien E1, Kaczorowski DC1, Brennand KJ5, Barry G4

1. Garvan Institute of Medical Research, Sydney, NSW, Australia. 2. St. Vincent’s Clinical School and School of Biotechnology and Biomolecular Sciences, University of New South Wales, Kensington, NSW, Australia. 3. Sydney Medical School, Brain and Mind Centre, The University of Sydney, NSW, Australia. 4. QIMR Berghofer Medical Research Institute, Herston, QLD, Australia. 5. Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

It has been known for decades that there is a strong association between cannabis use and the onset of schizophrenia but the key cellular links have to date eluded researchers. To examine potential underlying mechanisms we used neurons derived from human induced pluripotent stem cells (hiPSCs) that offer a platform for investigating dynamic changes in human neural cells. Here, we treated neurons derived from hiPSCs with Δ9-tetrahydrocannabinol (THC) and followed this by activating treated and untreated neurons with potassium chloride to mimic neural activity. RNA transcriptomic analyses revealed that THC administration, either as a single dose or 5 doses over 5 days, severely dampened the neuronal transcriptional response following KCl-induced neuronal depolarisation. Pathway analysis found that THC-treated neurons displayed significant synaptic, mitochondrial and glutamate signaling alterations that may underlie their failure to activate correctly. Interestingly, the blunted response in THC-treated neurons closely resembles what we previously observed in schizophrenia-associated iPSC-derived neurons. Furthermore, we show a significant alteration in genes activated by THC that are associated with glaucoma, autism and intellectual disability. These results uncover potential shared mechanisms to explain the significant phenotypic association between cannabis use and schizophrenia. We also reveal new insights into synaptic pathways that respond to THC treatment and how this has increased our understanding of an individual’s susceptibility to THC-induced schizophrenia.

Annie Quan (Children’s Medical Research Institute)
GABAPENTIN MODULATES HCN4 CHANNEL VOLTAGE-DEPENDENCE

Tae HS1, Smith KM2, Phillips MA1, Boyle KA1, Li M1, Forster IC1, Hatch R1, Richardson R1, Hughes DI1, Graham BA2, Petrou S1 and Reid CA1
1. Florey Institute of Neuroscience and Mental Health, Melbourne. 2. School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle. 3. Institute of Neuroscience and Psychology, University of Glasgow, Glasgow.

Gabapentin (GBP) is widely used to treat epilepsy and neuropathic pain. There is evidence that GBP can act on HCN channel-mediated li in brain slice experiments. However, evidence showing that GBP directly modulates HCN channels is lacking. The effect of GBP was tested using two-electrode voltage clamp recordings from human HCN1, HCN2 and HCN4 channels expressed in Xenopus oocytes. Whole-cell recordings were also made from mouse spinal cord slices targeting either parvalbumin positive (PV+) or calretinin positive (CR+) inhibitory neurons. The effect of GBP on li was measured in each inhibitory neuron population. HCN4 expression was assessed in the spinal cord using immunohistochemistry. When applied to HCN4 channels, GBP (100µM) caused a hyperpolarizing shift in the voltage of half activation (V1/2) thereby reducing the currents. GBP had no impact on the V1/2 of HCN1 or HCN2 channels. There was a robust increase in the time to half activation for HCN4 channels with only a small increase noted for HCN1 channels. GBP also caused a hyperpolarizing shift in the V1/2 of li measured from HCN4-expressing PV+ inhibitory neurons in the spinal dorsal horn. GBP had minimal effect on li recorded from CR+ neurons. Consistent with this, immunohistochemical analysis revealed that the majority of CR+ inhibitory neurons do not express somatic HCN4 channels. In conclusion, GBP reduces HCN4 channel-mediated currents through a hyperpolarised shift in the V1/2. The HCN channel subtype selectivity of GBP provides a unique tool for investigating HCN4 channel function in the central nervous system. The HCN4 channel is a candidate molecular target for the acute analgesic and anticonvulsant actions of GBP.

Nick Spencer (Flinders University)

OPTOGENETIC CONTROL OF THE ENTERIC NERVOUS SYSTEM AND PROPULSION OF FECAL CONTENT INDUCED BY LIGHT

Hibberd T1, Feng J2, Luo J2, Yang P2, Hu H*2 & Spencer NJ*1

1. Discipline of Human Physiology & Centre for Neuroscience, Flinders University, South Australia. 2. Department of Anesthesiology, School of Medicine, Washington University, St. Louis, USA.

Optogenetics has been demonstrated to successful control the excitability of the central nervous system, but not the enteric nervous system (ENS). Our aim was to determine whether optogenetics could be used to successfully control transit of content along the gastrointestinal (GI) transit. The ability to selectively control GI-motility and GI-transit without using non-specific drugs (that act all throughout the body) offers great hope for patients with impaired GI-transit, but, without having to endure the side effects of agonists that act in multiple organs. We generated a transgenic mouse line expressing channelrhodopsin in a specific class of excitatory neurons in the enteric nervous system (ENS). Cre-driven expression of the light-gated cation channel, channelrhodopsin-H134R (ChR2-H134R) was expressed in excitatory neurons immunoreactive to calretinin (CAL). Immunohistochemical analysis of colonic myenteric neurons revealed 97% CAL-immunoreactive neurons selectively expressed ChR2(H134R)-eYFP*. Mechanical recordings were made from intact whole colons in vitro (n=7). Both CAL-ChR2(H134R) and wild-type mice generated ongoing propagating neurotransgenic colonic motor complexes (CMCs), with a mean interval 280±37s (n=7). Focal illumination (1-5Hz, 10-60s) of blue light to the proximal, mid or distal colon evoked a significantly premature CMC in CAL-ChR2(H134R) mice (P=0.006, N=7), but never in wild-type littermates. Also, green light had no effect in CAL-ChR2(H134R) mice. Tetrodotoxin prevented optogenetic activation of CMCs (7/7 times tested, n=5). We provide the first demonstration that optogenetics can be successfully used to control the excitability of the ENS and induce propulsion of fecal content. Funded by NH&MRC #1067335 N.J.S and an NIH RO1 to H.Hu.

Tong Wang (Queensland Brain Institute, The University of Queensland)

SORTING NEXIN 27 REGULATES THE EXOCYTOSIS OF NMDA RECEPTORS

Wang T1, Yong XL1, Jang SE1, Yu X1, Collins BM2, Anggono V1

1. Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane,
### Sorting nexin (SNX)

SNX is a family of cytoplasmic and membrane proteins commonly involved in the endocytosis and trafficking of surface receptors. SNX27 is the only sorting nexin to contain a postsynaptic density 95/discs large/zona occludens (PDZ) domain, and is playing an important role in mediating PDZ-dependent endosomal sorting and recycling of cargo molecules to the plasma membrane. Mutations of SNX27 gene is linked to intellectual disability, epilepsy and growth retardation. Mice lacking SNX27 display impairments in glutamatergic neurotransmission and deficits in learning and memory. Previous studies have attributed synaptic dysfunction in SNX27 knockout mice to impairment in the trafficking of AMPA-type glutamate receptors. However, our recent finding found no evidence for direct interaction between SNX27 with AMPA receptor subunits. Instead, we found that SNX27 PDZ domain directly interacts with subunits of NMDA receptors and that this interaction is regulated by the phosphorylation of NMDA receptor near the carboxy-terminal PDZ ligands. Here, we report that SNX27 regulates the forward trafficking of GluN2A subunit of NMDA receptors in cultured hippocampal neurons. Overexpression of SNX27 upregulates surface expression GluN2A under basal conditions. In contrast, loss of SNX27 function abolishes activity-dependent insertion of GluN2A to the plasma membrane. Our results suggest that SNX27 plays critical roles in synaptic plasticity by enhancing the surface insertion of NR2A subunit containing NMDA receptors during synaptic potentiation.

Katherine Hankinson (University of Western Australia)

### INVESTIGATING THE EFFECTS OF A TAILORED MUSIC THERAPY MOBILE PHONE APP ON HUMAN CORTICAL EXCITABILITY AND FUNCTIONAL MOVEMENT IN HEALTHY ADULTS.

Authors: Katherine Hankinson¹, Jennifer Rodger¹, Michael Rosenberg², Alex Shaykevich³, Ann-Maree Vallence¹, Christopher Etherton-Beer⁴

1. University of Western Australia, School of Biological Science 2. University of Western Australia, School of Sport Science, Exercise and Health 3. Murdoch University, School of Psychology and Exercise Science 4. University of Western Australia, School of Medicine and Pharmacology

Music-movement therapy involves the therapeutic application of music and has been shown to effectively enhance movement in individuals with neurological disorders, including stroke and Parkinson’s Disease. However, not much is known about how music-movement therapy affects brain excitability. We developed a novel mobile software application, GotRhythm, that incorporates wireless sensors and real-time biofeedback to deliver a music-movement therapy to clinical populations. GotRhythm training involves repetitive movement to music and in time to a specified beat. In the present study, we tested GotRhythm on healthy young adult participants and measured motor learning and cortical excitability.

20 participants completed 30 mins of GotRhythm training or a control motor task. During training, the software collected data on rhythmic accuracy, a measure of motor learning. Cortical excitability was assessed using transcranial magnetic stimulation (TMS) of the left primary cortex to assess changes in motor evoked potentials (MEPs) recorded from a thumb muscle engaged in the task before and after the intervention. GotRhythm training was compared to a standard motor task (thumb abduction) previously shown to induce motor learning and increase cortical excitability following training.

Results indicate that a single session of GotRhythm significantly increased cortical excitability, after 30mins of training (p< .05). No evidence of motor learning (increase in accuracy) was found following GotRhythm training. We conclude that GotRhythm is effective in increasing cortical excitability and may, in the future, provide a useful tool for increasing neuroplasticity in a clinical population such as stroke patients.

Se Eun (Joanne) Jang (The University of Queensland)

### THE CALCIUM BINDING PROTEIN COPINE-6 MEDIATES AMPA RECEPTOR EXOCYTOSIS TO THE POSTSYNAPTIC MEMBRANE

Jang SE¹, Wang T³, Chandra M², Widagdo J¹, Collins BM², Anggono V¹

1. Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Queensland, Australia. 2. Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia.
AMPA-type glutamate receptors (AMPARs) mediate the majority of fast excitatory neurotransmission in the mammalian central nervous system. Activity-dependent trafficking of AMPARs is a major determinant of synaptic plasticity, which has long been considered as a cellular correlate of learning and memory. During long-term potentiation (LTP), the influx of Ca$^{2+}$ through NMDA receptors induces AMPAR insertion to the postsynaptic membrane. The mechanism that regulates Ca$^{2+}$-dependent recruitment of AMPARs remains unclear. Here we report a novel interaction between AMPARs and the Ca$^{2+}$- and lipid-binding protein, Copine-6, which plays a critical role in LTP, learning and memory. shRNA-mediated knockdown of Copine-6 significantly reduces the levels of surface and total AMPARs, but not of NMDA and transferrin receptors in cultured hippocampal neurons. Live-cell imaging analysis of pHluorin-GluA1 reveals a defect in the insertion AMPARs to the postsynaptic membrane in Copine-6 knockdown neurons. Importantly, we also demonstrate that Copine-6 is also required for activity-dependent exocytosis of AMPARs following glycine stimulation. Finally, we demonstrate that Copine-6 mutant that is defective in Ca$^{2+}$-binding ablates AMPAR forward trafficking in mammalian central neurons, a process that is critical for learning and memory consolidation.

Tianyi Zhu (The University Of Queensland)

UBIQUITINATION MEDIATES PROTEASOMAL DEGRADATION AND NUCLEAR TRANSLOCATION OF THE FAT MASS AND OBESITY-ASSOCIATED (FTO) PROTEIN

Zhu T$^1$, Yong XL$^1$, Xia D$^1$, Widagdo J$^1$, Anggono V$^1$
1. Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Queensland, Australia.

Genetic studies have consistently identified strong association between polymorphisms in the fat mass and obesity-associated (FTO) gene with body mass index and obesity in humans. FTO is a nuclear protein that catalyses the demethylation of N6-methyladenosine (m6A) modification of RNA. Outside the nucleus, FTO is known to act as an amino acid sensor and regulates the mTORC1 pathway, a key regulator of cell growth and mRNA translation. Recent works from our lab and others have demonstrated a critical role for FTO in regulating experience-dependent m6A dynamics and memory consolidation in the brain. However, the cellular mechanisms by which FTO protein is turned over or translocated between the nucleus and cytoplasm remain unknown. Here, we report that FTO is directly modified by post-translational ubiquitination on Lys-216. CRISPR-mediated knock-in of HeLa cells harboring the ubiquitin-deficient K216R mutation in the FTO gene reveal that FTO ubiquitination mediates its proteasomal degradation, resulting in enhanced stability of FTO in the knock-in cells. As a consequence, FTO K216R knock-in cells also exhibit higher phosphorylation levels of the ribosomal S6 kinase. In addition, we also demonstrate that the level of nuclear FTO expression is significantly lower in the knock-in cells and that K216R mutation completely abolishes the nuclear translocation of FTO upon amino acid starvation. Collectively, our results reveal the functional importance of FTO ubiquitination in controlling FTO levels and in fine-tuning the mTORC1 signalling pathway. This may have implications in understanding obesity, learning and memory.
Luca Aquili (Sheffield Hallam University)

TRANSCRANIAL DIRECT CURRENT STIMULATION OF THE DORSOLATERAL PREFRONTAL CORTEX AND TYROSINE ADMINISTRATION MODULATE INDICES OF COGNITIVE FLEXIBILITY

Dennison O 1, Aquili L 1.
1. Sheffield Hallam University, Department of Psychology, Sheffield, UK.

The dorsolateral prefrontal cortex and dopamine have been implicated in the control of cognitive flexibility. However, while a great deal of what we know regarding a causative relationship between cognitive flexibility and its neuronal underpinning comes from animal studies, human data have largely been correlational (i.e. imaging investigations). The current study aimed to examine whether putative increases in dopamine levels through tyrosine administration and blockage of these by cathodal tDCS of the dlPFC could causally be related to cognitive flexibility as measured by task switching and reversal learning. Using a crossover, double-blind, sham controlled, counterbalanced, randomized trial, we tested the effects of four types of pharmacological/tDCS parameters (i.e. 1: placebo+sham; 2: placebo+cathodal; 3: tyrosine+sham; 4: tyrosine+cathodal) on cognitive flexibility. We found that while none of the manipulations had an effect on task switching, there was a significant interaction effect between timing of testing and manipulation used on reversal learning. More specifically, changes in reversal learning performance from time 1 (baseline) to time 2 (post-manipulation) were significantly different when comparing placebo+cathodal to tyrosine+sham. That is, whilst performance worsened from time 1 to time 2 under placebo+cathodal, this improved under tyrosine+sham. Interestingly, performance was virtually identical between time 1 and time 2 when comparing placebo+sham to tyrosine+cathodal, indicating that the beneficial effects of tyrosine (as in the tyrosine+sham manipulation) could be blocked or reset by cathodal stimulation. Our results suggest a causative role for dopamine and the dorsolateral prefrontal cortex in regulating indices of cognitive flexibility.

Sarah Baracz (Macquarie University)

ADOLESCENT OXYTOCIN TREATMENT REVERSES THE EFFECTS OF EARLY LIFE STRESS ON METHAMPHETAMINE SEEKING BEHAVIOUR DIFFERENTLY DEPENDING ON SEX

Baracz SJ1,2, Everett NA1, McGregor IS2, Cornish JL1.
1Macquarie University, NSW, Australia 2University of Sydney, NSW, Australia.

Exposure to early life stress is associated with augmented vulnerability to abuse psychostimulant drugs, including methamphetamine. Early life adversity alters oxytocin regulation during one of its critical developmental periods, which contributes to increased susceptibility to developing drug dependence. Previous studies showed that oxytocin pre-treatment during adolescence protects against future drug-taking and drug-seeking in animals without a history of early life stress. The aim of our study was to determine whether an adolescent oxytocin treatment regime could reverse the adverse effects of early life stress and reduce susceptibility to methamphetamine addiction. Long Evans pups were separated from their mothers for either 15 or 360 mins on postnatal days (PND) 1 to 21. During adolescence (PNDs 28-42), rats received a daily injection of either oxytocin (1mg/kg) or saline. In adulthood, rats with implanted jugular vein catheters acquired methamphetamine intravenous self-administration (IVSA) over 22 days (0.03mg/kg/infusion first 10 days, 0.1mg/kg/infusion final 12 days). Rats then underwent drug-primed (0.3mg/kg and 1mg/kg) and stress-induced reinstatement (yohimbine hydrochloride, 0.625mg/kg and 1.25mg/kg) of methamphetamine-seeking following extinction. We demonstrated that stressed female rats treated with oxytocin showed reduced relapse behaviour after exposure to a stressor (0.625mg/kg) compared to vehicle controls. Stressed male rats treated with oxytocin showed a strong trend in the reduction of reinstatement responding after exposure to a methamphetamine-prime (1mg/kg) and a stressor (1.25mg/kg) compared to vehicle controls. Overall, this suggests a role for adolescent oxytocin treatment in reducing the impact of early adversity on vulnerability to engage in drug-seeking behaviours, which differs depending on sex.

Hui Chen (University of Technology Sydney)

EFFECT OF EXENDIN-4 TO IMPROVE OUTCOMES FOLLOWING MODERATE BRAIN CONTUSION
Objective: There is a need for pharmaceutical agents that can reduce neuronal loss and improve functional deficits following traumatic brain injury (TBI), as currently there are no proven drug treatment options available. Previous research has suggested that oxidative stress and mitochondrial dysfunction play a major role in ischemia induced neuronal damage such as occurs after traumatic injury. Therefore, this study aimed to investigate an existing drug known to have neuroprotective effects, exendin-4, in an animal model of moderate traumatic brain injury (TBI).

Method: A cortical contusion injury was induced in female Sprague-Dawley rats using a weight drop device. Exendin-4 (15 µg/kg/day, ip) were given to the rats with TBI immediately after the injury and continued for 2 weeks. Motor and cognitive function and brain tissue were examined at 24h and 6 weeks post-injury. Results: The rats with TBI showed some impairment in sensory, motor and memory function at 24h, which recovered by 6 weeks. Exendin-4 treatment improved sensory, motor and memory functions at 24h. The treatment reduced cortical contusion due to TBI at 24h and 6 weeks, however it did not affect gliosis and the activation of inflammatory cells. Oxidative stress appeared to be alleviated by exendin-4 treatment, however only increased mitochondrial functional marker protein transporter translocase of outer membrane 20 (Tom 20) at 24h post injury. Conclusion: Exendin-4 treatment immediately after the TBI can improve neurological functional outcome and tissue integrity by reducing oxidative stress.

THE ERM PROTEIN MOESIN IS ESSENTIAL FOR NEURONAL MORPHOGENESIS AND LONG-TERM MEMORY

Fitzsimons HL, Freymuth PS.

Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand

Moesin is a cytoskeletal adaptor protein that regulates organization of the actin cytoskeleton. Rearrangement of the actin cytoskeleton drives both neuronal morphogenesis and the structural changes in neurons that are required for long-term memory formation. Expression of Moesin is increased after a training session that results in formation of long-term memory in Drosophila, however whether it is required for this process has not been evaluated. Here, we investigate the role of Moesin in neuronal morphogenesis and in short- and long-term memory formation in the courtship suppression assay, a model of associative memory in Drosophila. We found that knockdown of Moesin led to severe defects in axon growth and guidance as well as reduced dendritic arborization in the fly brain. Furthermore, reduction of Moesin expression or expression of a constitutively active phosphomimetic in the adult Drosophila brain had no impact on short-term memory, but resulted in a significant impairment in long-term memory, an effect that was independent of its role in development. These results indicate a critical role for Moesin in both neuronal morphogenesis and long-term memory formation.

ACCELERATED HABITUAL BEHAVIOR RESULTING FROM L-DOPA EXPOSURE IS PREVENTED BY N-ACETYLCYSTEINE

Furlong TM 1,2, Keefe, KA2, Paxinos, G1.

1. Neuroscience Research Australia, Sydney, NSW, Australia. 2. The University of Utah, Salt Lake City, Utah, USA.

Exposure to amphetamine or cocaine alter the brain long-term to promote aberrant behaviour. For example, these dopamine agonists have been shown to accelerate habitual responding in animals in situations unrelated to obtaining drugs. L-dopa is also a dopamine agonist that increases dopamine signaling by augmenting vesicular dopamine content. However, the impact of L-dopa on behavioral control has yet to be established. In the first study, we demonstrated that L-dopa accelerates habitual responding. Rats were pre-exposed to L-dopa (6X 25 mg/kg or 50mg/kg, ip), trained to press a lever for a food outcome, and then tested using an outcome devaluation task. At test, control animals adjusted their behavior according to the current value of the outcome, reducing responding on the lever when the associated outcome was devalued by
specific satiety. In contrast, L-dopa exposed rats were insensitive to outcome value, demonstrating habitual responding. In the second study, we showed that habitual behavior following L-dopa exposure could be prevented by N-acetylcysteine (NAC, 50 mg/kg or 100 mg/kg, ip); a compound with both antioxidant and glutamate regulatory function. Our findings are similar to those seen following drugs of abuse and may have relevance to Parkinson’s disease where long-term L-dopa use is associated with the development of addiction-like behaviors. The capacity of NAC to reverse the progression to habitual responding suggests that NAC, or other compounds that regulate glutamate function, may be useful therapeutically. Further, it suggests that L-dopa results in neuroadaptions to glutamate systems similar to abused drugs.

Catherine Gorrie (University of Technology Sydney)

**BEHAVIOURAL AND EPIGENETIC CHANGES IN OFFSPRING IN A MOUSE MODEL OF MATERNAL VAPING**

Nguyen T¹, Li G¹, Chen H¹, Cranfield CG.¹ McGrath K¹, Gorrie CA¹
1. School of Life Sciences, University of Technology Sydney, Sydney, NSW, Australia.

Electronic cigarettes (also known as e-cigarettes) are battery-powered devices that can deliver nicotine through a vapor. There is a perception that e-cigarettes are less harmful than tobacco cigarettes. This perception may encourage pregnant women to use e-cigarettes during pregnancy as an alternative to smoking. However, there is no evidence that inhaled e-cigarettes are safe alternatives. This study aimed to investigate behavioral and epigenetic changes in e-cigarettes in offspring as a result of maternal vaping. Prior to and during pregnancy, 32 female Balb/c mice were treated either with ambient air (n=8), 0mg nicotine vapour (n=8), 18mg nicotine vapour (n=8) or cigarette smoke before pregnancy followed by 18mg nicotine vapour during pregnancy (n=8). Adult male offspring from each group (n=14) were then assessed by the elevated plus maze and the novel object recognition tests to investigate anxiety and short term memory respectively. Epigenetic analysis was assessed in brain sections of male offspring using RT² Profiler PCR Array Gene Expression. From the behavioural study, hyperactivity and short term memory deficits were found in groups exposed to e-cigarette vapors (P<0.05). Epigenetic analysis showed gene changes associated with mitosis, transcription and histone methylation, all of which have been linked to brain development, memory and behavior. Taken together the data suggests that in an animal model, maternal vaping induces neurological changes in the offspring that continued on into adulthood.

Lauren Harms (University Of Newcastle)

**THE EFFECTS OF MID-LATE GESTATIONAL MATERNAL IMMUNE ACTIVATION IN RATS ON SCHIZOPHRENIA-RELATED BEHAVIOUR**

Harms, L¹,² Gray, A¹, Tattoli, R¹, Michie, PT¹,² Hodgson, DM¹,²
6. School of Psychology, University of Newcastle, NSW, Australia.

1. Priority Research Centre for Brain and Mental Health Research, University of Newcastle, NSW, Australia.

2. Prenatal infection is a risk factor for schizophrenia in offspring and is believed to be mediated by maternal immune activation (MIA) in response to an infection. Previous studies in our lab investigating the effect of MIA during mid or late gestation found sensorimotor gating deficits and transient working memory impairments, but did not observe many other behavioural changes consistent with an animal model of schizophrenia. Therefore, the current study aims to determine whether MIA at another gestational time-point leads to significant changes to schizophrenia-related behaviour. Polyinosinic-polyribocytidylic acid (PolyI:C) was injected to induce MIA in pregnant Wistar rats at gestational day (GD) 14. Control dams were given saline injections. MIA-exposed rats were more sensitive to the locomotor-stimulating effects of the psychomimetic amphetamine (F(1, 29) = 8.00, \( p = .008 \)), an effect most pronounced after a high dose (2.5mg/kg) of amphetamine. In addition, MIA-exposed rats exhibited reduced novel object preference in the novel object recognition test of learning and memory (F(1, 38) = 9.57, \( p = .004 \)). Both effects were more pronounced in male rats. MIA exposure at GD14 did not affect sensorimotor gating, locomotor responsivity to the psychotomimetic MK-801, social interaction, sucrose preference, elevated plus maze or open field behaviour. These findings indicate that the MIA model may be useful for further investigation of schizophrenia-like cognitive deficits and psychotic-like behaviour, but is unlikely to be useful for the further investigation of negative symptom-like behaviour.
Kristin Hillman (University of Otago)

**REGULAR EXERCISE ENHANCES ANTERIOR CINGULATE-INSULA COHERENCE IN THE ADULT RAT BRAIN**

Jaquiery Z, Wall H, Hillman K.
*Department of Psychology, University of Otago, Dunedin, New Zealand.*

The ability to switch between the default mode network (DMN) and the central executive network (CEN) is important for task performance, particularly for tasks requiring executive function. Two different lines of human literature suggest that: 1) the DMN-to-CEN switch can be improved via exercise interventions; and 2) the DMN-to-CEN switch may rely on a third intermediary ‘salience network’ comprised of the anterior cingulate cortex (ACC) and the insular cortex (IC). Here we aimed to bring together these two lines of literature by testing whether an eight week exercise programme would alter ACC-IC functional connectivity in rat. Male Sprague-Dawleys (n=12) were implanted with chronic recording electrodes in the ACC and IC and assigned to an exercise programme (30 min/day, 5 days/week) or matched control conditions. In each week of the programme, rats completed a battery of behavioural tasks (open field, T-maze, operant box) and local field potentials were recorded. Power spectral density and coherence measures revealed progressive changes in ACC-IC coherence across time, across frequency-band, and between groups. Despite significant differences in ACC-IC coherence between groups, behavioural task performance did not differ. These data demonstrate that routine exercise can enhance baseline ACC-IC coherence, notably in low-frequency bandwidths. This is a previously unreported effect of exercise on the brain, and may help to explain the improved DMN-to-CEN switch reported in human exercise studies.

Philip Jean-Richard-dit-Bressel (UNSW)

**PHASIC ACTIVITY OF MIDBRAIN DOPAMINE NEURONS DURING RELAPSE TO ALCOHOL SEEKING**

Jean-Richard-dit-Bressel P, Yao JO-Y, McNally GP

Phasic excitation of midbrain dopamine (DA) neurons is sufficient and necessary for the acquisition of instrumental responding for reward, and is relatedly implicated in drug-seeking. We expressed a Cre-dependent genetically-encoded calcium indicator (FLEX-GCaMP6f) and implanted an optic fibre into the ventral tegmental area (VTA) of TH-Cre transgenic rats to examine the role of phasic activity in VTA TH neurons (VTATH) in relapse to alcohol seeking. Rats were trained to nosepoke for beer in Context A across 10 days; rats learned to make active nosepokes for beer, while ignoring an inconsequential inactive nosepoke. Reinforced nosepokes and magazine responses, but not unreinforced active nosepokes or magazine entries, resulted in VTATH Ca2+ transients. Responding was then extinguished (no longer reinforced) in Context B; the decrement in responding across extinction was accompanied by a reduction in VTATH Ca2+ transients to reinforcer cues. When tested for responding in Context A and B under extinction conditions, responding was higher in Context A than B, demonstrating renewal (context-induced relapse). Interestingly, this relapse was not accompanied by Ca2+ transients in VTATH. Rats were then given a reacquisition test (nosepokes reinforced in Context A); rats displayed a high level of responding (reinstatement-based relapse) as well as Ca2+ transients in VTATH to reinforced nosepokes and magazine entries. Taken together with chemogenetic findings from our lab, it is suggested that VTATH activity is responsible for encoding the reinforcer (and relevant cues) and driving responding when they are present.

Stephen Kent (La Trobe University)

**IS BIGGER BETTER? IMPACT OF A LARGE DOSE OF LPS AFTER A PERIOD OF CALORIE RESTRICTION**

Kirby A, Chong KL, Kivivali L, Kent, S.
*School of Psychology & Counselling, La Trobe University, Melbourne, Australia.*

A 50% reduction in food intake leads to a complete attenuation of sickness behaviour after a small dose of lipopolysaccharide (LPS). We aimed to expand upon these results using a 10-fold larger dose of LPS. C57BL/6J male mice implanted with biotelemetry devices were housed at 30±2°C under a 12:12 LD cycle and assigned to either ad libitum (AL; n=16) or CR50% (n=16) groups for 28 days. On day 29, either 500μg/kg LPS from Escherichia coli (serotype 0111:B4) or vehicle was injected. Sickness behaviour was assessed for 48 hours...
post-LPS. Fever peaked 5 hours post-LPS in AL mice (1.47±0.21°C) and at 3 hours in CR mice (1.87±0.62°C); however, body temperature (Ts) in CR mice returned to baseline levels more quickly. Ts change in AL and CR LPS groups did not differ in the first 6 hours post-injection. CR LPS mice differed from both saline groups at only 2 and 3 hours post-LPS (p<.01<.05). AL LPS mice differed from both saline groups at 2, 4, and 5 hours post-LPS (p<.05). All LPS mice lost weight (AL=10.8%, CR=5.3%; p<.001). AL LPS mice ate significantly less (M=0.38 ± 0.07g) compared to all other groups (p<.001) and CR mice ate all of their allotted food (range=1.32-1.38g). Contrary to past results, CR mice developed sickness behaviour post-LPS, albeit in an attenuated manner. It appears there is a dose-dependent relationship between LPS, CR, and sickness behaviour. Larger doses of LPS either induce additional febrile pathways or overwhelm the anti-inflammatory changes induced by CR.

Shaun Khoo (Concordia University)

MGLUR5 BUT NOT NMDA RECEPTOR ANTAGONISM REDUCES APPETITIVE PAVLOVIAN RESPONDING REGARDLESS OF CONTEXT

Khoo SY-S, Deyab, G., Chaudhri N.
Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, QC, Canada.

Glutamate receptors have attracted significant interest as a potential therapeutic target for disorders of appetitive motivation where reward-seeking is often driven by cues and/or contexts. We examined the involvement of glutamate in the expression of conditioned responding elicited by an appetitive conditioned stimulus (CS) in separate contexts, where one context had a history of unconditioned stimulus (US) delivery. Subjects were male Long-Evans rats (Charles River, n=16) that received conditioning sessions in which a 10 s CS (5 Hz clicker or continuous white noise; 10 trials per session; 260±120 s between cues) was paired with delivery of a sugar solution (5.5% fructose/4.5% glucose, 0.2 mL/CS trial). Each session was alternated with exposure to a second context where a neutral stimulus (white noise or clicker, counterbalanced with CS) was presented without sugar (10 sessions). At test, CS presentations in the absence of sugar elicited higher levels of port entry responding in the reward-associated context, compared to the neutral context. Rats were then retrained and retested in each context, and given pre-test, intraperitoneal injections of an NMDA receptor antagonist (MK-801, 0.1 mg/kg), an mGluR5 receptor antagonist (MTEP, 5 mg/kg) or 5% DMSO vehicle in counterbalanced order (1 ml/kg). Compared to vehicle, MK-801 had no effect on behaviour. However, blocking mGluR5 receptors with MTEP significantly reduced CS-elicited port responding in both contexts. Port entries occurring during inter-trial intervals were unaffected, suggesting a selective impact on CS-elicited responding. These results suggest that the mGluR5 receptor is involved in CS-elicited appetitive behaviour, regardless of context.

Belinda Lay (Concordia University)

DISTINCT NEURONAL ENSEMBLES WITHIN THE CENTRAL NUCLEUS OF THE AMYGDALA ENCODE REWARD EXPECTANCY

Lay, BPP1, Iordanova, MD1.
1. Center for Studies in Behavioural Neurobiology, Department of Psychology, Concordia University, Montreal, QC, Canada.

Correlational data from histochemical and physiological studies suggest that the central nucleus of the amygdala (CeA) is involved in encoding reward expectancies. Attempts at delineating the causal contribution of CeA neurons to this learning have targeted both activated and non-activated neurons, which likely have different functional roles. Fos is a widely used marker for neuronal activity and here, we used the Daun02 inactivation procedure to assess the causal role of activated c-fos-expressing CeA neurons in behaviour regulated by reward expectation. In c-fos-lacZ transgenic rats the c-fos promoter transcribes lacZ, which encodes the protein β-galactosidase. In turn, when the pro-drug Daun02 is microinjected into a specific neural site (e.g. CeA), β-galactosidase catalyses Daun02 into daunorubicin, which reduces neuronal excitability and inactivates c-fos-expressing neurons. In the present study, rats were trained to expect the delivery of a food reward upon the presentation of an auditory cue. Subsequently, rats received non-reinforced exposure to the reward-associated cue to generate reward expectation. Cell inactivation with Daun02 took place ninety
minutes following the start of the non-reinforced session, presumably when the reward memory was activated and the corresponding c-fos levels were at peak. This led to disruption in behaviour indicative of reward expectation compared to rats that received a vehicle infusion, which left those neurons intact. Additional preliminary data show that in the absence of the reward-associated neuronal ensemble, further retraining of the same cue-reward association was retarded. Further experiments are aimed at determining whether silencing the reward expectancy neuronal ensemble modulated cue recognition or attention.

Ekaterina Levichkina (The University of Melbourne)

TWO TYPES OF NEURONAL SYNCHRONY BETWEEN CORTICAL AREAS LIP AND MT IN SUPPORT OF A 2-STAGE MODEL OF SELECTIVE ATTENTION

Levichkina E1,3, Kermani M1, Saalmann YB1,2, Vidyasagar TR1
1. Department of Optometry & Vision Sciences, University of Melbourne, Parkville, Australia. 2. Department of Psychology, University of Wisconsin, Madison, USA. 3. Institute for Information Transmission Problems RAS, Moscow, Russia.

INTRODUCTION: Lateral intraparietal cortex (LIP) is known to possess a saliency map for orienting attention to relevant objects. The featural information of this map remains intact despite bottlenecks such as attentional blink and is available well after the initial spike response to the stimulus. We theorized that this information is retained in the coherent oscillations of LIP cells in synchrony with oscillations in cortical areas supplying the featural signals to LIP.

METHODS: To test this hypothesis we analysed dynamics of coherence of local field potentials (LFPs) between areas MT and LIP during performance of a delayed-match-to-sample task (DMS) by two macaques. This paradigm allowed feature-related responses to be separately analysed from activity related to top-down attentional modulation.

RESULTS: We identified frequency bands where featural or attentional coherence was significant (p<0.05) using statistical methods developed by Bokil et al. (2007) in 29 paired recordings. Feature-specific synchrony was apparent as high gamma coherence while attention-related coherence was mainly in the beta and low gamma bands. Featural coherence between LIP and MT showed nearly zero phase difference, while phase of attention-related coherence showed LIP leading MT. Featural coherence maximum occurred 200-300ms later than multiunit response maxima (Wilcoxon, p < 0.05) and thus could not be explained by spike leakage.

CONCLUSION: Featural information in the form of coherent oscillations between cortical areas MT and LIP is retained in the first part of the delay period and attentional coherence develops only after the featural coherence maximum is reached.

Xiang Li (Queensland Brain Institute)

THE FORMATION OF FEAR EXTINCTION MEMORY REQUIRES THE ACCUMULATION OF N6-METHYL-2'-DEOXYADENOSINE IN DNA

Xiang Li1, Qiongyi Zhao1, Wei Wei1, Quan Lin2, Christophe Magnan3, Michael R. Emami3, Luis E. Wearick da Silva4, Thiago W. Viola4, Paul Marshall1, Jordan Edmonds3, Sara Nainar3, Cathrine Broberg Vågbø5, Laura Leighton1, Esni Zajaczkowski1, Ke Ke3, Rodrigo Grassi-Oliveira4, Magnar Bjørås5, Pierre F. Baldi3, Robert C. Spitale3 and Timothy W. Bredy1

1Queensland Brain Institute, The University of Queensland, Brisbane, QLD, Australia; 2University of California at Los Angeles, Los Angeles, California, USA; 3Department of University of California Irvine, Irvine, CA, USA; 4Pontifical Catholic University of Rio Grande do Sul, Porto Alege, Brazil; 5Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway.

DNA methylation, once considered static and restricted to directing cellular lineage specificity during early development, is now recognized to be highly dynamic and reversible across the lifespan. Although it is known that there are more than 20 DNA modifications, nearly all research aimed at elucidating the role of these chemical modifications in the brain has focused on either 5-methylcytosine (5mC) or the recently rediscovered 5-hydroxymethylcytosine (5hmC), which is a functionally distinct oxidative derivative of 5mC. 5mC and 5hmC are highly prevalent in neurons relative to other cell types and both modifications are regulated in response to learning. Despite these exciting threads, a complete understanding of how DNA methylation controls neuronal
Australasian Neuroscience Society Annual Scientific Meeting 2017
International Convention Centre, Sydney, December 3rd – 6th 2017

gene expression to facilitate memory formation is severely lacking. Here we report that the novel eukaryotic DNA modification N6-methyl-2'-deoxyadenosine (m6dA), which is beyond cytosine methylation, drives activity-induced gene expression and is associated with fear extinction memory in the infralimbic prefrontal cortex (ILPFC) of adult C57/Bl6 mice. In primary cortical neurons, m6dA accumulates within the P4 promoter of the gene encoding brain-derived neurotrophic factor (bDNF) via a putative m6dA-specific methyltransferase, N6amt1. An N6amt1-dependent increase in the deposition of m6dA is associated with an active chromatin state, as well as the recruitment of the activating transcription factor Yin-Yang 1 and RNA polymerase II, which promote bDNF exon IV mRNA expression. The same process regulates learning-induced bDNF exon IV mRNA expression in the adult brain. Viral-mediated knockdown of N6amt1 in the ILPFC blocks the effect of extinction learning on m6dA deposition and related chromatin and transcriptional activity, resulting in a significant impairment in extinction memory.

Antigone Matsos (The University Of Sydney)

THE EFFECT OF IBUDILAST ON OXALIPLATIN-INDUCED COGNITIVE IMPAIRMENTS, TACTILE ALLODYNIA AND COLD HYPERALGESIA

Matsos A1, Loomes M1, Robertson NL1, Crouch G1, Dhillon H1, Vardy J2, Graeber M3, Hutchinson MR4, Johnston IN1
1. School of Psychology, The University of Sydney, Australia; 2. Sydney Medical School, The University of Sydney, Australia; 3. Brain and Mind Centre, The University of Sydney; 4. Adelaide Medical School, The University of Adelaide, Australia

Whilst chemotherapy agents show promising results as a robust cancer treatment, patients often experience peripheral neuropathies and cognitive impairments associated with such treatment long after chemotherapy has ceased. Changes in microglial activity in the central nervous system may be responsible for the development of these neurotoxic side effects. In an animal model of oxaliplatin induced neurotoxicity rats received a chronic course of oxaliplatin (OXP, 6mg/kg once/week for 3 weeks) with Ibudilast (IBU, 7.5mg/kg), a clinically proven immune therapy. IBU was administered either 30 minutes before every OXP treatment (experiment 1) or a single injection 2 weeks after the last OXP injection (experiment 2 and 3). Co-administration of OXP and IBU prevented the development of OXP induced tactile allodynia, cold hyperalgesia and recognition memory impairments. IBU treatment 2 weeks after OXP reversed OXP induced recognition memory impairments and tactile allodynia. Understanding the mechanisms that underlie the emergence of peripheral neuropathies and cognitive impairments is vital to implement behavioural and pharmacological strategies to prevent or reverse such acute toxicity in the brain brought about by chemotherapy.

Kohei Miyata (National Institute for Physiological Sciences)

THE EFFECTS OF VISUAL AND VOCAL INTERACTIONS BETWEEN INDIVIDUALS ON MOVEMENT SYNCHRONIZATION DURING JOINT DANCING

Miyata K1,2, Varlet M3, Miura A4, Kudo K1 and Keller P.3
1. Graduate School of Arts and Sciences, The University of Tokyo, Meguro Tokyo, Japan. 2. Japan Society for the Promotion of Science, Chiyoda Tokyo, Japan. 3. The MARCS Institute for Brain, Behaviour & Development, Western Sydney University, Milperra NSW, Australia. 4. Faculty of Sport Sciences, Waseda University, Tokorozawa Saitama, Japan.

In dance performance, rhythmic ability is paramount, such as the precise control of motor timing and coordination of body movements to musical beats. Previous studies have demonstrated that people have a tendency to synchronize spontaneously with each other’s movements (i.e. interpersonal entrainment) when they interact visually and vocally. This study aimed to investigate the effects of interpersonal visual and vocal interactions on rhythmic movement performance. We hypothesized that both visual and vocal interactions would result in interpersonal entrainment and modulation of individual movement performance, which was evaluated in a synchronization-continuation paradigm. Sixteen pairs of participants bounced their knees with metronome beats (synchronization task) and continued bouncing without metronome at the same tempo (continuation task). Paired participants were either facing each other or away from each other to manipulate visual interaction and either repeated the syllable “ta” (at knee flexion) or remained silent to manipulate vocal interaction. The results indicated interpersonal phase angles closer to zero and less variable when interacting
both visually and vocally, showing interpersonal entrainment. At the individual level, visual interaction increased the variability of auditory-motor (metronome beat-to-knee flexion) coordination in the synchronization task but did not affect individual movement variability in the continuation task. Interestingly, vocal interaction decreased the variability of auditory-motor coordination and individual movements in both sections of the synchronization-continuation task. Together, these results show that visual and vocal interactions lead to the occurrence of interpersonal entrainment, with individual movement performance being enhanced while interacting vocally but degraded when interacting visually.

Zhi Yi Ong (University of New South Wales)

ROLE OF PARAVENTRICULAR THALAMUS IN THE APPETITIVE AND MOTIVATIONAL ASPECTS OF FOOD INTAKE CONTROL

Ong ZY, McNally GP
School of Psychology, University of New South Wales, Sydney, NSW, Australia.

Paraventricular thalamus (PVT) is a midline thalamic nucleus implicated in the control of behaviours such as arousal, stress, fear, drug-seeking and more recently, food intake. Previous work demonstrated that PVT neurons are required for food intake control; however the role of PVT on the motivational and appetitive aspects of feeding behaviours was not explored. Here we examined whether activation of PVT contributes to the control of palatable food-seeking and food-motivated behaviours. Chemogenetic activation of PVT neurons reduced pellet-primed, cue-induced reinstatement of sucrose-seeking behaviours but had no effect on dark cycle chow intake nor on the motivation to work for sucrose pellets under a progressive ratio schedule of reinforcement. The impaired reinstatement of sucrose-seeking behaviour by PVT neuron activation was independent of the PVT à nucleus accumbens shell (NAcsh) pathway as chemogenetic activation of PVT à NAcsh did not affect reinstatement of sucrose-seeking. Together, results here highlight a role for PVT neurons in the control of sucrose-seeking behaviours. The lack of effect with PVT à NAcsh neuron activation suggests the contribution of other PVT projection targets in the regulation of palatable-food seeking behaviours.

Lei Qian (Queensland Brain Institute, School of Biomedical Science, University of Queensland)

APNEA-INDUCED INTERMITTENT HYPOXIA CAUSES CHOLINERGIC BASAL FOREBRAIN DEGENERATION IN ALZHEIMER’S DISEASE MODEL

Qian L1,2,3, Marks N1,3, Milne MR1,2,3, Sharma A1, Bellingham MC3, Coulson EJ1,2,3.
1. Queensland Brain Institute, 2. Clem Jones Centre for Ageing Dementia Research, 3. School of Biomedical Sciences, The University of Queensland, Brisbane Qld. 4072, Australia
To whom correspondence should be addressed: Prof. Elizabeth J. Coulson, E-mail: e.coulson@uq.edu.au

Neuronal death, leading to overall brain atrophy, is one of the fundamental characteristics of Alzheimer’s disease. Cholinergic neurons of the basal forebrain are particularly vulnerable in Alzheimer’s disease, and the consequent cholinergic neurotransmitter decline affects other neurotransmitter systems. Epidemiological studies indicate that sleep-disordered breathing is a strong risk factor for Alzheimer’s disease but the mechanisms remain unclear. Here we show that lesions of mouse cholinergic mesopontine tegmentum (cMPT) neurons, which control upper airway muscle tone during sleep, result in altered breathing, moderate hypoxia and mild cognitive impairment. We also demonstrate that APP/PS1 mice with cMPT neuronal lesions display severe cognitive impairment, and exacerbation of the pathological features of Alzheimer’s disease, including increased levels of amyloid-beta and inflammatory markers. Furthermore, the cMPT lesions cause selective degeneration of cholinergic basal forebrain (cBF) neurons, which are also characteristically lost in Alzheimer’s disease. We further reveal that this cBF neuronal loss is mediated by the p75 neurotrophin receptor and can be prevented by restoring blood oxygen levels during sleep. These findings provide a mechanism by which sleep apnea and Alzheimer’s disease could be causally linked through intermittent hypoxia-induced cBF degeneration.

Anna Maria Tartaglione (Istituto Superiore Di Sanità)

THE CRITICAL ROLE OF MICRONUTRIENTS IN NEURODEVELOPMENT: SHORT- AND LONG-TERM BEHAVIORAL OUTCOME IN A SELENIUM-DEFICIENT RAT MODEL
Research in animal models and human population shows that some essential elements such as selenium (Se) are particularly important during early stages of life to support rapidly maturation of cognitive functions. Conversely, at high concentrations essential micronutrients might also negatively influence brain development. Studies with animal birth cohorts might help to elucidate both protective and adverse effects of nutritional factors. Our main aim was to characterize the neurobehavioral effects of three diets with different Se content (two suboptimal diets, with a lower and higher Se level, respectively, and one Se-optimal diet) administered since the pre-conception stage up to adulthood. We performed a longitudinal assessment (since the very early neonatal stage to adulthood), by selecting different behavioral domains to evidence even subtle functional changes attributable to Se deficiency. Data indicate early behavioral changes in pups at the suboptimal (intermediate) Se dose persisting in adult rats. Specifically, adolescent rats experiencing dietary regimen with sub-optimal Se content show a hyperactive profile and impairment in spatial working memory. However, the underlying mechanisms of these effects are under investigation. The results of our research will be of potential high significance to elucidate the possible outcomes of an unbalanced diet on optimal brain development and assess the role of Se in these processes. The combination of biomarkers of nutrient intake and status in experimental models along with the use of innovative -omic techniques anchored to the behavioral phenotype will be pivotal to verify in prospective epidemiological studies the link between dietary intake and the neuropsychological outcomes.

Joanna Yau (University of New South Wales)

CALCIUM IMAGING OF BLA PRINCIPAL NEURONS DURING TWO FORMS OF PAVLOVIAN FEAR CONDITIONING

Yau JO-Y, Jean-Richard-dit-Bressel P, McNally, GP.
School of Psychology, University of New South Wales, Sydney, Australia.

Although the BLA has a well-established role in associative fear conditioning, the activity of specific classes of BLA neurons and their influence on learning is less understood. Recent evidence has shown that excitation of BLA principal neurons to the presence or absence of the fearful unconditioned stimulus (US) is sufficient to support learning (Sengupta & McNally, 2017, in preparation). Here, we used calcium imaging via fibre photometry to investigate the nature of BLA principal neuron activity during two widely used Pavlovian fear conditioning preparations: conditioned freezing and conditioned suppression. Glutamatergic BLA neurons of rats were targeted unilaterally using CaMKIIα-GCaMP6f and an optic fibre was implanted above the injection site to capture activity of these neurons. We recorded calcium transients during a differential fear conditioning (CSA-shock, CSB-no shock) using freezing (Experiment 1) and conditioned suppression (Experiment 2). There was a significant increase in normalised change in fluorescence signal (dF/F) to the shock onset that occurred at CSA offset, but not to the offset of the non-reinforced CSB. This was observed in both freezing and conditioned suppression preparations. Additionally, during conditioned suppression, we observed increased dF/F to pellet-reinforced lever presses and the magazine entry thereafter, but not to non-reinforced lever pressing or non-reinforced magazine entries. Taken together, these findings are consistent with the idea that BLA principal neurons are reactive to the shock US to support learning and that BLA principal neurons also have a role in appetitive learning.

Yinghua Yu (Xuzhou Medical University; University of Wollongong)

INCREASING SELECTED DIETARY FIBER INTAKE IMPROVES GUT MICROBIOTA FOR COGNITION-ASSOCIATED NEUROTRANSMISSION IN OBESE MICE

Yinghua Yu1,2, Peng Zhang1,2, Minmin Hu1,2, Renxian Tang1, Qingling Wang1, Hongqin Wang2, Kuiyang Zheng1, Xu-Feng Huang1,2

Affiliation:
Alzheimer’s disease (AD) is a severe and complex neurodegenerative disease affecting millions of people worldwide. The principal neuropathological hallmarks of AD are the formation of amyloid-β plaques and intracellular neurofibrillary tangles (NFTs), together with increased inflammation and disruption of the blood brain barrier (BBB) that lead to impairments in cognitive function. In this project, we characterised the Aβ-42-induced neuroinflammatory-, vascular- and tau-related changes in an in vivo mouse model of AD, established by a bilateral stereotaxic intrahippocampal injection of neurotoxic Aβ-42 into the CA1 region of the hippocampus in C57BL/6 mice. 30 days post-injection the tissue was collected and processed for free-floating fluorescence immunohistochemistry and confocal quantification to examine tau phosphorylation, and the expression of the main neuroinflammatory and neurovascular markers in the CA1, CA3 and Dentate Gyrus (DG) regions of the hippocampus. We observed an increased expression of phosphorylated tau in the CA1 region in Aβ-42-injected mice compared to the naïve control and ACSF-injected groups. We also found a significant neuroinflammatory response following Aβ-42 injection compared to controls, with an increased expression of Iba-1 and IP-10, markers of immune cell activation. Vascular changes were also observed as revealed by the significant up regulation of α-SMA in the CA1, CA3 and DG of the hippocampus of Aβ-42-injected mice. In conclusion, the detailed molecular and cellular characterisation of this AD mouse model demonstrates that a single Aβ-42 injection can mimic aspects of AD related to tau pathology, inflammation and BBB integrity, providing a basis for further research in the field. (250 words)

Purpose: Excess fat and lower dietary fiber intake contribute to the development of obesity which is associated with cognitive decline. Dietary fiber has beneficial effects in improving cognition, and has the ability to modulate gut microbiota and cannabinoid (CB) and serotonin (5-HT) tone. This study examined effects of four types of dietary fibres on gut microbiota and neurotransmitter receptor expression in mice and rats. Methods: 1) C57Bl6/J mice were on a chronic high-fat diet (HFD) for 22 weeks, the Y-maze test and novel object recognition test were performed for cognition, and gut microbiota in cecum content were examined by 16S sequencing; 2) The mice were given oat bran (1-3,1-4 beta-glucan) and curdlan (1-3,1-6 beta-glucan) pellet for 3 days. Gut microbiota were examined. 3) Rats were fed a HFD with or without galacto-oligosaccharides (GOS) and resistant starch (RS) for 4 weeks. The CB1, 5-HT1A and 5-HT2A receptors binding density in brain regions were examined by autoradiography. Results: Chronic HFD increased the abundance of Firmicutes and decreased Bacterioides in gut microbiota of obese mice with impaired recognition and spatial memory. Both oat bran fiber and curdlan decreased the abundance of Firmicutes and increased Bacterioides in mice. Furthermore, GOS and RS prevented HFD-induced alteration of CB1, 5-HT1A and 5-HT2A binding density in the mesolimbic system. Conclusion: Increasing dietary fiber intakes prevent alteration of gut microbiota and neurotransmitter/receptor binding in the brain regions involved in cognitive function. This maybe the neurobiological basis for improving altered cognitive function associated with obesity.

Beatrix Calvo-flores Guzman (Centre for Brain Research, University of Auckland)
Wei Zhen Chow (University of Newcastle)

**EXPERIMENTALLY-INDUCED PHOTOTHROMBOTIC STROKE IMPAIRED COGNITIVE PERFORMANCE ASSESSED IN THE RODENT PAIRED-ASSOCIATES LEARNING TASK**

Chow WZ1,2,3, Zhao Z1,2, Ong LK1,2,3, Walker FR1,2,3, Nilsson, M1,2,3.
1. School of Biomedical Sciences and Pharmacy and the Priority Research Centre for Stroke and Brain Injury, University of Newcastle, Callaghan, NSW, Australia. 2. Hunter Medical Research Institute, Newcastle, NSW, Australia. 3. NHMRC Centre of Research Excellence Stroke Rehabilitation and Brain Recovery, Heidelberg, VIC, Australia.

Cognitive functions, including memory and learning deteriorate progressively after stroke. Post-stroke cognitive impairment (PSCI) increases the risk of dementia - a third of stroke survivors is estimated to develop dementia within five years post-stroke. It is therefore critical to understand the neuropathology of PSCI and its functional outcome in pre-clinical model to guide potential therapeutic intervention. The paired-associates learning (PAL) is one of the established human cognitive tests used in various neurocognitive disorders, including Alzheimer's. We hereby demonstrated the use of a rodent version of PAL performed on an automated touchscreen platform to assess post-stroke cognitive performance in mice. Photothrombotic stroke model was performed to induce focal ischemia at the somatosensory and motor cortices in mice, and the cognitive performance was subsequently assessed at two different time-points - 1 week and 6 months post-stroke. Cognitive performance in the visuospatial domain was found to be significantly lower in stroked mice compared to non-stroke mice at both sub-acute and chronic phases of stroke recovery. In addition, we also showed that in non-stroke mice, older mice required longer time to acquire PAL task compared to young mice, demonstrating decreased learning ability due to ageing. The functional outcome findings will complement further histological studies on the neuropathology of PSCI. This study has for the first time to the best of our knowledge, validated the use of PAL to assess post-stroke cognitive performance in a mouse photothrombotic stroke model with a promising translational validity.

Ethan Cresswell (Newcastle University)

**ALTERED CHOLESTEROL HOMEOSTASIS IN THE AGEING BRAIN – A POTENTIAL CONTRIBUTOR TO AGE-RELATED CENTRAL NERVOUS SYSTEM DECLINE**

Cresswell ET1,2,3, Cummins MJ1,2,3, Smith DW1,2,3
1. Neurobiology of Aging and Dementia Laboratory, School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, New South Wales, Australia
2. Preclinical Neurobiology Research Program, Centre for Brain and Mental Health Research, University of Newcastle, Callaghan, New South Wales, Australia
3. Hunter Medical Research Institute, New South Wales, Australia

Ageing is a major risk factor for cognitive impairment and dementia. Unfortunately, the mechanisms causing these age-dependent declines remain widely unknown. The current study adds to the mounting data indicating ageing impacts regulation of cholesterol homeostasis in the central nervous system (CNS), which in turn could play a key role in the functional declines seen with age. Cholesterol is an essential lipid to CNS function, although excess free cholesterol is cytotoxic. Precise regulation of CNS cholesterol levels is therefore vital for normal function. However, it is proposed this stringent homeostatic control is altered with ageing.

Our previous RNA-seq studies showed up to 50% (34 of 68) of cholesterol-related genes were significantly altered with age in the CNS of old compared to young C57Bl6 mice. The current study expanded this investigation and analysed the effects of ageing on cholesterol related gene expression in five CNS regions (cortex, hippocampus, corpus collosum, as well as white and grey matter regions of the spinal cord). CNS regions were macrodissected from 8 young and 8 old C57Bl6 mice. Sensitive qPCR analyses were carried out to quantify the effects of ageing on genes involved in synthesis, storage, transport, and efflux processes of cholesterol homeostasis. We found cholesterol processes in all CNS regions studied were affected by ageing. However, the regions were differentially affected, with the spinal cord particularly impacted, especially those processes involved in synthesis and transport.

To further investigate the affects of ageing on CNS cholesterol, we are carrying out cell-specific expression
WHAT COULD PREVENT COGNITIVE DECLINE IN PEOPLE AT GENETIC RISK TOWARDS ALZHEIMER’S DISEASE?

De Frutos-Lucas J\textsuperscript{1,2} & Rodríguez-Rojo IC \textsuperscript{1,3}

1. Cognitive and Computational Neuroscience Laboratory, Center for Biomedical Technology, Madrid, Spain. 2. Biological and Health Psychology Department, Universidad Autónoma de Madrid, Spain. 3. Department of Cognitive Processes, Universidad Complutense de Madrid, Madrid, Spain.

Despite the great amount of effort that many research groups worldwide have devoted to find a cure for Alzheimer’s Disease (AD), no drug has been proven to be successful to either stop or revert cognitive decline in these patients. Therefore, the spotlight has moved towards prevention and many lifestyle factors have been proposed to have an impact on the risk, the age at onset, or the severity of the symptomatology. The apolipoprotein E (APOE) gene ε\textsuperscript{4} allele has been pointed as the most relevant genetic risk for late onset AD. In this study, we aimed to review the available literature on different lifestyle variables (including physical activity, diet, substance abuse, educational level and bilingualism among others) trying to understand the effect that they exert on healthy agers and also people with Mild Cognitive Impairment or AD. Beyond that, the main purpose of this work was to determine if such positive or negative effects also apply to the population at genetic risk towards AD or whether the carriage of this allele diminishes or exacerbates the protective or risk properties of the above-mentioned lifestyle variables. Among other findings, we have come to appreciate that physically inactive carriers are at a greater risk than inactive non-carriers and active carriers. Also, we have learnt that the protective effect of moderate alcohol consumption in the general population turns into a risk factor when it comes to carriers. However, more research needs to be conducted in order to explore possible interactions between all these lifestyle profiles.

COGNITIVE TRAINING OUTCOMES AND THE APOE ε4 MODULATION IN A SAMPLE OF HEALTHY ELDERS

Rodríguez-Rojo IC \textsuperscript{1,2}, López-Higes R \textsuperscript{2}, Prados JM. \textsuperscript{2}, Montejo P \textsuperscript{3}, Del-Río\textsuperscript{1,2}, Delgado-Losada ML \textsuperscript{2}, Montenegro M\textsuperscript{3}, López-Sanz D \textsuperscript{1,2}, Barabash A \textsuperscript{4,5}, De Frutos-Lucas J\textsuperscript{1,6}

1. Cognitive and Computational Neuroscience Laboratory, Center for Biomedical Technology, Madrid, Spain. 2. Department of Cognitive Processes, Universidad Complutense de Madrid, Madrid, Spain. 3. Department of Cognitive Processes, Universidad Complutense de Madrid, Madrid, Spain. 4. Laboratory of Psychoneuroendocrinology and Genetics, San Carlos Clinical Hospital, Madrid, Spain. 5. Institute of Sanitary Investigation, San Carlos Clinical Hospital, Madrid, Spain. 6. Biological and Health Psychology Department, Universidad Autónoma de Madrid, Spain.

Introduction: The ε4 allele of the apolipoprotein E (APOE) gene is a genetic risk factor for Alzheimer’s disease. This risk genotype impacts negatively on cognitive functioning, although little is known about how it interacts with the administration of cognitive training programs. Objective: To explore the differential APOE genotype modulation effect after a cognitive intervention in the following domains: language comprehension, executive functions and memory. Methods: Fifty older adults (> 65 years, 30 women and 20 men) participated in 30 training sessions along 12 weeks. Half of them were APOE ε4 carriers. The control group was matched in age, gender, normalized hippocampal volume, cognitive reserve, Mini-Mental State Examination (MMSE) score, and the Geriatric Depression Scale-Short Version (GDS-15). Results: We showed cognitive training benefits in the language comprehension domain (noncanonical sentences and sentences with two propositions), a domain that was not directly trained, although this effect was only found among APOE ε4 noncarriers. Conclusion: The genetic profile modulates the results derived from cognitive training in sentence comprehension.

STIMULUS REPETITION PATTERN IS A SIGNIFICANT PARAMETER IN EXTINCTION OF AUDITORY FEAR CONDITIONING

Faiz A, Windels F, Sah P
In auditory fear conditioning (FC) an aversive unconditioned stimulus (US) is contingently paired with a neutral conditioned stimulus (CS). As result, the CS evokes a conditioned response (CR, often measured as freezing). During FC the amygdala receives inputs from both cortical –and subcortical regions and its projections to the brain stem mediate freezing behaviour. Despite the large number of studies on FC, it is not yet known what the animal perceives as the CS. In this study mice were fear conditioned to an auditory tone consisting of 30 on-sounds (200 ms duration at 1 Hz, ‘pip’ on day 1 (D1). On day 2 (D2) mice underwent extinction using either the original CS (control group), 3 ‘pips’ delivered at 0.67 Hz, 3 pips as a train or a single pip of 200 ms duration. On following days (D3-D5) mice were tested using the original CS. The data show that the animals were able to respond to the fractions of the tone and froze till the duration of the conditioned tone. As expected, the control group extinguished within – and between sessions. Mice that were exposed to 3 pips on D2 extinguished to this tone within session but failed to extinguish between sessions. Finally, mice that were exposed to 1 pip or 3 pips as a train on D2 failed to extinguish within -and between sessions. The data suggest that the repetition pattern of the CS train is a significant parameter in distinguishing an established memory trace.

Shannyn Genders (La Trobe University)

CHARACTERISATION OF ALCOHOL-SEEKING BEHAVIOUR IN GALANIN RECEPTOR-3 KNOCKOUT MICE

Genders SG1, Scheller KJ1, Jaehne EJ2, van den Buuse M2, Lawrence AJ3, Turner BJ4, Brunner S4, Kolfer B4, Djouma E3.

1. School of Life Sciences, Dept. of Physiology, Anatomy and Microbiology, La Trobe University, Bundoora, Victoria, Australia. 2. School of Psychology and Public Health, Dept. of Psychology and Counselling, La Trobe University, Bundoora, Victoria, Australia. 3. Florey Institute of Neuroscience & Mental Health, University of Melbourne, Parkville, Victoria, Australia. 4. Laura Bassi Centre of Expertise-Therapeutic Application of Neuropeptides (THERAPEP), Research Program for Receptor Biochemistry and Tumour Metabolism, Department of Paediatrics, Paracelsus Medical University, Salzburg, Austria.

Galanin is a neuropeptide that has been critically implicated in mediating addiction. More specifically, administration of the galanin receptor-3 (GALr3) antagonist, SNAP 37889, has been shown to reduce alcohol-seeking behaviour in animal models. The recently developed GALr3 knockout (KO) mouse strain was utilised in the current study to investigate the effect of this novel KO on ethanol preference and motivation to obtain ethanol. In the two-bottle free choice paradigm, GALr3-KO mice consistently showed a significantly increased preference for ethanol when compared to wildtype (WT) littermates at concentrations of 5%, 10%, 15%, and 20% (males, 5.8-8.3% increase; females, 7.8-11.3% increase; p<0.05). Furthermore, male GALr3-KO mice displayed significantly increased motivation to obtain ethanol under operant responding protocol (162.2 ± 7.5 compared to 70 ± 4.2 lever presses; p<0.01). No differences between GALr3-KO and WT mice in blood ethanol concentrations at 1, 2 and 3 hours post 20% ethanol exposure suggest that this difference in consumption was not the result of altered ethanol metabolism. Further investigation found the increased consumption to be specific for ethanol, with GALr3-KO mice exhibiting similar preference for saccharin and sucrose over water as WT littermates. A battery of behavioural tests revealed no genotype differences in anxiety, cognition and locomotor behaviours, suggesting that the results obtained were not due to any behavioural deficits. Overall, our results show that ablation of GALr3 in mice increases alcohol consumption in various addiction paradigms which is in contrast to the previous effect observed with pharmacological studies blocking this receptor using SNAP 37889.

Jessica Herrington (Eccles Institute Of Neuroscience, John Curtin School Of Medical Research, Australian National University)

LEARNING COMPLEX TEXTURE DISCRIMINATION

Herrington J, Maddess T, Coy D, Carle CF, Sabeti F, Barbosa M
Eccles Institute for Neuroscience, John Curtin School of Medical Research, Australian National University.

Different isotrigon texture types are only discriminable from random binary patterns and each other by their third and higher-order spatial correlations. Their mean contrast and spatial frequency content is identical to
random noise. Our ability to make these discriminations has been proposed to be innate. We previously investigated learning of 17 isotrigon types in seven naïve subjects, where each type was tested in 14 sessions over 6 weeks. Significant learning was observed. Here we examined if 7 learning sessions conducted every 30 minutes on one day achieved similar learning. We used 13 naïve subjects with normal vision. We examined discrimination from random patterns of 5 of the original texture types, with 16 4AFC repeats/texture/session (5*13*7*16=7280 discriminations). Learning was similar to that achieved in the 6-week sessions. Three of the textures showed significant learning with mean discrimination improvement in probably of correct discrimination of 0.072 ± 0.001 to 0.188 ± 0.0137 (p=0.3 to 0.001). It appears that the number of discriminations, rather than the duration of the learning period is the key factor in learning differences in texture appearance based upon higher order spatial correlations. Initial performance was not chance so there appears to be some innate ability in naïve subjects.

Samuel Hogarth (La Trobe University)

ELUCIDATING THE INTERACTION BETWEEN BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND THE DOPAMINE D3 RECEPTOR IN METHAMPHETAMINE PSYCHOSIS

Hogarth SJ1, Jaehne E1, van den Buuse M1
1School of Psychology and Public Health, La Trobe University, Melbourne, Australia

Methamphetamine abuse is associated with heightened risk of a psychosis similar to paranoid schizophrenia. Brain-derived neurotrophic factor (BDNF) has been implicated in neuronal adaptation to methamphetamine as well as pathways mediating psychosis. BDNF also modulates expression of the dopamine D3 receptor (D3R), which is involved in psychosis and methamphetamine sensitization. The main aim of this study was to elucidate brain mechanisms involved in methamphetamine psychosis. Specifically, we investigated the role of the D3R in changes in methamphetamine sensitization in mice with reduced BDNF expression. We crossed BDNF heterozygous mice and D3R knockout mice to obtain wildtype, BDNF heterozygote, dopamine D3R knockout, and double mutant genotypes. Male and female mice were chronically treated with saline or methamphetamine during 6, 7 and 8 weeks of age on an escalating dose regime, after which they were left undisturbed for at least 2 weeks. Mice were then tested in automated photocells for changes in locomotor hyperactivity to an acute 3 mg/kg methamphetamine challenge. As expected, acute methamphetamine increased locomotor activity (P<.001) and this effect was enhanced in mice chronically pre-treated with the drug. In methamphetamine-pre-treated animals, the acute response to methamphetamine was significantly higher in BDNF heterozygotes compared to wildtype controls (P=0.004). This interaction seems reliant on D3 receptor availability as this enhancement of sensitization was lost in double mutant mice (P=0.897). Furthermore, these interactive effects were not observed in saline pre-treated mice, suggesting that the interplay between BDNF and downstream D3 signalling is particularly important during the sensitization phase of methamphetamine psychosis development.

Joshua Holmes (The University of Adelaide)

WESTERN STYLE DIET IMPACTS DEPRESSIVE-LIKE BEHAVIOUR BUT NOT ANXIETY-LIKE BEHAVIOUR DURING PROTRACTED WITHDRAWAL FROM SELF-ADMINISTRATION OF ALCOHOL.

Holmes JL1, Corrigan F2, Hutchinson MR1.
1Discipline of Physiology, School of Medical Sciences, University of Adelaide, Adelaide, South Australia.2Discipline of Anatomy and Pathology, School of Medical Sciences, University of Adelaide, Adelaide, South Australia.3Australian Research Council Centre for Nanoscale BioPhotonics, School of Medicine, University of Adelaide.

Clinical evidence suggests that abstinence from alcohol is associated with depression in many patients. It has been suggested that poor diet may influence this, via a gut to brain interactions. This study investigated the contribution that a western-style fast-food diet (WD) and free access to ethanol had on drinking behaviour and the development of depressive-like and anxiety-like behaviour during protracted withdrawal. Male C57BL/6 mice were given either a WD or regular chow ad libitum and were simultaneously exposed to water only or alcohol via the intermittent access 2-bottle (IA2B) choice paradigm. For the IA2B procedure mice were given two sipper tubes with access to 20% ethanol from one sipper tube every other day for 6-weeks. Following protracted withdrawal from alcohol access (10-14 days post alcohol) depressive and anxiety-like
Institute Annual Scientific Meeting 2017
International Convention Centre, Sydney, December 3rd – 6th 2017

behaviours were evaluated by the saccharin preference test and elevated plus maze respectively. No differences were noted in animals exposed to WD combined with alcohol compared to any other group in anxiety-like behaviour. However, WD combined with alcohol significantly decreased saccharin preference compared to WD/water and regular chow/alcohol groups (p<0.05). Indeed, 40% of animals in the WD/alcohol group had a significantly reduced saccharin preference (≤50% preference) compared to 12.5% of animals in the WD/water group and 0% in the regular chow/alcohol group. These results highlight the detrimental effects of alcohol combined with a WD on withdrawal symptoms. Future directions of this study will include analysis of the involvement of the immune to brain communication that may be influencing the observed behaviour.

Cynthia Lee (Neuroscience Research Australia)

INCREASED PLASMA BDNF LEVELS IN FEMALES WITH SCHIZOPHRENIA AND AN ASSOCIATION OF BDNF VAL/VAL GENOTYPE WITH DECREASED BRAIN VOLUME IN SCHIZOPHRENIA


8. 1. Schizophrenia Research Institute, Sydney, Australia. 2. Schizophrenia Research Laboratory, Neuroscience Research Australia, Sydney, Australia. 3. School of Psychiatry, University of New South Wales, Sydney, Australia. 4. Discipline of Psychiatry, University of Adelaide, South Australia, Australia. 5. Northern Adelaide Local Health Network. 6. Ramsay Health Care (SA) Mental Health. 7. Flinders University, South Australia, Australia. 8. Augusta University, Augusta, GA, USA. 9. Virginia Commonwealth University, Virginia, USA.

Brain-derived neurotrophic factor (BDNF) plays a critical role in neuroplasticity. BDNF is a potential biomarker for cognitive change in schizophrenia and functional polymorphisms of the human BDNF gene have been shown to impact cognition and brain volume in healthy humans. This study examines the relationships between peripheral BDNF levels and BDNF genotype, regional brain volumes and cognitive abilities, in people with schizophrenia and healthy controls. We measured circulating plasma BDNF levels by ELISA, BDNF val66met genotype by PCR based allele discrimination assay, and the cognitive abilities of verbal memory, language, working memory, processing speed and perceptual organisation, in 97 people with schizophrenia and 87 healthy controls. Brain volumes were measured in 61 patients and 65 controls using a 3T MRI with three regions of interest chosen based on their known relationship to both BDNF and cognitive deficits in schizophrenia. Plasma BDNF levels in women with schizophrenia were significantly elevated compared to men with schizophrenia and to female healthy controls. In controls, BDNF genotype significantly predicted dorsolateral prefrontal cortex (DLPFC) volume, with val homozygotes having larger DLPFC volume than met carriers. Conversely, in patients with schizophrenia, BDNF val homozygotes had decreased parietal lobe and hippocampal volumes compared to met carriers. Plasma BDNF levels did not significantly predict brain volume or cognitive performance in either diagnostic group. This study shows sex-specific differences in plasma BDNF levels and differential associations between BDNF val66met polymorphism and brain volumes in healthy adults compared to people with schizophrenia.

Mona Lei (Centenary Institute, University Of Sydney)

LOSS OF MYELIN LIPIDS AND ENHANCED ANXIETY IN SPHINGOSINE KINASE 2 KNOCKOUT MICE

Mona Lei, Adeena Shafique, Timothy A Couttas, Hua Zhao, Tim Karl and Anthony S Don.

1 Prince of Wales Clinical School, University of New South Wales, NSW 2052, Australia; 2 School of Medicine, Western Sydney University, NSW 2560, Australia; 3 Neuroscience Research Australia (NeuRA), NSW 2031, Australia; 4 Centenary Institute and Sydney Medical School, University of Sydney, NSW 2006, Australia

Background: The sphingolipid sphingosine 1-phosphate (S1P) is an essential neuroprotective signalling molecule. It signals through its own family of five G-protein coupled receptors. A loss of S1P is evident in preclinical stages of AD pathology and particularly in brain regions that develop neuronal atrophy. S1P receptors are good pharmacological targets therefore understanding the functions of S1P in normal brain physiology is important in the context of AD therapy.

The enzymes sphingosine kinase 1 and 2 catalyse the synthesis of S1P. SphK2 is the predominant isoform in the brain. We conducted a comprehensive analysis of the role of SphK2 in memory, cognition and
**preservation of myelin integrity.**

**Methods:** We tested 23 mice SphK2\(^{-/-}\) and C57BL/6 mice aged between 10-12 months. Cognitive examination included tests for spatial memory, anxiety, fear memory and motor function. Brain regions underwent biochemical analysis which consisted of lipidomic analysis and western blotting to examine myelin lipids and proteins. Liquid chromatography-mass spectrometry was also used to investigate S1P levels and sphingolipid species.

**Results:** We observed enhanced anxiety in the SphK2\(^{-/-}\) mice compared to the controls using the fear conditioning paradigm but no deficit in fear extinction. We did not observe any deficits in spatial memory in the SphK2\(^{-/-}\) mice using the cheeseboard paradigm. A significant loss of myelin lipids and classical myelin protein marker levels in the SphK2\(^{-/-}\) mice was identified.

**Conclusion:** S1P synthesised by SphK2 is required for myelin integrity. The loss of myelin integrity provides a possible explanation for enhanced anxiety in the SphK2\(^{-/-}\) mice.

Amy Li (University of New South Wales)

**ROLE OF THE BASOLATERAL AMYGDALA GLUTAMATERGIC NEURONS AND THEIR VENTRAL STRIATAL PROJECTIONS IN FEAR AND EXTINCTION LEARNING**

Li A\(^1\) & McNally GP\(^1\).

1. School of Psychology, University of New South Wales, Sydney, Australia.

A core feature of anxiety disorders is the inability to suppress excessive fear and anxiety in the absence of real and immediate danger. Although the neural mechanisms and circuits that regulate fear inhibition and safety are poorly understood, the basolateral amygdala (BLA) and its projections to the prefrontal cortex have been implicated in the regulation of fear. We used a rodent model of Pavlovian fear conditioning and extinction to investigate the roles of global as well as distinct subpopulations of BLA glutamatergic neurons in fear and extinction learning. To study the global population, we optogenetically silenced BLA glutamatergic neurons at the time of the expected but absent footshock US and showed that this augmented fear extinction as evidenced by increased resistance to fear relapse. To study distinct subpopulations we targeted BLA glutamatergic neurons that project to the nucleus accumbens (Acb) using optogenetic terminal inhibition in the accumbens and retrograde gCaMP imaging of BLA projection neurons. We found that optogenetic inhibition of BLA glutamatergic terminals in the accumbens did not affect the acquisition of learned fear but did augment the extinction of learned fear.

Liying Lin (School Of Pharmacy And Medical Sciences, University Of South Australia)

**CHRONIC CORTICOSTERONE ADMINISTRATION ALTERS THE BALANCE BETWEEN BDNF AND PROBDNF IN MICE**

Lin L\(^1\), Luo S\(^2\), Al-Hawwas M\(^1\), Zhou XF\(^1\), Bobrovskaya L\(^1\)

1. School of pharmacy and medical science, University of South Australia, Australia. 2. Department of breast surgery, Xiangya hospital, Hunan province, China.

In this study, we aimed to establish the effects of chronic corticosterone administration on the levels of mature brain-derived neurotrophic factor (mBDNF) and its precursor protein proBDNF in mice tissues. C57BL male and female mice received drinking water (n=8), corticosterone in drinking water (100µg/ml, n=8) or vehicle in drinking water (1% ethanol, n=8) for 4 weeks. At the end of experimental protocol the open field test and Elevated plus Maze test were performed. Brain and adrenal tissues were collected and mBDNF and proBDNF were measured by ELISA assays. We found that the mice fed with corticosterone developed anxiety-like behaviours as evidenced by reduced time in the central zone in the open field test compared with the vehicle group (p=0.009). Both proBDNF and mBDNF were significantly decreased in the corticosterone and vehicle groups compared to the control group in prefrontal cortex (P<0.0001), hippocampus (P<0.0001), hypothalamus (P=0.0013), and adrenal (P=0.0007). The ratio of proBDNF/mBDNF in prefrontal cortex and adrenal tissues in the corticosterone group was increased compared to the ethanol group; whereas, the ratio of proBDNF/mBDNF in hypothalamus was reduced in both ethanol (p=0.0013) and corticosterone (p=0.033).
groups compared with the control group. Our data suggest that the ratio of proBDNF/mBDNF is differentially regulated in different tissues. Ethanol and corticosterone down regulate both BDNF and proBDNF and alter the balance of proBDNF/mature BDNF in most tissues. In conclusion, the ethanol and corticosterone may cause abnormal regulation of BDNF and proBDNF which may lead to mood disorders.

Yu Liu (Unsw)

ROLE OF THE TUBERAL HYPOTHALAMUS IN CONTEXT-INDUCED REINSTATEMENT OF ALCOHOL SEEKING


Contexts play an important role in promoting and preventing relapse to drug taking. Contexts associated with self-administration promote relapse whereas contexts associated with the absence of self-administration promote abstinence. The tuberal hypothalamus mediates both these roles with lateral regions (LH) contributing to relapse and medial regions (MDH) contributing to abstinence. These experiments assessed the role of LH and MDH neurons in promoting abstinence using calcium imaging, optogenetics, and chemogenetics in the awake freely moving animal. Animals trained to respond for alcoholic beer in a distinctive context (A) and extinguished in a second, different context (B) showed low levels of alcohol seeking when tested in the extinction context (ABB) but expressed relapse when tested in the training context (ABA). Chemogenetic inhibition of LH neurons prevented this relapse. In contrast, chemogenetic excitation of LH, but not MDH neurons, increased this relapse. There was evidence for calcium transients in LH neurons during relapse but these were not observed in LH GAD1 neurons. Calcium imaging of MDH neurons is ongoing.

Zeyue Liu (Peking Union Medical College)

INVERSE CHANGES IN TELOMERE LENGTH BETWEEN BLOOD AND BRAIN IN MAJOR DEPRESSIVE DISORDER

Zeyue Liu1, Yan Shen1, Jianbo Xiu1, Qi Xu1.
1 National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences & Neuroscience Center, Chinese Academy of Medical Sciences and Peking Union Medical College, Tsinghua University, Beijing, 10005, China.

As a mood disorder, previous studies confirmed that telomere length in peripheral blood of depressive patients was reduced. However, it is less clear whether or not telomere length in mood-regulated brain regions is changed. Using real time quantitative PCR, we measured telomere length in five brain regions (hippocampal, amygdala, paraventricular nucleus, nucleus accumbens and prefrontal cortex) of depressive-like mice (N=22) and normal control mice (N=12). Telomere length in peripheral blood of mice and patients were also measured. We observed significant increase in the prefrontal cortex and amygdala of depressive-like mice, compared with normal control. Peripheral blood telomere length of mice and patients were both significantly shortened, consistent with previous researches. In our study, telomere length in brain regions and peripheral blood was systematically measured in a typically depressive model, chronic unpredictable stress model (CUS). This finding provides a new cue about the relationship between variable telomere length of MDD related brain regions and MDD, and hints that telomere length may plays a special role in MDD, but not just present events about ageing. But the detail mechanisms are required further exploration.

Jiaqi Luo (The Florey Institute Of Neuroscience And Mental Health)

NEUROLIGIN-1 REGULATES MOTIVATION AND GOAL-DIRECTED BEHAVIOUR

Luo J1, Brose N2, Nithianantharajah J1.
1 The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Victoria, Australia.
2 Max Planck Institute for Experimental Medicine, Göttingen, Germany

The complex protein machinery at the synapse underlies information processing in the brain. Human mutations in the neuroligin family of postsynaptic cell adhesion molecules, including the neuroligin-1 gene (NLGN1) have been documented in various psychiatric disorders in which cognitive dysfunction is a core symptom. To systematically investigate the role of neuroligin 1 in cognitive behaviour, we assessed male and female neuroligin-1 null mutant mice (Nlgn1−/−) on a battery of cognitive tests using the rodent touchscreen system. We observe that Nlgn1−/− mice exhibit impaired motivational processing, but interestingly show
normal performance on a range of tests for learning and memory. Our findings highlight a distinct role for neuroligin-1 in regulating reward salience in goal-directed behaviours, essential for decision making.

Paul Marshall (Queensland Brain Institute)

**ACTIVATION-INDUCED CYTIDINE DEAMINASE REGULATES THE EXTINCTION OF CONDITIONED FEAR**

Paul Marshall¹, Vikram Ratnu¹, Wei Wei¹, Xiang Li¹, Esmi Zajaczkowski¹, Jordan Edmunds¹, Laura Leighton¹, Timothy Bredy¹

*Queensland Brain Institute, University of Queensland, Brisbane, QLD, Australia*

Base editing has been correlatively linked to the mediation of behaviour in organisms ranging from flies to humans, with the highest levels of the enzymes mediating this process found in primates humans, and in some instance in a brain-specific manner. Mechanistically, this occurs through the deamination of specific base nucleotides, including cytosine in the case of activation-induced cytidine deaminase (AID) and adenosine in the case of adenosine deaminase, RNA-specific (ADAR). Previously, we found in primary cortical neurons that blocking the expression of AID impairs BDNF exon IV expression by modifying DNA methylation and CREB occupancy within its promoter. To extend these observations, AID shRNA was transfected into the infralimbic cortex of fear conditioned and extinction trained mice. Contrary to expectations, as it is known that a reduction in BDNF expression impairs fear extinction, we found that knockdown of AID significantly enhanced fear extinction memory. We have therefore established a functional role for AID in the regulation of adaptive behaviour the mechanism underlying this effect remains to be determined.

Kathryn Mathews (University of Sydney)

**HIPPOCAMPAL GROWTH FACTORS ARE ALTERED IN PARKINSON’S DISEASE WITH DEMENTIA**

Virachit S ¹,², Mathews KJ ³,⁴, Cottam V ³,⁴, Werry E ⁵, Galli E ⁶, Rappou E ⁶, Lindholm P ⁶, Saarma M ⁶, Halliday GM ¹,²,⁴,⁷, Shannon Weickert C ¹,⁸,⁹, Double KL ³,⁴

¹. Neuroscience Research Australia, Randwick, Australia  ². School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, Australia  ³. Discipline of Biomedical Science, Sydney Medical School, University of Sydney, Australia  ⁴. Brain and Mind Centre, University of Sydney, Australia  ⁵. Faculty of Health Sciences, University of Sydney, Australia  ⁶. Institute of Biotechnology, University of Helsinki, Finland  ⁷. Central Clinical School, University of Sydney, Australia  ⁸. School of Psychiatry, Faculty of Medicine, University of New South Wales, Sydney, Australia.  ⁹. Schizophrenia Research Institute, Darlington, Australia.

Growth factors are associated with mitogenesis, differentiation, neuroprotection and neurorestoration, and neuroplasticity. Alterations in hippocampal expression of growth factors may therefore contribute to neuronal vulnerability, which in turn may impact dementia risk in Parkinson’s disease. However, to date there have been no human studies quantifying these factors specifically in the Parkinson’s disease dementia (PDD) hippocampus compared with healthy control hippocampi. We analysed levels of seven growth factor proteins in the hippocampus of 10 PDD and 9 age-matched controls using enzyme-linked immunosorbent assays. Levels of glial cell line-derived neurotrophic factor (GDNF) were significantly reduced (19%, p=0.04), while fibroblast growth factor 2 (FGF2; 40%, p=0.01) and cerebral dopamine neurotrophic factor (CDNF; 41%, p=0.02) were significantly increased. Using immunohistochemistry, the expression of FGF2 was primarily found in an increased proportion of resting glial cells (p=0.005), whereas the neuronal expression pattern of GDNF and CDNF was unchanged. These results demonstrate specific alterations in hippocampal growth factors in PDD. These changes may contribute to both hippocampal tissue stability and dysfunction; alternatively they may represent compensatory responses in this brain region.

Chun Hui (Johnny) Park (The Florey Institute of Neuroscience and Mental Health)

**SEX DIFFERENCES IN RELAPSE FOLLOWING FEAR EXTINCTION IN JUVENILE RATS.**

Park CHJ¹,², Ganella DE¹,², Kim JH¹,².

¹. Behavioural Neuroscience Division, The Florey Institute of Neuroscience and Mental Health, Melbourne, VIC 3052 Australia.  ². Florey Department of Neuroscience and Mental Health, University of Melbourne, Melbourne, VIC 3052 Australia.
By 6 years of age, girls are more than twice as likely to have experienced an anxiety disorder than boys. This indicates sex differences in fear learning may emerge from early childhood. Thus, our study investigated sex differences in the fear relapse behaviours, a core pathology of anxiety disorders, in juvenile rats (postnatal day 17±1). All rats first received three white-noise - footshock pairings and the freezing to the white-noise was then extinguished. In experiment 1, rats were tested for freezing to the white-noise in the same or different context to extinction. Female rats showed renewal of extinguished fear when tested in the different context to extinction (p < 0.05), whereas male rats did not (p > 0.05). In experiment 2, rats received either a reminder footshock or context exposure the day after extinction and were tested for freezing to the white-noise the next day. Female rats that received the reminder showed reinstatement of extinguished fear (p < 0.05), whereas the male rats did not (p > 0.05). In experiment 3, rats were tested for freezing to the white-noise either the day after or 5 days from extinction. Female rats tested after 5 days showed spontaneous recovery of extinguished fear (p < 0.05), whereas the male rats did not (p > 0.05). Our findings indicate the fear relapse behaviours in juvenile male and female rats may be qualitatively different. These differences may underlie the higher likelihood to experience anxiety disorders in young female children compared to male children.

Naveen Sendhilnathan (Columbia University Department of Neuroscience)

CEREBELLAR SIMPLE SPIKES RESPOND DIFFERENTLY AFTER REWARDED AND UNREWARDED TRIALS DURING VISUOMOTOR ASSOCIATIVE LEARNING

Naveen Sendhilnathan1,2,4,5,7, Anna Ipata2,4,5, Mulugeta Semework2,4,5 and Michael E. Goldberg2,3,4,5,6,7
1 Doctoral program in Neurobiology and Behavior, Columbia University, New York, NY; 2 Dept. of Neuroscience, Columbia University, New York, NY; 3 Dept. of Neurology, Psychiatry, and Ophthalmology, Columbia University College of Physicians and Surgeons, New York, NY; 4 Mahoney Center for Brain and Behavior Research, Columbia University, New York, NY; 5 New York State Psychiatric Institute, New York, NY; 6 Kavli Neuroscience Institute, Columbia University, New York, NY; 7 Zuckerman Mind, Brain and Behavior Institutes, Columbia University, New York, NY.

The cerebellum (CB) is known to play role in motor learning. However, recent tract-tracing and fMRI connection studies show it is reciprocally connected to prefrontal cortex, suggesting cognitive roles for CB. In addition, there is some evidence that patients with cerebellar lesions have cognitive deficits. In keeping with this anatomical and clinical data, we have recently shown that Purkinje cells in monkey mid-lateral cerebellum track the learning of arbitrary visuomotor associations where the kinematics of the movement remained unchanged. The monkeys press a rod with each hand, after which a fixation point appears and then one of a pair of symbols that the monkey has never seen before, one of which is associated with reward. Simple spike activity of Purkinje cells tracks the monkey’s learning, responding differently after rewarded and non-rewarded trials. This differential response occurs only for a few hundred milliseconds during the trial, but different cells respond during different epochs during the trial, so that across the sample, the change in activity of the cells continuously tiles the entire trial duration. A given cell will respond during the identical epoch when the monkey learns different symbols. The effect was not related to the monkey’s reaction time, nor was it present when the monkey made a mistake responding to an overtrained symbol. We conclude that the cerebellum has a role in learning far beyond the improvement of motor performance.

Elysa Sokolenko (University of Melbourne)

IDENTIFYING THE CELL TYPE MEDIATING NMDA RECEPTOR HYPOFUNCTION EFFECTS ON WORKING MEMORY

Sokolenko EM1, Nithianantharajah J2, Jones NC1
1. Department of Medicine (Royal Melbourne Hospital), University of Melbourne, Melbourne Brain Centre, Parkville, Victoria, Australia. 2. The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, Australia.

Working memory deficits are perhaps the most studied of the cognitive impairments in schizophrenia. Antagonists of the NMDA receptor (NMDAR) can recreate these deficits in both healthy people and rodents, observations which led to the NMDAR hypofunction theory of schizophrenia. Transgenic mice lacking the NMDA receptor from either parvalbumin positive (PV+) interneurons (PV-Cre; NR1f/f) or pyramidal cells in the...
forebrain (CaMKIIα-Cre; NR1f/f) and their wildtype (wt) littermates were trained to perform the Trial Unique Nonmatching to Location (TUNL) task of working memory. Once trained, mice were administered MK-801 (0, 0.1 or 0.3 mg/kg) prior to testing. Task performance was assessed in terms of accuracy and perseveration (no. of correction trials/no. of incorrect responses). In wt mice, treatment with MK-801 produced significant decreases in accuracy and increases in perseverative responses. These MK-801-induced effects were actually more pronounced in the PV-NR1 KO mice compared to their wt littermates, but were no different when comparing CaMKIIα-NR1 mice and their wt littermates. These results suggest that neither PV+ interneurons nor CaMKIIa+ pyramidal cells are the exclusive cell type mediating the effects of NMDAR antagonists on working memory. However, NMDAR deletion from PV+ interneurons appears to sensitize circuits to the behavioural effects of NMDAR antagonists on this behaviour. The effect of NMDAR hypofunction on working memory may be mediated by other interneuron subtypes.

Nannan Sun (Institute of Basic Medical Sciences, Chinese Academy Of Medical Sciences & Peking Union Medical College)

**LPS-INDUCED DEPRESSIVE-LIKE BEHAVIOR IN MICE ACCOMPANIED WITH PERIPHERAL MONOCYTE RECRUITMENT TO THE BRAIN.**

Sun NN, Xiu JB, Xu Q.
State Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China.

Background: Lipopolysaccharide (LPS) primes peripheral myeloid cells, activates microglia, and induces depressive-like behavior. Here we propose that LPS-associated release and redistribution of primed myeloid cells is an important link between immune dysfunction and the development of mood disorders.

Objective: Therefore, the objectives of this study were to (1) evaluate whether there was monocyte recruitment to the brain corresponded with LPS-induced depressive-like behavior and (2) examine whether these infiltration had brain region-dependent manners.

Methods: Mice were treated with LPS (intraperitoneal, 830 μg/kg) to induce a depressive-like behavior. And GFP+ bone marrow (BM)-chimeric mice were used to determine the neuroanatomical distribution of peripheral monocyte cells recruited to the brain during LPS.

Results: In comparison to saline, LPS reduced time spent in the center of the open field, however, there was no significant difference in motor activity (figure a). As a result, high degrees of stable chimerism (about 80%) was established 4 weeks after reconstitution, which was verified by determining the percentage of GFP+ cells in the BM and the blood samples (figure b, c). With GFP+ BM-chimeric mice, we found that LPS increased recruitment of GFP+ macrophages to the brain hippocampus and amygdala, which had brain region-dependent manners (figure d). Conclusion: These findings indicate that monocyte recruitment to the brain in response to LPS may represent a novel cellular mechanism that contributes to the development of depression.

Stephanie Carmen Tran (La Trobe University)

**PLAG1 EXPRESSION IN ADULT MOUSE BRAIN AND EFFECT OF PLAG1 KNOCKOUT ON SELECTED BEHAVIOURS**

Tran SC, Dye L, Gasperoni J, Wong J, Grommen SVH, De Groef B
Laboratory of Comparative Endocrinology and Neurobiology, La Trobe University, Bundoora, Victoria, Australia

The proto-oncogene pleomorphic adenoma gene 1 (Plag1) encodes a zinc finger transcription factor that has been implicated in tumorigenesis in certain types of cancer. However, its physiological role has not been thoroughly investigated. We investigated PLAG1 expression in the hippocampus and hypothalamus of adult mice using a combination of X-gal staining and immunohistochemistry. PLAG1 is predominantly expressed by neurons (stained with the neuronal marker NeuN), with also some expression in astrocytes (stained with the astrocytic marker GFAP). In addition, we subjected adult male and female Plag1 knock-out (KO) mice (n=36) to a series of behavioural tests to compare their locomotor activity (open-field test), anxiety (open-field and elevated plus maze), spatial memory (Y-maze), sensory gating (pre-pulse inhibition) and fear responses (contextual and cued fear conditioning) to age- and gender-matched wild-type (WT) mice. The WT and KO mice of both sexes were not significantly different in both locomotor activity and memory. However, the open-field and elevated plus maze did reveal that KO females displayed more anxiety-like behaviour compared to male KOs. KO mice spent more time frozen in the context trial of the fear conditioning test;
however, time frozen decreased when the shock-associated cue was played. Female KOs spent less time frozen when the associated cue was played in the fear conditioning test compared to female WTs. Male and female KOs both had decreased average mean startle responses compared to their WT counterparts. This could indicate that Plag1 KO mice have hearing impairment, in addition to increased anxiety-like behaviour in females.

Cong Wang (Queensland Brain Institute)

STATE-SPECIFIC NEURAL ACTIVITY IN THE MEDIAL PREFRONTAL CORTEX AND HIPPOCAMPUS THAT ENCODES FEAR LEARNING AND EXTINCTION BEHAVIOR

Cong W1, Roger M1, Peter S1, Pankaj S1.
1. Synaptic Plasticity Laboratory, Queensland Brain Institute, University of Queensland, Brisbane, Queensland, Australia.

The medial prefrontal cortex (mPFC) and the hippocampus (HPC) have been shown to play an important role in modulation of fear learning and extinction, which are involved in many mental disorder symptoms, such as PTSD. However, the neural activity and connectivity of the subregions of the mPFC and the HPC during fear learning and extinction and how they encoding the corresponding behavior are not widely studied.

We implanted two microdrives with 4 tetrodes in the prelimbic (PL), infralimbic (IL) of mPFC and the ventral, dorsal CA1 of HPC respectively in male SD rats (n=6). Then we recorded single-unit activity and local field potential simultaneously in freely-moving rats during fear learning and extinction. The conditioned rats showed a significant increasing freezing behavior (from 22.0±5.1% to 65.6±8.9%) with a rising theta activity power in the PL and IL. After extinction, the rats showed a descending freezing rate (down into 36.1±8.3%) with an increasing HPC power and a higher level of synchronized oscillation between the IL and HPC. We found that the increasing activity in the PL and IL during fear learning and in the HPC during extinction, suggesting the separate roles of the subregions’ neural activities for encrypting into different behavioral patterns. Our results also showed a significant increasing of the specific correlation (with a synchronized oscillation around 6-7Hz) between IL and HPC during extinction, indicating that this rising functional connectivity between IL and HPC might be one of the crucial keys to inhibit freezing behavior.

Lisa Zhou (University of Otago)

STROKE TO THE PREFRONTAL CORTEX DISRUPTS CHOLINERGIC SIGNALLING AND IMPAIRS ATTENTION.

Zhou LYY1, Barwick DK 1, Gowing EK1, Young S3, Clarkson AN1,2.
1. Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin. 2. Faculty of Pharmacy, The University of Sydney, Sydney, Australia. 3. Department of Pathology, Dunedin School of Medicine, University of Otago

Cholinergic signalling to the prefrontal cortex (PFC) plays a critical role in attention and memory, with damage to either resulting in dementia or impaired stroke recovery. Use of nicotinic alpha-seven agonists, such as PHA568487, improve memory and post-stroke recovery, however, the efficacy of starting the treatment early versus late remains unknown. Therefore, we investigated whether attention was impaired following stroke to the PFC using the five-choice serial reaction time task (5-CSRTT) and whether treatment with PHA568487 ameliorated this impairment. Nine month male C56BL/6J mice were assigned a treatment group (sham-saline, stroke-saline, stroke + PHA568487 early (three-days post-stroke) and stroke + PHA568487 late (three-weeks post-stroke)) based on learning rates in the 5-CSRTT (N=11-16 per group). Focal stroke to the PFC was induced using the photothrombosis method. Animals received daily intraperitoneal injections of 0.8mg/kg PHA568487 in saline. From 3.5 weeks post-stroke, animals were tested on attentional probes by altering the inter-trial interval (ITI) or stimulus duration and data compared using pairwise comparisons. Compared to shams, stroke animals showed impairments in the ITI (correct responses and omissions; P<0.05) and stimulus duration probe (correct responses; P<0.05). Treatment with either early or late PHA568487 showed no significant differences in both ITI and stimulus duration probes compared to stroke controls (P>0.05). Stroke to the PFC results in impaired attention on the 5-CSRTT that was not ameliorated following treatment with PHA568487. By better understanding how stroke influences attentional and other memory deficits, we will be able to investigate potential therapies for clinical use.

Thomas Burton (The University of Sydney)
ABRUPT TRANSITIONS IN BEHAVIOURAL CONTROL DURING DISCRIMINATION LEARNING AND RULE REVERSAL

Burton TJ1,2, Sawatari A1.
1. Discipline of Physiology, School of Medical Sciences, The University of Sydney. 2. The Bosch Institute Animal Behavioural Facility, The University of Sydney.

Processes such as goal-directed learning and flexible decision-making allow us to survive and thrive in dynamic and uncertain situations. Little is known about how these functions operate and integrate with each other in complex scenarios under which behaviour is normally generated and experienced. To investigate how learning and decision-making behaviour manifest in a more naturalistic setting, we have developed detailed and automated assessments of a range of cognitive processes in the home cage of group-housed mice.

Groups of 4 adult male C57BL/6 mice were co-housed in an IntelliCage and accessed water by engaging in an instrumental visual cue discrimination [VD] task. On any given trial, animals were faced with two choices (nosepoke left or right) with the correct choice randomly assigned and signalled by an LED cue. Once an individual reached performance criterion (2 consecutive sessions of >80% correct), the rule was reversed [RR] so that the visual cue now signalled the incorrect choice. Individual cumulative records of performance and behaviour underwent a thorough change point analysis to characterise the acquisition profiles for the VD and RR tasks. Change point analyses revealed that mice consistently exhibit distinct behavioural phases during acquisition and reversal of the discrimination task (p<0.0001). Furthermore, transitions between these phases appear to be abrupt, suggesting rapid changes in decision-making strategies as animals learn and adapt to change. Our novel approach allows for a highly detailed examination of choice behaviour evolution across entire epochs of discrimination learning and adaptation to change in complex and naturalistic scenarios.

Erin Campbell (Florey Institute of Neuroscience and Mental Health)

INDIVIDUAL DIFFERENCES IN REINSTATEMENT OF ALCOHOL-SEEKING BEHAVIOUR FOLLOWING PUNISHMENT-IMPOSED ABSTINENCE

Campbell EJ1, Flanagan J1, Marchant NJ1,2, Lawrence AJ1.
1. The Florey Institute of Neuroscience and Mental Health, Parkville, Victoria 3052, Australia. Florey Department of Neuroscience and Mental Health, The University of Melbourne, Victoria 3010, Australia. 2. Anatomy & Neurosciences Department, VUmc, Amsterdam, The Netherlands

In humans, individual variation in the expression of particular traits contributes to the onset of neuropsychiatric disease states, including drug addiction. One definition of addiction is persistent drug use despite adverse consequences; however, this only occurs in approximately 20% of drug users. Here we model compulsive alcohol use in rats by punishing the drug-reinforced operant response. First, we train rats to self-administer alcohol in one context (A), then punish their alcohol-reinforced lever responses in a different context (B) using contingent foot shock punishment. Finally, we test rats for reinstatement in either context A or B without alcohol or foot shock. Rats are tested one-day post punishment, or after a 30-day abstinence period in either context. We show individual variation in alcohol-seeking behaviour in the punishment-associated context but only after prolonged abstinence to alcohol (p<0.05). Interestingly, we show no difference in latency to first lever press in either the alcohol-associated context or the punishment-associated context (p>0.05). Our results highlight the importance of examining individual variation when assessing reinstatement of alcohol-seeking behaviour. Additionally, it is likely that the response to punishment and the motivation for alcohol are mediated by independent factors. Interestingly, we see this individual variability in alcohol-seeking behaviour in a strain of inbred alcohol preferring iP rats. Future research should focus on the cause of this individual variability and the underlying neural mechanisms involved.

Bret Church (University Of Sydney)

STRUCTURE-BASED DESIGN OF KYNURENINE AMINOTRANSFERASE INHIBITORS: INFORMING AN UNDERSTANDING OF THE KYNURENINE PATHWAY

Church WB1, Nematollahi A1, Sun G1, Jayawickrama GS1.
1. Group in Biomolecular Structure and Informatics, Faculty of Pharmacy, University of Sydney, NSW 2006, Australia
The kynurenine pathway is a fundamental biochemical pathway, which begins with tryptophan and, in the brain, many of the ensuing metabolites are identified as either neuro-protective or neuro-active. We have been specifically interested in kynurenine aminotransferase (KAT) activity in this pathway, which converts kynurenine (KYN) to kynurenic acid (KYNA), and consider enzymes with this activity as potential drug targets to ameliorate neuroregeneration and psychosis. An additional interest in this work is sexual dimorphism, observed in schizophrenia. We have now designed the most potent reversible inhibitors for both human KAT-1 and KAT-2, using structure-based methods, and here report our protein crystallographic studies (PDB IDs 5TF5, 5EUN), and our understanding of the inhibitory activity of the designed compounds specifically working on KAT-2. A fragment-library design approach has also informed this work which started with a diverse 1,000 compound set which was screened using surface plasmon resonance, against immobilised KAT-2. Our current lead compounds have IC50s approximately 50 mM. We also report our specific results for the activity of the estrogens with the overall status of our current inhibitors. Our experiments continue to inform our design, with the potential for discovering further novel inhibitors.

Xin Du (Monash University)

A ROLE FOR BDNF IN MEDIATING ADOLESCENT GABAERIC INTERNEURON EXPRESSION

Du X1, Serena, K2, Wu YWC2, Hwang W2, Schroeder A2, Grech AM1, Hill RA*1
1Behavioural Neuroscience Laboratory, Department of Psychiatry, Monash University, Clayton, Victoria, 3168, Australia
2Psychoneuroendocrinology Laboratory, Florey Institute for Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, 3010, Australia

GABAergic interneuron dysfunction underlie cognitive deficits in schizophrenia. Brain-derived neurotrophic factor (BDNF) plays critical roles in the early development of cortical GABAergic interneurons. Recently our laboratory and others have shown changing trajectories of development in specific subpopulations of GABAergic interneurons extending into adolescence. BDNF expression also changes significantly across adolescent development as levels of sex steroid hormones rise rapidly. However the role of BDNF in regulating GABAergic changes during adolescence remains unclear. Here, we performed a week-by-week Western analysis of the protein expression of three major GABAergic interneurons, parvalbumin (PV), somatostatin (SST) and calretinin (Cal), in the medial prefrontal cortex (mPFC) from prepubescence (PND21) to adulthood (PND 84). In order to assess the roles of BDNF and sex we compared WT as well as BDNF heterozygous (het) male and female mice. We also examined cell density via immunohistochemistry at weeks 4, 6 and 12. Compared to wild-types, PV protein expression and cell density were reduced in male BDNF heterozygous mice but only cell density was differentially altered in female BDNF heterozygotes according to subregions of the mPFC. SST protein upregulation was delayed in female but not male BDNF heterozygotes. Cal expression did not differ from WT in either sex, despite reduced cell density especially in males. Our results showcase the sexually-dimorphic influence of BDNF on GABAergic interneuron maturation across adolescence. These data may explain the sex differences apparent in the age of onset, symptomatology and response to treatment evident in schizophrenia.

Asheeta Prasad (UNSW Sydney)

VENTRAL PALLIDUM OUTPUT PATHWAYS IN CONTEXT-INDUCED REINSTATEMENT OF ALCOHOL SEEKING.

Asheeta A. Prasad1 and Gavan P. McNally1
School of Psychology, UNSW Australia

Ventral pallidum is a well-established locus for the reinforcing effects of drugs of abuse and reinstatement of drug seeking. However, VP neurons are at the origin of multiple output pathways, with strong projections to ventral tegmental area (VTA), subthalamic nucleus (STN) and the lateral hypothalamus (LH), among others, and the roles of these VP output pathways in reinstatement of drug seeking remain poorly understood. Here we addressed these issues using a combination of neuroanatomical tracing, chemogenetic and optogenetic approaches. First, using dual retrograde tracing, we show that VP neurons projecting to the LH are recruited during context-induced reinstatement of extinguished alcohol seeking in rats. To determine the causal roles of VP-LH pathway in context-induced reinstatement we used complementary approaches of chemogenetic

**BRAIN-DERIVED NEUROTROPHIC FACTOR REVERSES DOPAMINE-MEDIATED DEFICITS IN PREPULSE INHIBITION IN EARLY LIFE STRESS MODELS OF SCHIZOPHRENIA**

Jaehne EJ\(^1\), Chong E\(^2\), van den Buuse M\(^1\)

1. School of Psychology and Public Health, La Trobe University, Melbourne, Australia

Brain-Derived Neurotrophic Factor (BDNF) expression is reduced in brain tissue from patients with schizophrenia; BDNF may therefore be a potential therapeutic target. We used two developmental animal models of schizophrenia, maternal immune stimulation and social isolation rearing, to investigate the role of BDNF in the regulation of prepulse inhibition (PPI), a model of sensorimotor gating reduced in schizophrenia. Pregnant Long-Evans rats were treated with the viral mimetic, poly I:C, and male offspring were compared in adulthood to control offspring. PPI testing included the BDNF receptor agonist, 7,8-dihydroxyflavone (7,8-DHF, 10 mg/kg), as well as the dopamine receptor agonist, apomorphine (APO, 1 mg/kg), or the dopamine releaser, methamphetamine (METH, 2 mg/kg) to induce a schizophrenia-like PPI disruption. A second cohort of rats were reared in social isolation from adulthood and compared to group-housed controls.

Acute administration of APO caused a significant reduction of PPI which was not significantly altered in poly I:C rats. However, in poly I:C offspring only, 7,8-DHF significantly reversed the effect of APO on PPI (Control baseline 54 ± 2%, APO 20 ± 4%, APO+DHF 16 ± 2%; poly I:C baseline 54 ± 3%, APO 16 ± 3%, APO+DHF 35 ± 3%; p < 0.05). A similar trend was observed after treatment with METH as well as in social isolation rats. These findings suggest that 7,8-DHF has the ability to reverse dopamine-mediated deficits in PPI in early life stress models of schizophrenia. This highlights the therapeutic potential of targeting BDNF signalling for the treatment of schizophrenia.

Jesse Bourke (University of Newcastle)

**SIMPLE SPEECH ASYMMETRIES? NOT EVEN: LEFTWARD LATERALISATION IN PSYCHOLINGUISTICS, PSYCHOACoustICS, AND NEUROANATOMY**

Bourke JD\(^1\), Todd J\(^1\), Schall U\(^1\), Cooper G\(^1\), Michie P\(^1\), Forstmann BU\(^2\), Rasser P\(^1\)

1. University of Newcastle, Australia. 2. University of Amsterdam

Leftward neuroanatomical asymmetry of the planum temporale (PT), a triangular area of secondary auditory cortex (part of the classically defined Wernicke’s area), has been implicated as an important substrate of the leftward functional asymmetry of speech. Mediating factors driving the leftward speech bias remain unclear, with two primary perspectives argued: **acoustical sound-properties** (rapid temporal cues) versus **linguistic language-representations** (consonant vowel syllables etc.) of speech. Our current research project aims to disentwined acoustical and linguistic factors of speech and their relationship to PT asymmetry. In a sample of 63 healthy participants (aged 18-46), we measured PT asymmetry using MRI (surface-based-analysis; Freesurfer), psychophysical sensitivity to rapid temporal cues using gap detection threshold (GDT) tasks, and language lateralisation using dichotic listening tasks (DLT). Our results closely replicated previous findings of PT size and asymmetry, GDT abilities, and DLT performance. Comparing the measures, we found significant positive correlations of strength of leftward PT laterality (surface area and volume, but not thickness) with psychophysical sensitivity to acoustical cues as well as strength of leftward language lateralisation. No meaningful relationships emerged between the acoustical and linguistic measures. Therefore, despite neuroanatomical leftward asymmetry of the PT being argued to be related to acoustical processing or linguistic processing, it is apparently related to both, yet independently so. Thus, whilst functional and neuroanatomical asymmetries are apparently robust, they are not so simple, and a consolidating approach of acoustical and linguistic explanations of speech asymmetries is supported.

Danielle Burgess (The University of Queensland)

**PERICONCEPTIONAL ALCOHOL ALTERS THE SOCIAL AND DEPRESSIVE-LIKE PHENOTYPES IN RAT OFFSPRING.**
Burgess DJ\textsuperscript{1}, Cuffe JSM\textsuperscript{2} and Moritz KM\textsuperscript{1,3}

1. School of Biomedical Sciences, The University of Queensland, Brisbane, QLD, 4072. 2. School of Medical Sciences, Griffith University, Australia, 4222. 3. Child Health Research Centre, The University of Queensland, Brisbane, 4072.

Long term moderate and high dose ethanol (EtOH) consumption during pregnancy results in altered offspring hypothalamus-pituitary-adrenal axis (HPA) activity and neuro-behavioural outcomes in human and animal studies. Up to 50% of woman admit to drinking prior to pregnancy recognition, resulting in early embryonic exposure. This study aims to investigate periconceptional ethanol (PC:EtOH) exposure on neuro-behavioural outcomes in rat offspring. Female Sprague-Dawley rats were given ad libitum access to a liquid diet containing 12.5% v/v EtOH from 4 days before conception (E-4), until embryonic day 4 (E4) (PC:EtOH) or a matched control diet. Dams littered down naturally and at 3 months of age, male and female offspring underwent forced swim and social interaction testing. PC:EtOH exposure resulted in a significant increase in immobility (p<0.05) in both male and female offspring in the forced swim test. Regardless of treatment, sex did not influence the amount or rate of immobility within this test. PC:EtOH also significantly increased affiliative behaviour (p<0.05) within the social interaction, however only within female offspring. There was no impact of PC:EtOH on non-affiliative behaviour in either male or female offspring. This study demonstrates that PC:EtOH has significant effects on neuro-behavioural outcomes. The increased immobility in the swim test in both males and females is indicative of a depressive-like phenotype. The increased affiliative behaviour in female offspring may suggest less inhibitive behaviour. As mental illness is sexually dimorphic within the human population, these results support the necessity of further neurological investigation into the impacts of PC:EtOH exposure.

Dylan Fox (Monash University)

THE MARMOSET AS A MODEL FOR UNDERSTANDING COMPLEX VISUAL BEHAVIOUR

Fox DM\textsuperscript{1}, Mundiniano IC\textsuperscript{1}, Bourne JA\textsuperscript{1}

9. Australian Regenerative Medicine Institute, Monash University, Melbourne

Every day we perform a large range of skilled hand movements without much thought. Phylogenetic, developmental, and behavioural evidence suggest that the reach and grasp evolved separately under somatosensory control and was later refined with visual adaptations in the primate lineage. The marmoset is positioned such that it displays adept visuomotor control of hand movements but descending spinal pathways do not enable independent digit movements resulting in grips that are less precise than those observed in Old World monkeys such as the macaque, apes and humans. The marmoset model offers a unique composition of basic circuitry and skilled hand use to explore the evolution of visually-guided actions. In this study, four adult marmosets (>18 Months) were trained to perform a series of visually-guided tasks designed to assess their control over accurately locating and retrieving static and moving objects in their environment. The kinematics of their reaching and grasping behaviours were recorded for offline analysis. Predictive modelling revealed that kinematic variables such as the grip aperture and digit velocity could reliably predict the accuracy of marmoset reaching to grasp actions. It also showed that marmosets were capable of pre-shaping their hand according the size of the target object. Despite the lack of independent digit control, the marmoset displays a high complexity in their visually-guided actions performing anticipatory rate hand configurations mid-flight to the dimensions of a target object. This further supports the notion that the evolution of refined vision enabled the expansion of skilled visuomotor behaviours.

Adrienne Grech (Monash University)

SEX AND GABAERGIC SUBTYPE-SPECIFIC CHANGES IN HIPPOCAMPAL PROTEIN EXPRESSION IN A TWO-HIT DEVELOPMENTAL MODEL

Grech A\textsuperscript{1}, Ratnayake U\textsuperscript{2}, Hill RA\textsuperscript{1} and Van den Buuse, M\textsuperscript{3}.

1. Department of Psychiatry, School of Clinical Sciences at Monash Health, Monash University 2. The Florey Institute of Neuroscience and Mental Health 3. School of Psychology and Public Health, La Trobe University

The two-hit hypothesis suggests that a combination of genetic and environmental insults during critical
1.2 million people involved in their care. Current research into therapies and treatments have been hampered.

The University of Melbourne, Australia.

1. Marshall J

CHARACTERISATION OF AN ALZHEIMER’S DISEASE MOUSE MODEL EXPRESSING AMYLOID IN THE PRESENCE OF TAU: AN EXTENDED REPLICATION STUDY

Jessica Marshall (The Florey Institute of Neuroscience and Mental Health, Melbourne, Australia.)

Alzheimer’s disease (AD) in Australia is the second leading cause of death; affecting over 300,000 patients with 1.2 million people involved in their care. Current research into therapies and treatments have been hampered.


Baker Heart and Diabetes Institute, Melbourne, Australia. 2. School of Medicine, Dentistry and Health Sciences, The University of Melbourne, Australia. 3. The Florey Institute of Neuroscience and Mental Health, Melbourne, Australia. 4. The Garvin Institute of Medical Research, Sydney, Australia.
by the availability of an appropriate and physiologically accurate mouse model of AD. We have performed a comprehensive battery of behavioural and physiological tests on an AD mouse model developed by Heraud et al in 2014. We crossed 5xFAD (overexpressing human APP and Presenilin-1) and Tg30 (overexpressing tau) mice so that we could investigate the effects of tau accumulation in the presence of amyloid pathology, which closely resembles human AD. In agreement with Heraud and colleagues we observed a decline in Rotarod performance at 6 months of age compared to wildtype. This corresponded with a 40% decrease in survival rates by 10 months. While we noted a decrease in Morris Water Maze performance at 8 months, Y-maze, large open field and novel object recognition tests were not yet significantly affected at this time point. Additionally, body weight was lower in the 5xFAD*Tg30 animals, with a significant difference in tibialis anterior skeletal muscle weight and tibia length. Our results successfully reproduced many aspects of the 2014 Heraud paper, while adding further additional characterisation of the model. This initial characterisation study suggests that the model contains many aspects associated with frailty as well as the potential initiation of cognitive decline. Further characterisation is warranted to study the cognitive function in these mice with alternative tests and time points.

Jay Nakamura (Monash University)

TARGETTED MATERNAL IMMUNE ACTIVATION AND TRIAL-UNIQUE, NON-MATCHING TO LOCATION TASK PERFORMANCE

Nakamura JP1, Du X1, van den Buuse M2, Hill RA1
1Department of Psychiatry, Monash University, Melbourne, Australia
2School of Psychology and Public Health, La Trobe University, Melbourne, Australia

Maternal immune activation (MIA) is a well-established risk factor for schizophrenia, however, the mechanism underlying its action is unknown. The prevailing neurodevelopmental model of schizophrenia suggests the etiology of the disease and events leading to the behavioural changes seen later in life occur during early/prenatal brain development. Of interest are the fast spiking parvalbumin positive sub-population of inhibitory interneurons. The function of these interneurons has previously been shown to be disrupted in patients with schizophrenia and experiments in animal models have demonstrated the importance of these specific interneurons in the generation of high frequency oscillations in the brain to facilitate healthy cognitive performance. We induced MIA in mouse dams using the viral mimetic, poly-I:C, during an important gestational window of parvalbumin positive interneuron development (GD 13, 14, 15) and investigated the behavioural phenotype of the resulting offspring. Mice were tested using the Trial Unique, Non-Matching to Location (TUNL) touchscreen task, Y-maze, elevated plus maze, and pre-pulse inhibition (PPI) acoustic startle chambers. Our targeted protocol of 3 consecutive injections of poly-I:C led to task-specific alterations in TUNL and PPI performance. This behavioural data along with future tissue analysis aims to uncover the time sensitive mechanism by which MIA disrupts neurodevelopment. Understanding how and when inflammation alters the development of critical neuronal populations may guide future targets for preventative intervention.

Maria Roitman (The University of Melbourne)

INVESTIGATING A GENE NETWORK OF FEAR RESPONSE IN A MOUSE MODEL OF POST-TRAUMATIC STRESS DISORDER

Roitman M1, Brodnicki TC2, Mackin L2, Murphy M1, Wilson YM1, Gunnersen JM1.
1. Neuron Development and Plasticity Laboratory, Anatomy and Neuroscience Department, University of Melbourne, Parkville, VIC, Australia. 2. Immunogenetics Research Group, St Vincent’s Institute of Medical Research, Fitzroy, VIC, Australia.

Post-traumatic stress disorder (PTSD) is a debilitating condition which develops in response to a stressor. PTSD is characterised by enhanced memory of the trauma (fear acquisition) and impaired ability to forget the trauma (extinction). Our previous data showed that mice of the congenic inbred strain, Str1, exhibit higher innate stress-responsiveness, enhanced context fear acquisition and a context fear extinction deficit compared to their low stress-responsive parental strain C57B/6J (B6). To extend this observation, B6 and Str1 mice (N=9-10/group/gender) underwent auditory fear conditioning and extinction. Genetic mapping of the Str1 locus, inherited from the highly stress-responsive parental strain DBA/2J (D2), implicates the E2F-.
associated phosphoprotein (EAPP) gene in elevated stress-responsiveness. Thus, after undergoing either an acute stress paradigm or behavioural testing, mRNA was extracted and droplet digital PCR (ddPCR) was used to measure stress-induced changes in gene expression levels of EAPP in the brains of D2 and B6 mice. Compared to female B6 mice, female Str1 mice exhibited enhanced fear acquisition, impaired auditory fear extinction, enhanced fear renewal after auditory extinction, and a delayed onset of context fear extinction after auditory extinction. No behavioural difference between Str1 and B6 males was observed. EAPP mRNA was more highly expressed after acute stress in B6 females and males than in D2 mice of either gender. Increased freezing in Str1 females compared to controls indicates that the Str1 locus is involved in fear memory. Changes in EAPP expression after acute stress suggest that this gene is an important mediator of differential stress-responsiveness.

Diana Sketriene (University Of Melbourne)

N-ACETYL-CYSTEINE REDUCES COMPULSIVE-LIKE BEHAVIOUR IN DIET-INDUCED OBESE RATS

Sketriene D, Battista DC, Lawrence AJ, Brown RM
The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria, Australia.

Pathological overeating displayed by obese individuals shares similarities with compulsive drug taking behaviour observed in drug-addicted subjects. This raises the possibility that drug addiction treatments may show utility in the treatment of compulsive overeating. N-Acetylcysteine (NAC) is a cysteine pro-drug which has experienced some success in clinical trials to reduce the use of cocaine, marijuana and cigarettes, as well compulsive behaviours such as gambling and trichotillomania. We assessed the impact of NAC on addiction-like behaviour towards highly palatable food in a rat model of diet-induced obesity. Diet-induced obesity-prone (OP) and resistant (OR) rats were subjected to an operant conditioning paradigm where they were given the opportunity to lever press for high-fat high-sugar food pellets (S+). This alternated with periods of signalled reward unavailability (S-). Responding during S- periods is reflective of compulsive-like food-seeking behaviour; OP rats had greater difficulty regulating reward-seeking behaviour during S- periods compared to OR rats. This persistent S- responding in OP rats was ameliorated by daily injections of NAC (100mg/kg, i.p.) administered 2h prior to the operant session for 10 days. By the end of the treatment period, there was no significant difference in S- responding between NAC treated OP rats and OR rats whereas vehicle treated OP rats remained significantly higher (p<0.05). These findings show that NAC can modify aspects of food-seeking behaviour in OP rats and supports the potential use of this compound in compulsive overeating and related forms of obesity.

Julia van der Hoven (UNSW Sydney Dementia Research Unit)

THE IMPACT OF B CELL DEPLETION ON THE PHENOTYPE OF TAU-FTLD MICE.

van der Hoven JJ1, Suh LS1, van Hummel AE1,2, Przybyla M1, Ittner AA1 & Ittner LM1,2,3
1. Dementia Research Unit, School of Medical Science, UNSW Sydney, Sydney, Australia.
2. Motor Neuron Disease Unit, School of Medical Science, UNSW Sydney, Sydney, Australia.
3. Transgenic Animal Unit, Mark Wainwright Analytical Centre, UNSW Sydney, Sydney, Australia.

In recent years, the involvement of the immune system, including antibodies, and attributable neuroinflammation in neurodegenerative disorders have been under rigorous investigation. In order to explore the role of antibodies in frontotemporal lobar dementia and Alzheimer’s disease, we have carried out behavioral testing experiments in the novel TAU58/2 transgenic mouse model, which mimics the histopathological and behavioural features of AD and FTLD. TAU58/2 mouse line was crossbred with a B6.129S2-Lghm<sup>tm1Cgn</sup>/J mouse line (muMt<sup>−</sup> mice) to produce homozygous mice lacking mature B cells and no expression of membrane bound IgM , another cohort of TAU58/2 mice were treated with a B cell-depleting antibody from weaning (BD), eliminating B cells from the pre-B cell developmental stage onwards. Behavioural experiments were performed to assess the defined behavioural deficits of the TAU58/2 mouse line when crossed with the muMt<sup>−</sup> mouse line or treated with the B cell depleting antibody. Elevated plus maze for disinhibition behaviorRotarod testing for motor phenotype 4 TAU58/2 x MuMt<sup>−</sup> BD mice Morris Watermaze 8 TAU58/2 x MuMt<sup>−</sup> BD mice muMt<sup>−</sup> or.

B cell depletion not have a significant impact on the behavioural phenotype of the TAU58/2 mouse.
Lauren Whyte (SAHMRI)

REDUCTION IN OPEN FIELD ACTIVITY IN APP NL-G-F MICE IS NOT MODIFIED BY HETEROZYGOUS DELETION OF HEXB

Whyte LS\textsuperscript{1,2}, Lau AA\textsuperscript{3}, Hemsley KM\textsuperscript{2}, Hopwood JJ\textsuperscript{4} and Sargeant TJ\textsuperscript{2}.
1. The University of Adelaide, School of Medicine, North Terrace, Adelaide, Australia. 2. Lysosomal Diseases Research Unit, Nutrition and Metabolism Theme, South Australian Health and Medical Research Institute, North Terrace, Adelaide, Australia.

The recent development of knock-in mouse models of Alzheimer’s disease provides distinct advantages over traditional transgenic models that rely on over-expression of amyloid precursor protein. This study aimed to further characterise the behavioural phenotype of App\textsuperscript{NL-G-F} knock-in mice (C57BL/6J) at six-months of age. Given the dysregulation of gangliosides, including GM2, observed in Alzheimer’s disease, and that modulation of Hexb alters behaviour in another Alzheimer’s mouse model, we then investigated whether heterozygous deletion of Hexb was sufficient to exacerbate the behavioural phenotype in App\textsuperscript{NL-G-F} mice. Hexb\textsuperscript{+/-} (C57BL/6J; 129S) mice were crossed with App\textsuperscript{NL-G-F} mice. We assessed memory using y-maze, novel object recognition and Morris water maze tests, and measured locomotor/exploratory activity in an open field. Despite no memory deficit in App\textsuperscript{NL-G-F} (C57BL/6J) mice at six-months, we report a significant reduction in open field activity. In the mixed C57BL/6J; 129S strain, there were no memory deficits detected, and the activity phenotype took longer to develop, presenting in App\textsuperscript{NL-G-F} mice at 9 months of age, but not at 6 months. Significant hypoactivity was also seen in App\textsuperscript{NL-G-F}; Hexb\textsuperscript{+/-} mice at 9 months, but the Hexb\textsuperscript{+/-} allele did not exacerbate the phenotype. Whilst heterozygous deletion in Hexb alone was not sufficient to significantly alter activity from wildtype, an interesting pattern emerged, whereby Hexb\textsuperscript{+/-} mice demonstrated an intermediate activity between wildtype and App\textsuperscript{NL-G-F} mice. In conclusion, we report a novel phenotype in App\textsuperscript{NL-G-F} mice of reduced locomotor/exploratory activity in an open field, which is not significantly impacted by heterozygous deletion in Hexb.

Alexandra Suchowerska (Unsw Sydney)

DEVELOPMENTAL PROFILING OF THE ACTIN ASSOCIATED PROTEIN TROPOMYOSIN HIGHLIGHTS CHANGES IN LOCALISATION OF DIFFERENT ISOFORMS AT CENTRAL NERVOUS SYSTEM SYNPASES WITH AGE.

Suchowerska AK1, Gunning PW2, Hardeman EC2 and T. Fath1
1 Neurodegenerative and Repair Unit, School of Medical Sciences, University of New South Wales, Sydney, Australia.
2Cellular and Genetic Medicine Unit, School of Medical Sciences, University of New South Wales, Sydney, Australia.

The actin cytoskeleton in the post-synaptic compartment of synapses is crucial to supporting synaptic maturation, structure and function. The family of tropomyosins are one of many actin-associated proteins known to regulate the postsynaptic actin cytoskeleton and are considered as gate-keepers of actin filament dynamics. We have previously shown the spatial segregation to the pre- and post- synapse of different tropomyosin isoforms at central nervous system synapses, indicative of specific regulation of actin filament populations in different subcellular compartments. Here, we biochemically confirm previous reports of a segregation of Tpm1.12 to the pre-synaptic compartment and Tpm4.2 to the post-synaptic compartment. We also show that in C57Bl6 wild-type mice, there is a developmental shift in the expression of tropomyosin isoforms: as the total pool of Tpm4.2 in the brain decreases with age, there is an increase in localisation of Tpm4.2 to the post synaptic compartment in aged mice. Together these data demonstrate the instrumental role of Tpm4.2 in regulating the post synaptic actin cytoskeleton, particularly in aged mice.
Clinical Disorders and Injury of the Nervous System

Vladimir Balcar (The University of Sydney)

CD36 GENE POLYMORPHISM IS ASSOCIATED WITH ALZHEIMER’S DISEASE

Šerý O1,2, Janoutová J3, Ewerlingová L1,2, Hálová A1,2, Lochman J1, Janout V3, Khan NA3, Balcar VJ2,5
1. Laboratory of Neurobiology and Molecular Psychiatry, Department of Biochemistry, Faculty of Science, Masaryk University, Brno. 2. Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Brno, Czech Republic. 3. Department of Epidemiology and Public Health, Faculty of Medicine, University of Ostrava, Czech Republic. 4. Physiologie de la Nutrition et Toxicologie, UMR U866 INSERM, Université de Bourgogne/Agro-Sup, Dijon, France. 5. Bosch Institute and Discipline of Anatomy and Histology, School of Medical Sciences, Sydney Medical School, The University of Sydney NSW 2006, Australia

In a case control genetic association study of several single nucleotide polymorphisms in 859 patients with Alzheimer’s disease (AD) and controls, we have identified the A allele of rs3211893 polymorphism of CD36 gene as significantly increasing the risk of AD. CD36 gene encodes a membrane glycoprotein (type B scavenger receptor) present in many types of cells and having multiple cellular functions ranging from angiogenesis to transport and gustatory perception of fatty acids. Additionally, we have investigated, using the same sample of control subjects and AD patients, polymorphisms in ApoE gene and confirmed that the previously identified AD-associated ApoE variants indeed increased the risk of AD as reported in earlier studies. Based on the current knowledge of CD36 biochemistry we propose that the AD risk-imparting variants of CD36 alter cholesterol homeostasis, exacerbate oxidative stress and/or induce pathological inflammatory cascades. The SNP rs3211892 has previously been associated with cardiovascular diseases and other conditions but the present study is the first evidence of its significant association with the risk of Alzheimer’s disease1. 1. Šerý et al. Biochimie 135 46-53 (2017)
This work has been supported by Agency for Healthcare Research, Czech Republic (AZV ČR) - grant project No. 16-31207A.

Alessandro Castorina (University of Technology Sydney)

EFFECTS OF NEUROPATHIC INJURY ON THE EXPRESSION PROFILE OF PACAP FAMILY MEMBERS IN THE RAT BRAIN

Alessandro Castorina, James WM Kang, Monica Vogiatzis, Eszter Kalman, Kevin A. Keay
School of Medical Sciences, The University of Sydney, NSW, Australia, 2006

There is growing evidence that neuropathic injury affects emotional coping behaviors in a vulnerable subset of patients. These patients develop traits described as behavioral ‘disability’, including altered social behavior and sleep. The neuropeptide PACAP, its homolog VIP, and their associated receptors (PAC1, VPAC1 & VPAC2), comprise an endogenous system implicated in emotional coping responses. Whether the PACAP system is altered in the brain following neuropathic injury remains unexplored. To evaluate this possibility, we profiled PACAP system expression levels in the brains of nerve-injured rats with and without disability. Altered social behaviour following chronic constriction injury of the sciatic nerve (CCI) was used to determine disabled and non-disabled male, Sprague-Dawley rats. Their brains were removed and the hypothalamus, dorsal and ventral hippocampus and periaqueductal grey region were isolated and the expression of PACAP/VIP peptides, PAC1, VPAC1 and VPAC2 receptors was measured using qPCR and Western blots. CCI, evoked regionally-specific changes in the PACAP system. CCI up-regulated the PACAP family in the hypothalamus, however in the periaqueductal grey up-regulation was seen primarily in disabled rats. To contrast, down-regulation of the PACAP family was identified in the dorsal but not in the ventral hippocampus. Our gene and protein data suggest the occurrence of post-transcriptional events. Neuropathic injury alters the expression of PACAP family components in regions of the rat brain pivotal in the regulation of emotional coping behaviours. In the periaqueductal grey this regulation may underpin the expression of disability.

Paul Dawson (Mater Research Institute, The University of Queensland)

NON-SYNDROMIC INTELLECTUAL DISABILITY AND ASSOCIATED GENE NETWORKS

Soohyun Lee1, Stephen Rudd2, Jacob Gratten2, Peter M Visscher3, Johannes B. Prins1 and Paul A. Dawson1
1. Mater Research Institute, The University of Queensland, Woolloongabba, Queensland 4102, Australia. 2. QFAB Bioinformatics, Queensland Bioscience Precinct, The University of Queensland, Brisbane, Queensland 4072, Australia. 3.
Non-syndromic intellectual disability (NS-ID) is a genetically heterogeneous disorder, with more than 200 candidate genes identified to date. Despite the increasing number of novel mutations detected, less than 20 genes (≤ 10%) are clinically screened to determine the genetic contribution of intellectual disability. Furthermore, there is no consistency in terms of the number of reportable genes across medical institutions, leaving families without appropriate genetic counselling. The aetiology of NS-ID remains unknown and is recognised to be under-researched. Through a systematic search of PubMed and Medline, we curated 245 NS-ID candidate genes harbouring non-synonymous variants, insertions or deletions from case reports or from linkage or pedigree analyses were identified. Gene networks and protein-protein interactions were analysed using GeneGO MetaCore™ and DAPPLE databases, respectively. From the list of 245 NS-ID candidate genes, we identified common pathways of axon guidance, synaptogenesis, cell adhesion and neurotransmission, all of which are key neurodevelopmental processes for the establishment of mature neuronal circuitry in the brain. These genes are evolutionarily constrained, consistent with expectations for a disorder such as NS-ID that is associated with reduced fecundity. In addition, we report enrichment of dopaminergic and glutamatergic pathways for those candidate NS-ID genes which are common to syndromic intellectual disability (S-ID) and/or disorders that exhibit intellectual disability. This study suggests modulation of neurotransmission, particularly dopaminergic and glutamatergic systems as key contributors to synaptic dysfunction in NS-ID. Collectively, the candidate genes and molecular pathways reported in this study provide reference information for future genetic studies of NS-ID.

Emily Don (Macquarie University)

ZEBRAFISH EXPRESSING MUTANT HUMAN FUS REPRODUCE HISTOPATHOLOGICAL FEATURES OF ALS

1. Macquarie University Centre for Motor Neuron Disease Research, Department of Biomedical Sciences, Faculty of Medicine & Health Sciences, Macquarie University, North Ryde, NSW 2109, Australia.
2. Northcott Neuroscience Laboratory, ANZAC Research Institute; Molecular Medicine Laboratory, Concord Hospital; Sydney Medical School, University of Sydney, NSW 2139, Sydney, Australia.
*These authors contributed equally

Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease that affects the both the upper and lower motor neurons of the nervous system. Currently there are limited treatment options for ALS and the average lifespan post diagnosis is 3-5 years. Whilst the cause of ALS is poorly understood, it is known that approximately 10% cases result from heritable gene mutations. Mutations have been described in over 20 different genes, with mutations in C9orf72, TARDBP, SOD1 and FUS accounting for the majority of cases. Our aim is to create animal models of ALS based on these gene mutations with the long-term goal of therapeutic testing. Zebrafish are an established model organism that have been used in multiple studies to investigate ALS. Therefore, we generated stable transgenic zebrafish lines expressing either wild-type or mutant human FUS. The transgenic zebrafish were then examined for histopathological features and signs of neurodegeneration using confocal microscopy, axon measurements, neuromuscular junction staining and tracking swimming behaviour. The zebrafish expressing mutant human FUS recapitulated distinctive histopathological features of ALS pathology, including significant mislocalisation of FUS to the cytoplasm and the formation of stress granules. However, interestingly, they did not develop an overt behavioural phenotype or any neuronal defects. The human mutant FUS expressing transgenic zebrafish could be a valuable tool for studying the effects of FUS proteinopathies and testing therapeutics targeted at correcting these.

Mitsuaki Hirano (Department of Psychiatry, Graduate school of medicine, Nagoya University)

CLINICOPATHOLOGICAL DIFFERENCES BETWEEN MOTOR ONSET AND PSYCHIATRIC ONSET OF HUNTINGTON DISEASE.

Hirano M1, Iritani S1, Fujishiro H1, Torii Y1, Habuchi C1, Sekiguchi H2, Yoshida M2, Ozaki N1
1. Department of Psychiatry, Graduate school of medicine, Nagoya University, AICHI, JAPAN. 2. Department of Neuropathology, Institute for Medical Science of Aging, Aichi Medical University, AICHI, JAPAN.

Huntington disease (HD) is an autosomal dominantly inherited neurodegenerative disorder characterized by presence of chorea, behavioural and psychiatric symptoms, and dementia. HD is caused by a pathologic cytosine-adenine-guanine (CAG) repeat expansion, on the 5' end of the Huntingtin gene. Though the most of patients would present with motor
onset as initial symptoms, not a few patients would present with psychiatric onset and they visit psychiatrists at first medication. But the biological studies comparing patients between with psychiatric symptoms at the onset of HD and with those having motor symptoms are sparse and the clinicopathological correlation remains to be elucidated. We did chart review of 28 autopsied HD cases that detected by neuropathological estimation and/or by CAG repeat length [psychiatric onset group (including cognitive symptoms) =10, motor onset group=10, and mixed onset group=8]. Each of mean age of onset is 26.4, 48.1, and 47.8. Each of mean age of death is 43.8, 64.8, 57. Psychiatric onset group indicate younger onset (mean26.4±10.8) than the other groups significantly and tendency that longer CAG repeat length (mean 52.8±10.3) but without significantly. In neuropathological observation, the number of small size neuron in Nucleus accumbens was smaller in HD than controls significantly, but there are no significant differences among those groups. We should pursuit the biological pathogenesis of clinical symptoms in HD for more accurate diagnosis and treatment in early stage.

Shuji Iritani (Dep. Of Psychiatry, Nagoya University)

ESTABLISHMENT OF THE PSYCHIATRIC BRAIN BANK IN CENTRAL JAPAN

Iritani S, Torii Y, Habuchi C, Hirano M, Fujishiro H, Sekiguchi H. Department of Psychiatry, Graduate school of medicine, Nagoya University, AICHI, JAPAN

In 2016, Japan Agency for Medical Research and Development （AMED） have budgeted for the establishment of Japan brain bank network as the national project. This project is aiming to provide brain tissue officially for brain researchers. Former in Japan, there has not been systematic brain bank ever, and brain resources has been significantly short on the investigating the pathogenesis of neuropsychiatric disease. There are some reasons on this situation including Japanese culture for dead body, the legal constrained environment and the much lowering the autopsy rate in the Japanese hospital. Under this project, we have been collecting the brain tissue in three psychiatric hospitals since 2009, preserved over 70 cases as brain tissue ever, and started to deliver for some brain research institutions. The resource characteristics of this brain banking are indicated mainly as 1) neurological disease cases with significant psychiatric symptoms, 2) dementia disease with BPSD cases, 3) psychiatric disease with the extensive laboratory and clinical information. because the all the autopsy cases were passed away in each psychiatric hospitals. One of our neuropathological investigation using this brain resources indicated that cases with presenting psychiatric symptoms firstly after over 50 years old would tend to indicate neurodegeneration findings more frequently including tauopathy or TDP-43 proteinopathy than those cases under 50 years old. Anyway, we Japanese psychiatrist is now emulating the western, American or Australian brain bank networks to establish systematic Japanese psychiatric Brain Bank.

Hyung-seok Kim (Department Of Forensic Medicine, Chonnam National University Medical School)

HISTOPATHOLOGICAL REVIEW OF PHOTOTHROMOSIS-INDUCED INTERNAL CAPSULE INFARCT

Ra-Young Park1, Ji-Hoon Jo2, Man-Seok Park1, Hyung-Seok Kim4 Department of 1Radiology, 2Biomedical Science, 3Neurology, and 4Forensic Medicine. Chonnam National University Medical School, Gwangju, Korea

AIMS: Stroke involving the cerebral white matter (WM) has increased in prevalence, but most experimental studies have focused on ischemic injury of the gray matter. This study was performed to investigate the WM in a unique rat model of photothrombotic infarct targeting the posterior limb of internal capsule (PLIC), focusing on the identification of the fundamental histopathologic feature causing different neurologic outcomes.

METHODS: Light microscopy with immunohistochemical stains and electron microscopic examinations of the lesion were performed between 3 hours and 21 days post-ischemic injury.

RESULTS: Initial pathological change develops in myelinated axon, concomitantly with reactive change of astrocytes. The first pathology to present is nodular loosening to separate the myelin sheath with axonal wrinkling. Subsequent pathologies include rupture of the myelin sheath with extrusion of axonal organelles, progressive necrosis, oligodendrocyte degeneration and death, and reactive gliosis. Increase of glial fibrillary acidic protein (GFAP) immunoreactivity is an early event in the ischemic lesion. WM pathologies result in motor dysfunction. Motor function recovery after the infarct was correlated to the extent of PLIC injury proper rather than the infarct volume.

CONCLUSIONS: Pathologic changes indicate that the cerebral WM, independent of cortical neurons, is highly vulnerable to the effects of focal ischemia, among which myelin sheath is first damaged. Early increase of GFAP immunoreactivity indicates that astrocyte response initially begins with myelinated axonal injury, and supports the biologic role related to WM injury or plasticity. The reaction of astrocytes in the experimental model might be important for the study of...
Australasian Neuroscience Society Annual Scientific Meeting 2017
International Convention Centre, Sydney, December 3rd – 6th 2017

**LPS-INDUCED PERIPHERAL INFLAMMATION AGGRAVATES THE OUTCOME OF CEREBRAL STROKE IN AGED MICE**


**Department of Neurobiology, A.I. Virtanen Institute of Molecular Sciences, University of Eastern Finland**

Cerebral stroke is one of the leading causes of disability and death. Aging influences the outcome of stroke by inducing changes in the cerebral blood vessels, reducing the number of mitochondria and increasing oxidative stress. Aging is associated with co-morbidities such as atherosclerosis, diabetes and infections, all of which have inflammatory condition as an important component. Systemic inflammation is known to aggravate ischemic induced neuronal death specifically in aged mice. In this study we aimed to elucidate the mediators of the increased vulnerability to ischemia induced neuronal damage in aged mice subjected to peripheral inflammation. Systemic inflammation was induced by intraperitoneal injection of 100 µg/kg of LPS in 4- and 20-month-old C57Bl/6J mice 30 minutes before permanent middle cerebral artery occlusion. LPS induced inflammation increased the lesion size specifically in aged mice as measured by MRI and led to impaired performance in motor function tests. Levels of plasma pro-inflammatory cytokines were increased specifically in aged ischemic animals with peripheral inflammation. Next generation sequencing for micro-RNAs from peri-ischemic samples of the ischemic mice showed a significant down regulation in miR-127 in the brains of the aged ischemic animals and concomitant increase in the expression of constitutive proteasome PSMB5, a predicted target of miR-127. This study shows the importance of age and inflammation status on the outcome of cerebral stroke, and clarifies the importance of miR-127 and its downstream targets in aging and stroke.

**DEVELOPMENT OF SCANNING ULTRASOUND OPENING OF THE BLOOD-BRAIN-BARRIER FOR ALZHEIMER’S DISEASE**

In Alzheimer’s disease (AD) two types of insoluble protein aggregates occur, extracellular amyloid-β plaques and intracellular neurofibrillary tangles containing hyperphosphorylated tau. In this work we developed a technique to temporarily, safely and repeatedly open the blood-brain-barrier (BBB) in mice and showed that it enhances clearance of amyloid-β in APP23 transgenic mice and tau in pR5 transgenic mice. Intravenous injections of microbubbles are combined with ultrasound pulses, which exerts mechanical force on blood vessels in the brain leading to BBB opening which can facilitate the delivery of therapeutics or have action on its own through enhancement of microglia phagocytosis. This work demonstrates the potential of scanning ultrasound as a potential therapeutic for Alzheimer’s disease.

**PACAP IS DECREASED IN SPECIFIC NUCLEI OF THE DEVELOPING BRAINSTEM AFTER HYPOXIC EXPOSURE BUT NOT NICOTINE.**

**Huang J**, Waters KA, Machaalani R

1. Department of Medicine, & Bosch Institute, University of Sydney, NSW, Australia

2. The Children’s Hospital, Westmead, NSW 2145, Australia

Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptor 1 (PAC1) either increase or decrease in the adult brain pending on duration and strength of hypoxic and nicotine exposures. However, how they are affected in the young developing brain has not yet been determined. Using piglet models of intermittent hypercapnic hypoxia (IHH- clinically mimicking rebreathing when prone sleeping or as experience with obstructive sleep apnea) and nicotine, this study aimed to determine their effects on PACAP and PAC1 protein expression in the medulla. IHH was delivered for 1 (n= 7), 2 (n= 6), 3 (n= 6) and 4 (n= 7) days, while nicotine (n= 7) was continuous for the first 14 days of life. An additional group of combined nicotine and 1day IHH was studied to determine the combined effects. A decrease in PACAP was seen after the acute 1DHX exposure (none after repeated exposures) and was in the dorsal motor nucleus of vagus (DMNV; p= 0.024), nucleus of the solitary tract (NTS; p= 0.024) and the gracile nucleus (GRAC; p= 0.001). A
| **Australasian Neuroscience Society Annual Scientific Meeting 2017**
| **International Convention Centre, Sydney, December 3rd – 6th 2017**

| **BEHAVIOURAL AND PHYSIOLOGICAL EFFECTS OF α9-nAChR DELETION IN PAIN, STRESS AND AFFECTIVE BEHAVIOUR**

Mohammadi SA, Burton TJ, Christie MJ.
*The University of Sydney, NSW, Australia*

**OBJECTIVE:**
α9-nAChR are a target for novel therapeutic analgesics. However, the evidence for the involvement of the α9-nACh receptor in pain is mixed, and a definitive mechanism of α9-nAChR-mediated analgesia has yet to be defined. Furthermore, the potential for adverse effects due to blocking α9-nAChRs for pain relief are not yet known.

**METHODS:** We compared behavioural and physiological differences in α9-nAChR knockout (KO) mice with their wildtype (WT) counterparts. We used models of chronic neuropathic and inflammatory pain together with a variety of behavioural tests for mechanical and thermal pain. We compared the histology of the nerves of WT and KO animals in naïve and injured states to determine the effect of α9-nAChRs in disease progression. Furthermore, we looked at the receptors’ normal function in physiological feedback such as circadian rhythms and stress-regulation which may inform potential side-effect liabilities of α9-nAChR-blockers.

**FINDINGS:** We found that the lack of functional α9-nAChRs caused less extreme and less persistent mechanical hyperalgesia, but that all other pain modalities were normal. We found no difference in the histology of nerve-injured WT and KO animals. KO animals exhibited altered circadian activity patterns, altered affective behaviour following stress and loss of reward, and dysregulation of corticosterone after stress.

**CONCLUSION:** We have found weak evidence for the contribution of α9-nAChRs in chronic neuropathic or inflammatory pain, and we saw no evidence of disease modifying effects of α9-nAChR. Phenotyping of α9-nAChR-null mice revealed a role of the receptor in normal regulatory physiology, which may become compromised following treatment with α9-nAChR-blockers.

Inaki-Carril Mundinano (Monash University)

**CONSCIOUS VISION IN THE ABSENCE OF V1: A CASE REPORT**

Mundiñano IC1, Da Souza M2, Chen J2, Sarossy MG3, Goodale MA2, Bourne JA1.
1.Australian Regenerative Medicine Institute, Monash University, Victoria, 3800, Australia. 2. The Brain and Mind Institute, The University of Western Ontario, London, ON, Canada. 3. Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, Melbourne, Victoria, Australia.

Here we report for the first time a case of a child (B.I.) who suffered bilateral occipital-lobe injury in the first two weeks postnatally due to medium-chain acyl-Co-A dehydrogenase deficiency. At 7 years of age, B.I. underwent a battery of ophthalmological and neurophysiological tests, as well as structural and diffusion MRI radiological examination. Despite the extensive bilateral occipital cortical damage, B.I. has extensive conscious visual abilities, is not blind, and can use vision to navigate his environment. Furthermore, he can readily and consciously identify happy and neutral faces and colours, tasks associated with ventral stream processing. These findings suggest significant re-routing of visual information. To identify the putative pathway/s responsible for this ability, MRI tractography of secondary visual pathways to area MT originating from the lateral geniculate nucleus (LGN) and the inferior pulvinar (PI) were analysed. Results revealed an increased PI-MT pathway in the left hemisphere, suggesting that this pulvinar relay could be the neural pathway affording the preserved visual capacity following an early-life lesion of V1. This finding corroborates anatomical evidence from monkeys showing an enhanced PI-MT pathway following an early-life lesion of V1, compared to adults.

Ruth Napper (University Of Otago)

**LONG-TERM EFFECTS OF DEVELOPMENTAL ALCOHOL EXPOSURE ON THE CIRCUITRY OF THE MATURE BRAIN.**
Binge exposure to alcohol during early neonatal life in the rat, equivalent to human third trimester brain development, results in apoptotic cell death in many brain regions within 12 hours of exposure. The present study investigated the long-term effect of acute apoptotic death on quantitative aspects of brain circuitry in the mature ethanol-exposed brain. Rat pups were exposed to alcohol, on postnatal days 4-9 or on a single day, as 2 feeds 2 hours apart, via gavage. Gavage and suckle controls were also used. Mature rats were perfused under general anaesthesia (sodium pentobarbitone) and the brain serially sectioned at 40µm in the coronal plane. Unbiased stereological methods were used to determine total neuronal number within specific brain regions involved in cerebellar and hippocampal brain circuits. There was a significant deficit of neurons in key cerebellar and hippocampal subregions in the alcohol-exposed animals compared to controls but the quantitative relationships within specific circuits did not always show changes. Within the cerebellum, the ratio between Purkinje cells to granule cells was 1:500; to deep cerebellar nuclear neurons was 13:1; and to inferior olivary neurons was 130:1. Ratios within hippocampal circuits were altered in alcohol-exposed animals. This suggests that there may be compensatory plasticity including, additional cell death, in some brain regions to optimize brain circuitry and function after development alcohol exposure, but this is not uniform. The acute effect of developmental exposure may not reflect long-term changes which must be studied in animal models, to better understand potential human outcomes.

Shyuan Ngo (The University of Queensland)

HYPERMETABOLISM IS ASSOCIATED WITH LOWER MOTOR NEURONE BURDEN, FUNCTIONAL DECLINE, AND PREDICTS SURVIVAL IN AMYOTROPHIC LATERAL SCLEROSIS (ALS).

Steyn F1,2,3, Ioannides Z1,3, van Eijk RP4, van den Berg LH5, Henderson RD1,5, McCombe PA1,5 and Ngo ST1,2,3,5,6
1. University of Queensland Centre for Clinical Research, Brisbane, Queensland, Australia. 2. Wesley Medical Research, The Wesley Hospital, Auchenflower, Queensland, Australia. 3. Department of Neurology, Royal Brisbane & Women’s Hospital, Brisbane, Queensland, Australia. 4. Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht, Netherlands. 5. Queensland Brain Institute, Brisbane, Queensland, Australia. 6. Australian Institute for Bioengineering and Nanotechnology, Brisbane, Queensland, Australia.

Alterations in whole body metabolic homeostasis are thought to impact ALS outcome. We investigated the prevalence of hypermetabolism in ALS in a prospective case-control study, and the relationship between hypermetabolism, clinical features of disease, and survival. We enrolled 58 patients with clinically definite or probable ALS and 58 control participants who were matched for age and gender. Predicted energy expenditure was determined relative to body composition. Measured energy expenditure was determined by indirect calorimetry, and expressed as a percentage of predicted energy expenditure to obtain a metabolic index (MI). MI≥120% was defined as hypermetabolism. Logistic regression was used to determine the difference in prevalence of hypermetabolism between cases and controls. Linear mixed models were used to explore the longitudinal relationship between MI and clinical features. Kaplan-Meier curves between normometabolic vs. hypermetabolic ALS patients were compared using the log-rank test. We found an increased prevalence of hypermetabolism in ALS patients relative to controls (41% vs. 12%, adjusted odds ratio=5.4; p<0.01). Hypermetabolism was associated with a greater lower motor neurone burden (p=0.03), and a greater decline in ALSFRS-R (p=0.02). MI was inversely associated with survival; 18-month survival in normometabolic vs. hypermetabolic individuals was 93.8% vs. 20.9% (p<0.001). Higher MI in ALS patients who have significant lower motor neurone burden suggests that hypermetabolism may be linked to the loss of motor units. The association between hypermetabolism, rate of functional decline, and shorter survival indicates important prognostic properties. Assessment of energy expenditure could be useful for stratifying patients for clinical trials.

Jin Sung Park (Kolling Institute)

SINGLE HETEROZYGOUS ATP13A2 MUTATIONS CAUSE CELLULAR DYSFUNCTION ASSOCIATED WITH PARKINSON’S DISEASE

Jin-Sung Park1, Christine Klein2, and Carolyn M. Sue1
1. Department of Neurogenetics, Kolling Institute, Royal North Shore Hospital and University of Sydney, St. Leonards, New South Wales 2065, Australia. 2. Institute of Neurogenetics, University of Lübeck, Lübeck, Germany
Thymoquinone (TQ), the active principle of *Nigella sativa* seed, has been shown to have antioxidant, anti-inflammatory, antibacterial, hepatoprotective, antimutagenic, and antitumor activities. A recent study has demonstrated that TQ has a protective effect against radiation-induced oxidative stress in cultured human peripheral blood mononuclear cells.

Deficits in diabetics have been associated with reduced levels of brain-derived neurotrophic factor (BDNF) and impaired neurogenesis. Thymoquinone increases BDNF and neurogenesis in the hippocampus and ameliorates cognitive deficits in diabetic rats.

**Thymoquinone Enhances BDNF and Neurogenesis in the Hippocampus and Ameliorates the Cognitive Deficits in Diabetic Rats**

Rao MS and Smitha S

*Department of Anatomy, Faculty of Medicine, Kuwait University, Kuwait*

Thymoquinone (TQ) has been shown to have antioxidant, anti-inflammatory, antibacterial, hepatoprotective, antimutagenic, and antitumor activities. A recent study has demonstrated that TQ has a protective effect against radiation-induced oxidative stress in cultured human peripheral blood mononuclear cells.

Deficits in diabetics have been associated with reduced levels of brain-derived neurotrophic factor (BDNF) and impaired neurogenesis. Thymoquinone increases BDNF and neurogenesis in the hippocampus and ameliorates cognitive deficits in diabetic rats.

**Establishing a High Throughput Model for Investigating Axonal Degeneration**

Simone MJ1, Witting PK1

1. Discipline of Pathology, Charles Perkins Centre, The University of Sydney, Sydney, NSW, 2006

When a neuronal axon is damaged or separated from its cell body, it undergoes a regulated process of rapid fragmentation known as Wallerian Degeneration. Once thought to be a passive process initiated through starvation of the axon of proteins and organelles from the primary cell body, Wallerian Degeneration is now known to be an active and highly regulated process of Axonal Destruction. To investigate the molecular mechanisms regulating Wallerian Degeneration, we have developed a robust, high throughput axonal model using a cloned N-type human SH-SYSY cell line (readily transfected to express fluorescent tagged proteins) that is chemically differentiated to a neuronal phenotype using Neurobasal Media, B27 supplements and Retinoic Acid. Differentiated cells aggregate to form a single cell cluster, and extend their axons to greater than 1000 μM. Once fully differentiated, cells can be used in various axonal degenerative models including dissection, hypoxia-reperfusion injury and toxic insult. Experimental models assessing axonal/cell rescue either by the transfection of genes known to delay axonal degeneration, or by treatment with...
Differential Lipid Histopathology in Alzheimer’s Disease

Smith CC\(^1\), O’Rourke MB\(^2\), Kril JJ\(^1\), De La Monte SM\(^3\), Sutherland GT\(^1\).

1. Discipline of Pathology, Charles Perkins Centre, Sydney Medical School, The University of Sydney, New South Wales, Australia. 2. Mass Spectrometry Core Facility, Charles Perkins Centre, The University of Sydney, New South Wales, Australia. 3. Departments of Pathology, Neurology, Neurosurgery, and Medicine, Rhode Island Hospital, Alpert Medical School of Brown University, Providence, Rhode Island, USA.

Alzheimer’s disease (AD) is the most common neurodegenerative disorder. The symptomology of AD reflects region-specific neuronal loss, but there are also significant white matter changes. Biochemical studies of post-mortem tissue from susceptible brain regions in AD are confounded by neuronal loss and secondary changes. Alternatively, it might be possible to use differentially affected areas to model the natural history of AD. Here, Matrix-Assisted Laser Desorption/Ionisation-Imaging Mass Spectrometry (MALDI-IMS) was used to investigate myelin lipid biochemistry at the histological level in AD and matched controls. Formalin-fixed, frozen sections from the mildly affected primary visual cortex, moderately affected precuneus and superior temporal gyrus, and severely affected inferior temporal gyrus (ITG) of three AD cases and five controls were supplied by NSW Brain Banks. Lipids were analysed by MALDI-IMS in positive ion mode, using 2,5-dihydroxybenzoic acid as the matrix on a Bruker Ultraflextreme. Data were processed with SCiLS Lab 2014b, and visually analysed for regions exhibiting significant lipid changes. Principal components analysis indicated significant changes to the lipid-species makeup of the ITG between AD and controls, and visual comparisons of different regions within AD showed an increasing magnitude of lipid changes from mildly to severely affected areas. Specifically, there was a broad reduction in the expression of sphingo- and phospho-lipids in both white and grey matter. This suggests that AD is associated with lipid abnormalities and the severity correlates with the regional tau pathology. MALDI-IMS is a valuable discovery tool for investigating the role of the lipidome in AD pathogenesis.

Expression of Oligodendrocyte-Myelin in the Superior Temporal Gyrus of a Postmortem Schizophrenic Brain of 22q11.2 Deletion Syndrome

Torii Y\(^1\), Iritani S\(^1\), Fujishiro H\(^1\), Habuchi C\(^1\), Sekiguchi H\(^1\), Masaki K\(^3\), Hayashida S\(^3\), Kira J\(^3\), Ozaki N\(^1\).

1. Department of Psychiatry, Graduate School of Medicine, Nagoya University, AICHI, JAPAN. 2. Center for Postgraduate Clinical Training and Career Development, Nagoya University Hospital, AICHI, JAPAN. 3. Department of Neurology, Graduate School of Medical Sciences, Kyushu University, FUKUOKA, JAPAN.

Recent studies based on the neuroimaging and molecular biological analysis suggest that the pathogenesis of schizophrenia would be related to myelin-oligodendrocyte abnormalities. 22q11.2 deletion syndrome is known to be at high risk for schizophrenia and have deleted region including RTN4R gene, which encode the receptor for proteins expressed in oligodendrocyte-myelin. However, the neuropathological abnormality of myelin-oligodendrocyte has not been sufficiently verified in postmortem brain tissue of schizophrenia of 22q11.2 deletion syndrome. In this study, we have investigated the expression of myelin-oligodendrocyte directly in the brain tissue of the superior temporal gyrus (STG) in the schizophrenic subject with 22q11.2 deletion (27 years of illness duration, 59 years of age at death, male) stained with antibody against MOG and Nogo-A immunohistochemically, and compared to those of normal controls and/or schizophrenia without 22q11.2 deletion. The decreased expressions of MOG and the decreased thickness of MOG-positive fiber-like structures were observed in the middle layer of neocortex of STG of the schizophrenic brain with 22q11.2 deletion, compared to those of normal controls. These findings were also observed in the schizophrenia without 22q11.2 deletion. On the other hand, density of Nogo-A positive neurons in layer III of the neocortex in the STG was increased in the case with 22q11.2 deletion, compared to the schizophrenia without 22q11.2 deletion. These neuropathological findings might suggest that 22q11.2 deletion would be related to myelin-oligodendrocyte abnormalities in these regions of schizophrenia.

Z-Drugs as Novel Therapeutic for Stroke Recovery

Caine Smith (The University of Sydney)

Expression of Oligodendrocyte-Myelin in the Superior Temporal Gyrus of a Postmortem Schizophrenic Brain of 22q11.2 Deletion Syndrome

Torii Y\(^1\), Iritani S\(^1\), Fujishiro H\(^1\), Habuchi C\(^1\), Sekiguchi H\(^1\), Masaki K\(^3\), Hayashida S\(^3\), Kira J\(^3\), Ozaki N\(^1\).

1. Department of Psychiatry, Graduate School of Medicine, Nagoya University, AICHI, JAPAN. 2. Center for Postgraduate Clinical Training and Career Development, Nagoya University Hospital, AICHI, JAPAN. 3. Department of Neurology, Graduate School of Medical Sciences, Kyushu University, FUKUOKA, JAPAN.

Recent studies based on the neuroimaging and molecular biological analysis suggest that the pathogenesis of schizophrenia would be related to myelin-oligodendrocyte abnormalities. 22q11.2 deletion syndrome is known to be at high risk for schizophrenia and have deleted region including RTN4R gene, which encode the receptor for proteins expressed in oligodendrocyte-myelin. However, the neuropathological abnormality of myelin-oligodendrocyte has not been sufficiently verified in postmortem brain tissue of schizophrenia of 22q11.2 deletion syndrome. In this study, we have investigated the expression of myelin-oligodendrocyte directly in the brain tissue of the superior temporal gyrus (STG) in the schizophrenic subject with 22q11.2 deletion (27 years of illness duration, 59 years of age at death, male) stained with antibody against MOG and Nogo-A immunohistochemically, and compared to those of normal controls and/or schizophrenia without 22q11.2 deletion. The decreased expressions of MOG and the decreased thickness of MOG-positive fiber-like structures were observed in the middle layer of neocortex of STG of the schizophrenic brain with 22q11.2 deletion, compared to those of normal controls. These findings were also observed in the schizophrenia without 22q11.2 deletion. On the other hand, density of Nogo-A positive neurons in layer III of the neocortex in the STG was increased in the case with 22q11.2 deletion, compared to the schizophrenia without 22q11.2 deletion. These neuropathological findings might suggest that 22q11.2 deletion would be related to myelin-oligodendrocyte abnormalities in these regions of schizophrenia.

Petra Van Nieuwenhuijzen (University of Sydney)

Z-Drugs as Novel Therapeutic for Stroke Recovery

Caine Smith (The University of Sydney)
van Nieuwenhuijzen PS, Chan V, Clarkson AC, Chebib M
1. Faculty of Pharmacy, The University of Sydney, Camperdown, 2006, Australia
2. Department of Anatomy, Brain Health Research Centre and Brain Research New Zealand, University of Otago, Dunedin 9054, New Zealand

Stroke is a leading cause of disability in Australia. Stroke survivors often require lifelong help and experience a low quality of life. To date there is only one acute treatment option available and not many people are able to receive this treatment due to time constraints and other pre-existing factors. We are exploring the role of GABA-ergic drugs and in particular the cyclopyrromes as novel treatment options in stroke recovery.

Using a photothrombotic stroke model in mice, we induced stroke in the M1 region of the motor-cortex, mice were divided into different treatment groups consisting of both acute (1 dose, 1 hr after stroke) and long-term (continuous infusion for 4 weeks starting 2 weeks after stroke) treatment and received either: Vehicle, Zaleplon, Zolpidem or Diazepam.

The acute treatment group were assessed 1 week after stroke on grid-walking and cylinder tasks. In the long-term group mice were assessed weekly until 6 weeks after stroke. After their final behavioural test, mice were sedated and transcardially perfused with PFA and histologically processed with cresyl violet to assess infarct size.

Our study shows that targeting specific GABA_a receptors results in a significant improvement in motor recovery in both acute and chronic treatment groups, while infarct size was only improved in the acute treatment group.

Marloes Van Roijen (University of Sydney)

NEUROPATHOLOGIC ASSESSMENT OF ALZHEIMER PATHOLOGY IN SCHIZOPHRENIA

Marloes van Roijen1, Shelley L Forrest1, Clair De Sousa1, Toni McCrossin1, Donna Sheedy1, Jillian J Kril1
1 New South Wales Brain Tissue Resource Centre, Discipline of Pathology, Sydney Medical School, The University of Sydney, Australia.

The NSW Brain Tissue Resource Centre (NSWBTRC) facilitates high quality donated brain tissue to the neuroscience community. As psychotic or schizophrreniform symptoms in old age may be a manifestation of Alzheimer’s disease (AD), the NSWBTRC expanded the neuropathological characterisation protocol to include the assessment of Alzheimer pathology using the National Institute on Ageing–Alzheimer’s Association neuropathologic (ABC) criteria in all neurologically normal control and neuropsychiatric cases. The criteria includes the quantification of the burden of total beta-amyloid (Ab) load (Thal Phase), neurofibrillary tangles (Braak and Braak) and neuritic plaques (CERAD). In this study 167 controls and 45 schizophrenia cases were examined using immunohistochemistry for phosphorylated-tau and Ab, and modified Bielschowsky Silver. Of the 212 cases, 70% were male. Mean age (±SD) of control cases was 61 ± 7 years (range 18-104 years), and 54 ± 13 years (range 26-84 years) for schizophrenia cases. AD neuropathological change was absent in 64% of control cases, low in 31% and intermediate in 5% of cases. In schizophrenia cases, AD neuropathological change was absent in 78% of cases, low in 20% and intermediate in 2% of cases. Group differences were seen in schizophrenia cases for Ab plaques (p=0.03), neurofibrillary tangles (p<0.001) and neuritic plaques (p=0.013). This study has better characterised the NSWBTRC cohorts, which will enhance research outcomes and facilitate the standardisation of brain tissue for distribution.

Munawwar Abdulla (Unsw Sydney)

TOXICITY OF PACLITAXEL ON DISSOCIATED PRIMARY DORSAL ROOT GANGLION NEURONS IN VITRO

1. Neuropathic Pain Research Group, Translational Neuroscience Facility, School of Medical Sciences, the University of New South Wales, Sydney, NSW, 2051, Australia. 2. Department of Medical Oncology, Prince of Wales Hospital, Australia and Prince of Wales Clinical School, University of New South Wales, Sydney, NSW, 2051, Australia.

Chemotherapy-induced peripheral neuropathy (CIPN) is a debilitating and dose-limiting side effect of many chemotherapy regimens and is becoming a more prevalent issue as the longevity of cancer patients continues to increase. Sensory symptoms include pain, tingling, burning, or numbness and usually spread in a glove-and-stocking distribution. At present, there are no effective medications to treat or prevent CIPN, and the mechanisms by which symptoms are induced have not been fully elucidated. Paclitaxel (PTX) is a commonly used chemotherapeutic that induces neuropathy in a high percentage of patients. Emerging evidence implicates the involvement of sensory neurons...
in the dorsal root ganglion (DRG). To investigate the neurotoxic effects of PTX in vitro, dissociated primary DRG neurons from 5-week-old C57BL/6 mice were cultured for 1 (short-term) and 3 (established) days before being treated with 10-250 nM of PTX for 24-48 hrs, and the neuronal growth was measured. PTX induced at least 60% reduction in the amount of neurite outgrowth per neuron in the short-term model, but there was no significant difference in the established model, indicating that PTX prevents neurite outgrowth rather than inducing neurite degradation. However, there were significant morphological changes in both cultures, including the formation of retraction bulbs at the ends of the neurites, which indicate the blockage of neurite outgrowth. These in vitro models of PTX-induced neurotoxicity would enable testing of candidates for either neuroprotection or treatment of CIPN.

Karl Aoun (The University of Sydney)

COPPER-GTSM IN THE COPPER-DEFICIENT MAMMALIAN MODEL OF THE PARKINSON’S DISEASE BRAIN: AN EXPLORATORY STUDY

Aoun K1, Suryana E1, Meikle S2, Parmar A3, Rahardjo G1, Zahra D1, Arthur A3, Safavi-Naeini M1, Emvalomenos GM3, Finkelstein DI1, Barnham KJ4,5, Sedjajthera A4, Bray L4, Perrones K4, McAllum E4, Hare DJ4, Double KL1.

1. Discipline of Biomedical Science and Brain and Mind Centre, Sydney Medical School, The University of Sydney, Sydney, NSW 2006, Australia
2. Brain and Mind Centre, University of Sydney; Faculty of Health Sciences, University of Sydney
3. Life Sciences, ANSTO
4. Florey Institute of Neuroscience and Mental Health, 30 Royal Parade, Parkville, VIC, 3052, Australia
5. Bio21 Institute and Department of Pharmacology and Therapeutics, The University of Melbourne, Melbourne, VIC, 3010, Australia

Dopaminergic vulnerability to degeneration in Parkinson’s disease (PD) is associated with multiple pathological mechanisms including metal dyshomeostasis, dopamine auto-oxidation and protein aggregation. We have previously reported a significant deficit in copper, and dysfunction of the copper-dependent antioxidant superoxide dismutase 1 in PD-vulnerable brain regions, suggesting that copper supplementation may convey neuroprotective effects. Though administration of a copper-containing drug is reported to rescue the phenotype in four animal models of PD, the approach has not been investigated in models expressing the copper deficiency observed in PD. Here we investigate the potential disease-modifying effects of copper delivery in a transgenic model of low brain copper with an 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesion. Following a 45mg/kg dose of MPTP, thirty-two copper-deficient Slc31a1tm2.1Djt/J Ctrl1/– mice were gavaged 3mg/kg of copper(II)-bis(thiosemicarbazone) (Cu-GTSM)/day or vehicle over a 21-day period. Another group of eight unlesioned Ctrl1+/+ mice received vehicle only. At day 22, nigral copper levels...
were quantified using Inductively Coupled Plasma Mass Spectrometry and striatal dopamine levels and metabolism were assessed using High-Pressure Liquid Chromatography. While the MPTP lesion alone did not significantly alter nigral copper levels, Cu-GTSM treatment increased nigral copper concentrations by 62.4% and 58.1%, respectively, compared to untreated controls (p-value=0.000), and vehicle-gavaged MPTP-lesioned mice (p-value<0.001). Striatal levels of dopamine and 3,4-Dihydroxyphenylacetic acid (DOPAC) were respectively reduced by 60.5% and 50.8% after MPTP lesion (p-value=0.01; p-value=0.006), however following Cu-GTSM treatment of MPTP lesioned mice, striatal levels of dopamine and DOPAC were no longer significantly different to unlesioned vehicle treated controls, suggestive of a mild neuroprotective effect.

Micah Daniel Austria (The University of Auckland)

CHARACTERISING PERIVASCULAR CELLS IN ALZHEIMER’S DISEASE HUMAN TISSUE MICROARRAYS

Austria MD1, Singh-Bains M1, Faull RLM2, Draganow M2
1Department of Anatomy and Medical Imaging, University of Auckland, Auckland, New Zealand
2Department of Pharmacology, University of Auckland, Auckland, New Zealand

Alzheimer’s disease (AD) is the most common neurodegenerative disorder. Increasing evidence implicates the involvement of non-neuronal cells in the pathogenesis of AD, with current studies focusing on the different components of the neurovascular unit. The aim of this project is to characterise neurovascular disruption in AD post-mortem human brain tissue by examining non-neuronal cells involved in the neurovascular unit of the middle temporal gyrus (MTG). Immunohistochemistry was carried out on human brain tissue microarrays (TMAs) from the MTG. Each TMA consisted of at least 21 control and 21 AD cases from the Human Brain Bank. Antibodies to alpha-smooth muscle actin (α-SMA) and platelet-derived growth factor receptor-beta (PDGFRβ) were used to investigate perivascular cells (smooth muscle cells and pericytes, respectively) of the neurovascular unit. The immunolabelled TMAs were imaged using the V-slide scanner automated imaging system. Densitometric analysis for staining intensity and load was carried out on the acquired images using Metamorph. Parametric methods were used to compare the mean intensity and load of each marker for the AD and control cohorts. Preliminary findings show a significant increase in α-SMA expression (p<0.01) in probable arteriolar smooth muscle cells in AD, which is not attributable to a change in arteriole number. Furthermore, there is a significant reduction of PDGFRβ intensity (p<0.001) and load (p<0.01) in pericytes surrounding capillaries in AD. These findings suggest that perivascular cells are differentially compromised in the MTG in AD, as evidenced by differences in the expression patterns for capillary pericytes, and arteriolar smooth muscle cells.

Vladimir Balcar (The University of Sydney)

POLYMORPHISM rs3810950 OF CHAT (THE GENE ENCODING THE ACETYLCHOLINE SYNTHESISING ENZYME CHOLINE ACETYLTRANSFERASE) IS ASSOCIATED WITH ALZHEIMER’S DISEASE.

Hálová A1, Janoutová J2, Ewerlingová L1, Janout V1, Kashem MA1,4, Gerguri T4, Pravda L3, Balcar VJ1,5, Šerý O1,5
1. Laboratory of Neurobiology and Molecular Psychiatry, Department of Biochemistry, Faculty of Science, Masaryk University. 2. Department of Epidemiology and Public Health, Faculty of Medicine, University of Ostrava, Czech Republic. 3. Bosch Institute and Discipline of Anatomy and Histology, Sydney Medical School, The University of Sydney, Australia. 4. National Centre for Biomolecular Research, Faculty of Science and CEITEC, Masaryk University, Brno. 5. Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Brno, Czech Republic

Cholinergic hypothesis of Alzheimer’s disease (AD) is based on the findings that a reduced cholinergic activity in the CNS correlates with the cognitive decline observed in patients with AD. The hypothesis contributed to the development of agents potentiating cholinergic neurotransmission; however, these drugs only slowed down the cognitive decline and could not prevent it. Consequently, the central cholinergic deficit has been accepted as a part of the etiology of AD but not as its primary cause. We have been studying the rs3810950 polymorphism of CHAT and analyzed its relationship to AD in a group of 1186 persons residing in Czech Republic. We have found that the AA genotype increased the risk of AD by at least 21 control and 21 AD cases from the Human Brain Bank. Antibodies to alpha-smooth muscle actin (α-SMA) and platelet-derived growth factor receptor-beta (PDGFRβ) were used to investigate perivascular cells (smooth muscle cells and pericytes, respectively) of the neurovascular unit. The immunolabelled TMAs were imaged using the V-slide scanner automated imaging system. Densitometric analysis for staining intensity and load was carried out on the acquired images using Metamorph. Parametric methods were used to compare the mean intensity and load of each marker for the AD and control cohorts. Preliminary findings show a significant increase in α-SMA expression (p<0.01) in probable arteriolar smooth muscle cells in AD, which is not attributable to a change in arteriole number. Furthermore, there is a significant reduction of PDGFRβ intensity (p<0.001) and load (p<0.01) in pericytes surrounding capillaries in AD. These findings suggest that perivascular cells are differentially compromised in the MTG in AD, as evidenced by differences in the expression patterns for capillary pericytes, and arteriolar smooth muscle cells.
### IMPACT OF ALCOHOL ON RAT NEURAL STEM CELLS AND ON ADULT HUMAN BRAIN: FROM WESTERN BLOTS AND PROTEOMICS TO INTERACTOME STUDIES.

Kashem MA, Sultana N, Balcar VJ
*Bosch Institute and Discipline of Anatomy and Histology, School of Medical Sciences, Sydney Medical School, The University of Sydney NSW 2006, Australia*

Neural stem cells from rat embryos were exposed to ethanol (25 to 100 mM) for up to 96 hours. The numbers of neuron-like MAP-expressing cells were reduced with no obvious changes in morphology. The protein analyses by Western blotting (WB) and proteomics (MALDI-TOF/Mass Spectroscopy) identified 29 proteins as altered by ethanol (50 mM for 96 hours), among these were nucleophosmin (NPM) and dead end homolog-1 (DND1), proteins involved in transcription and translation. NPM was reduced by 45% and DND1 was up by 75%. Next, using WB we found that, in the human prefrontal cortex exposed to chronic excessive alcohol, NPM was decreased by 43% and DND1 increased by 80%. We then applied proteomics/interactomics to identify proteins with which NPM and DND1 can interact. NPM interacted with 55 proteins related to cell growth (4), cell structure (4), metabolism (18), oxidative stress response (5), signalling (8), apoptosis and DNA damage (2), epigenetics (2), transcription (7) and as transmembrane proteins (5). DND1 interacted with 24 proteins related to cell growth (1), cell structure (2), metabolism (13), oxidative stress (3), signalling (3) and transcription (2). Particularly striking are the numerous interactions with metabolic enzymes; these include those of NPM with Na⁺,K⁺-ATPase and DND1 with 4-aminobutyrate (GABA) transaminase. Na⁺,K⁺-ATPase provides energy for neuronal activity and drives neurotransmitter-inactivating transporters. GABA transaminase regulates the most important inhibitory neurotransmitter GABA. We conclude that the changes in NPM and DND1 could help to explain the effects of alcohol and alcoholism on brain from early development to adulthood.

---

### TAU INDUCED EXCITOTOXICITY PROMOTES LOCAL APOPTOTIC EVENTS AT THE SYNAPSE.

**Benetatos J, Bodea LG, Götz, J.**
*Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, University of Queensland, Brisbane, Queensland 4072, Australia.*

Aberrant accumulation of the mainly axonal microtubule associated protein tau in the somatodendritic compartment is a critical event promoting NMDAR mediated excitotoxicity in Alzheimer’s disease pathogenesis. We sought to determine if PTEN, an NDMAR activity driven regulator of synaptic plasticity was altered in synapses after tau accumulation. Using the rTg4510 mouse model which overexpresses human tau harbouring the frontotemporal dementia (FTD) mutation P301L, we found increased synaptosomal activity of the lipid phosphatase PTEN, previously implicated in regulating long-term synaptic depression. The increase of PTEN activity in the synapses of mice was recorded as early as 2 months of age and was maintained at both 6 and 12 months, while the total brain lysate measurements of PTEN activation revealed an increased activity only at the later time point. Interestingly, PTEN activity in synaptosomes occurred prior to the cleavage of caspase 3 and exposure of phosphatidylserine, both well described molecular hallmarks of apoptosis. These apoptotic events occurred first specifically at synapses, as demonstrated by western blot and flow cytometry analysis of purified synaptosomes and total brain lysates. These results indicate a synapse-specific type of apoptosis that is induced by tau mediated excitotoxicity.

---

### LONG TERM EXPRESSION OF TRANSGENIC TDP-43 CAUSES PROGRESSIVE GAIT DEFICITS IN AN INDUCIBLE MOUSE MODEL OF MND AND FTLD-TDP

**Chan G1, van Hummel A1,2, Ittner LM2,3, Ke YD1.**
1. *Motor Neuron Disease Unit, School of Medical Sciences, University of New South Wales, Sydney, Australia*
2. *Dementia Research Unit, School of Medical Sciences, University of New South Wales, Sydney, Australia*
3. *Neuroscience Research Australia, Sydney, Australia.*

The nuclear transactive response DNA binding protein 43 (TDP-43) undergoes several hallmark changes in motor neuron...
disease (MND) and frontotemporal lobar degeneration (FTLD-TDP) including cytoplasmic mislocalisation and aggregation. Furthermore, pathological mutations in the TDP-43-encoding TARDBP gene are known to cause MND and FTLD-TDP, and these mutations have been utilized to generate mouse models of disease. In particular, the A315T mutation in TDP-43 has been identified as a disease-causing mutation in MND patients. We have previously demonstrated that constitutive expression of human TDP-43\textsuperscript{A315T} in mice leads to a complex disease phenotype recapitulating key aspects of MND and FTLD-TDP. Here, this iTDP-43\textsuperscript{A315T} mouse model was further characterised functionally over time, using digital gait analysis with the DigiGait™ system (Mouse Specifics Inc.). Some deficits were already detectable at 1 month of age in iTDP-43\textsuperscript{A315T} while others only presented as mice aged. Accordingly, analysis of individual stride phases revealed significantly reduced stride time (=time to complete a full step cycle), stride length (=distance covered per step), swing time (=time paws are in the air during each step) and propel time (=time spent to move forward), together with increased compensatory stride frequency in iTDP-43\textsuperscript{A315T} compared to control mice. Furthermore, we found progressive gait asymmetry and paw placement abnormalities that started with the hind limbs and progressed to the fore limbs as iTDP-43\textsuperscript{A315T} mice aged. Taken together, iTDP-43\textsuperscript{A315T} mice presented with progressive gait problems.

Li Shan Chiu (Perron Institute for Neurological and Translational Science)

**CATIONIC ARGININE-RICH PEPTIDES HAVE NEUROPROTECTIVE POTENTIAL IN EXPERIMENTAL TRAUMATIC BRAIN INJURY.**

Chiu LS\textsuperscript{1,2}, Anderton RS\textsuperscript{1,2,4,5}, Clark VW\textsuperscript{1,3}, Cross JL\textsuperscript{1,3}, Knuckey NW\textsuperscript{1,3}, Meloni BP\textsuperscript{1,3}

1. Perron Institute for Neurological and Translational Sciences, Western Australia 2. Centre for Neuromuscular and Neurological Disorders, The University of WA 3. Department of Neurosurgery, Sir Charles Gairdner Hospital, WA. 4. School of Health Sciences, The University Notre Dame Australia, WA, 5. Institute for Health Research, The University Notre Dame Australia.

Cationic arginine-rich and poly-arginine peptides (referred to as CARPs) have potent neuroprotective properties in in vitro excitotoxicity and animal models of stroke. Traumatic brain injury (TBI) shares many pathophysiological processes as stroke, including excitotoxicity. Therefore, we evaluated our lead peptide, poly-arginine R18, with the COG1410 and APP96-110 peptides, which have neuroprotective actions following TBI. In a cortical neuronal glutamic acid excitotoxicity injury model, R18 was highly neuroprotective and reduced neuronal calcium influx, while COG1410 and APP96-110 displayed modest neuroprotection and were less effective at reducing calcium influx. In an impact-acceleration closed-head injury model (Marmarou model), R18, COG1410, and APP96-110 were administered intravenously (300 nmol/kg) at 30 minutes after injury in male Sprague-Dawley rats. When compared to vehicle, no peptide significantly improved functional outcomes, however the R18 and COG1410 treatment groups displayed positive trends in the adhesive tape test and rotarod assessments. Similarly, no peptide had a significant effect on hippocampal neuronal loss, however a significant reduction in axonal injury was observed for R18 and COG1410. In conclusion, this study has demonstrated that R18 is significantly more effective than COG1410 and APP96-110 at reducing neuronal injury and calcium influx following excitotoxicity, and that both R18 and COG1410 reduce axonal injury following TBI. Additional dose response and treatment time course studies to further assess the efficacy of R18 in TBI are ongoing.

Lucette Cysique (Unsw)

**A LOWER CD4/CD8 RATIO IS PREDICTIVE OF SUBCORTICO-FRONTAL BRAIN ATROPHY IN VIRALLY-SUPPRESSED HIV-INFECTED PERSONS**

**Authors:**

Nichols M\textsuperscript{1}, Gates TM\textsuperscript{2,3}, Soares J\textsuperscript{1}, Moffat K\textsuperscript{2}, Brew BJ\textsuperscript{2,3}, Rae C\textsuperscript{1}, Cysique LA\textsuperscript{1,3}

\textsuperscript{1} Neuroscience Research Australia, School of Medical Science, UNSW Australia 2 St. Vincent’s Hospital Sydney, 3 St. Vincent’s Applied Medical Research Centre

**Objective:** Determining the magnitude and predictors of atrophy in virally suppressed HIV infection.

**Methods:** 92 HIV+ and 50 HIV- participants (Mean age=57) underwent high-resolution anatomical MRI, neuropsychological evaluation, and HIV laboratory tests. MRIs were processed via FreeSurfer. Total cortical volume, total white matter (WM) volume, basal ganglia, lateral ventricles, fronto-striatal and fronto-parietal WM volumes were measured with reference to total intracranial volume. Neurocognitive performance was summarized using Global Deficit Score (GDS) method and neurocognitive impairment (NCI) was defined dichotomously as GDS>0.5. History of HIV-related NCI (yes/no) was also obtained. HIV status group differences and the effects of age and NCI on volumes of interest were
assessed using multivariate analyses of variance, while multiple linear regression was used to assess the effects of HIV biomarkers.

**Results:** The HIV+ group demonstrated predominant subcortical grey ($d=0.50-0.60$) and WM ($d=0.43-0.69$) atrophy, while the cortex was relatively spared ($d=0.23$). Historical and current NCI were separately associated with caudal-middle-frontal and superior-frontal WM atrophy. Caudal-middle-frontal WM volumes were smaller in those with both historical and current NCI than when only one or neither were present. The inferior-parietal WM was smaller in the HIV+ group compared to controls as a function of age. Finally, lower CD4/CD8 ratio was associated with volume loss across several subcortical grey and WM regions.

**Conclusion:** Virally suppressed HIV+ individuals show moderate subcortical grey and WM atrophy and mild cortical atrophy, which is the product of historical and ongoing brain damage principally related to chronic immune activation.

---

**Dysregulated miRNA expression in Parkinson’s disease results in impaired endocytosis, mitochondrial dysfunction and lysosome dyshomeostasis**

**Duly AMP1, Guennewig B1, Cooper A1,2.**

1. The Garvan Institute of Medical Research, Sydney, Australia. 2. St Vincent’s Clinical School, Faculty of Medicine, and School of Biotechnology and Biomolecular Sciences, Faculty of Science, The University of New South Wales, Sydney, Australia

Parkinson’s disease (PD) is a progressive neurodegenerative disorder with ~85% of patients classified as idiopathic (iPD). To identify pathways rendered dysfunctional early in the disease, and thus with enhanced therapeutic potential, small RNA sequencing was performed on patient brain regions yet to display significant pathology. The up-regulation of miR-142-3p in iPD patients led to the identification of several miR-142-3p target genes, correspondingly down-regulated in patients, that include SYNJ1 (a familial PD gene), WASL and MOB4, all of which are associated with synaptic vesicle endocytosis. Elevated miR-142-3p expression in vitro impaired endocytosis, implicating that iPD, like familial PD, may involve endocytic defects. This work also revealed an endocytosis-associated inhibition of early stages of autophagy, potentially explaining autophagy impairments observed in post-mortem iPD brain tissue.

**SERAC1**, another miR-142-3p target gene, was also down-regulated in idiopathic patients. Elevated miR-142-3p expression induced decreased SERAC1 expression, resulting in altered lipid metabolism and producing (1) mitochondrial dysfunction and elevated ROS production (2) lysosomal dyshomeostasis. Finally, the miR-132/212 cluster, whose reduced expression results in impaired neuronal function, was also identified as a target of miR-142-3p, and was down-regulated in vitro and in patients. Dysregulation of endocytosis, autophagy, mitochondrial and lysosomal homeostasis...
are all features observed in PD; and elevated miR-142-3p expression may provide a common mechanism contributing their perturbation in iPD. Targeted reduction of miR-142-3p expression in patients could be a potential therapeutic target for the treatment PD.

Ariel Dunn (University of Newcastle)

**EFFECT OF ENVIRONMENTAL RISK FACTORS ON ELECTROPHYSIOLOGICAL FEATURES RELATED TO SCHIZOPHRENIA**

Dunn, A\(^1\), Harms, L\(^1\), Todd, J\(^1\), Fulham, R\(^1\), Wong, A\(^1\), Hodgson, DM\(^1\), Schall, U\(^2\), Michie, P\(^1\)

1. School of Psychology, University of Newcastle, NSW, Australia.
2. School of Medicine and Public Health, University of Newcastle, NSW, Australia.
3. Priority Research Centre for Brain and Mental Health Research, University of Newcastle, NSW, Australia.

Maternal immune activation (MIA) in response to gestational infection is a risk factor for the development of schizophrenia in offspring. Previous studies have shown that MIA in rats or mice, induced by the non-infectious viral mimic Poly(I:C), produces a wide-range of schizophrenia-like behavioural alterations in the offspring. The current study investigated the impact of MIA on two electrophysiological features altered in schizophrenia, gamma activity and mismatch negativity (MMN). Furthermore, our study investigated these features in both male and female rodents. Pregnant Wistar rats were exposed to either Poly(I:C) (MIA) or saline during late gestation (gestational day 19). Offspring underwent surgery in adulthood to implant skull electrodes which were used to assess the neurophysiological phenotypes of MMN and gamma activity. MMN was measured using an oddball and many-standards control paradigm, while gamma activity was measured via an auditory steady-state response task (ASSR). Reliable ASSRs were found from 40 to 80 Hz. No significant treatment or sex effects were found for ASSRs. MMN responses were found and female animals had higher overall responses than males early in the MMN waveform, a novel finding. However, no significant treatment effects were found for any MMN component. A multiple hit model of MIA, or a two-hit model of a variety of risk factors might be employed in the future to produce more observable alterations. Our novel findings of sex differences suggest that animal research should consistently include both sexes to improve the validity of current models of schizophrenia.

Michael Geaghan (University Of Newcastle, Australia)

**MICRORNA-MRNA INTERACTIONS IN LYMPHOCYTES FROM INDIVIDUALS WITH SCHIZOPHRENIA**

Geaghan MP\(^1,2\), Cairns MJ\(^1,2,3\)

1. University of Newcastle, Australia, Callaghan, Australia. 2. Centre for Brain and Mental Health, Hunter Medical Research Institute, New Lambton, Australia. 3. Schizophrenia Research Institute, Sydney, Australia.

Schizophrenia is a severe neuropsychiatric disorder, affecting approximately 1% of the population, and characterised by positive and negative symptoms, and cognitive deficits. Over the last few decades, high throughput technologies including arrays and next-generation sequencing have identified numerous genetic and transcriptional signatures associated with schizophrenia, including a class of small, non-coding RNAs known as microRNAs (miRNAs). miRNA have been found differentially expressed in both peripheral and post-mortem grey matter tissue in schizophrenia, and one of the most significant schizophrenia-associated variants occurs within the MIR137 genetic locus. In this study, we utilised RNA sequencing to examine both miRNA and mRNA expression from peripheral blood mononuclear cells (PBMCs) obtained from 36 individuals with schizophrenia and 15 healthy controls. This analysis revealed 16 miRNAs and 23 genes that were differentially expressed (adjusted p<0.05). Most miRNAs (13 out of 16) were downregulated, while the vast majority of mRNAs (22 out of 23) were upregulated. Furthermore, several miRNAs were predicted to be regulators of differentially expressed genes, including let-7a-5p, a member of the let-7 miRNA family that has been previously observed dysregulated in schizophrenia. Our analysis of miRNA-mRNA interactions within this tissue revealed an enrichment of genes involved in immune responses. These results contribute to a growing body of evidence that suggest peripheral miRNA and mRNA expression is altered in schizophrenia. Moreover, our integrative analysis suggests that there are disrupted regulatory modules that could modify the immune system’s activity in the brain in a manner significant for the pathophysiology of the disorder.

Sian Genoud (Brain and Mind Centre, University of Sydney)

**ALTERATIONS IN BIOMETALS AND METALLOPROTEINS IN THE SOLUBLE FRACTION OF THE PARKINSON’S DISEASE BRAIN**
INTENTION TREMOR
MULTIPLE SINGLE UNIT ACTIVITY IN VENTRAL INTERMEDIATE THALAMUS OF ESSENTIAL TREMOR PATIENTS DURING INTENTION TREMOR

Andrea Giorni1,2, Peter A Silburn1,2,3, Terry Coyne1,2,3, FranÇois Windels1,2 and Pankaj Sah1,2
1. Queensland Brain Institute, University of Queensland, St Lucia, Brisbane, Australia
2. Asia-Pacific Centre for Neuromodulation, Queensland Brain Institute, University of Queensland, St Lucia, Brisbane, Australia
3. St Andrews War Memorial Hospital, Spring Hill, Brisbane, Australia

The pathophysiology of Essential Tremor (ET) is unclear, and Deep Brain Stimulation (DBS) of the Ventral Intermediate nucleus of the Thalamus (Vim) is widely used to control refractory underlying tremor. Single unit and field potential recordings were made in the Vim of four patients undergoing surgery for implantation of DBS electrodes for ET. Simultaneous accelerometer recordings were obtained from the contralateral hand. All patients were awake during recordings. The Local Field Potential (LFP) activity was coherent with tremor at the tremor frequency (4-5 Hz), with a tendency of tremor to lead the LFP with a variable delay (~250 - 650 ms). Hand tremor showed a periodic fluctuation at ~0.5 Hz. Spike sorting yielded 66 units (27 single cell activity and 39 multi cell activity) recorded during rest (n= 54) and tremor (n= 32). There were no differences in firing rate, action potential duration, burst index, mean spikes per burst, burst rate and proportion of spikes in bursts between rest and tremor (Wilcoxon rank-sum test p>0.05). At rest, some units were phase locked at 15-20 Hz (Rayleigh test, corrected p<0.01). Four of the 23 units active during tremor were phase locked at the tremor frequency. While Vim neurons showed a different phase locking tendency between tremor and baseline, no differences were seen in classical firing indexes to separate these units. These results describe neuronal activity patterns in the Vim related to tremor. Periodical fluctuation of the tremor frequency we describe is to our knowledge a new pathological feature of intention tremor.

Angela Hanton (University Of Queensland)
DYsREGULATION OF THE TERMINAL COMPLEMENT PATHWAY IN SOD1G93A TRANSGENIC MICE

Hanton A1, Noakes PG1,2, Woodruff TM1 and Lee JD1,3
1. School of Biomedical Sciences, the University of Queensland, QLD.
2. Queensland Brain Institute, the University of Queensland, QLD.
The terminal pathway of the innate immune complement system has recently been implicated in the pathogenesis of motor neuron disease (MND). Our previous studies have showed that SOD1<sup>G93A</sup> rats and mice treated with the selective C5a receptor (C5aR1) antagonist PMX205 have extended survival and improved motor functions, suggesting that C5aR1 has a pathogenic function. However, the contribution of the other components of terminal pathway, namely the second C5a receptor, C5aR2, and the terminal complement component C9, to MND progression is still unknown. The current study therefore aimed to initially identify the potential involvement of C5aR2 and C9 by investigating their expression and localisation at both mRNA and protein levels in the SOD1<sup>G93A</sup> mouse model of MND. Lumbar spinal cord and tibialis anterior muscle from SOD1<sup>G93A</sup> mice and their wild-type littermates were obtained at 3 different ages during disease progression, and expression and localisation of C5aR2 and C9 were examined using qPCR, western blotting and immunohistochemistry. We found consistent upregulation of C5aR2 mRNA levels in the lumbar spinal cord and tibialis anterior muscle of SOD1<sup>G93A</sup> mice during disease progression, while interestingly, C9 mRNA and protein levels were downregulated. Immunolocalisation also showed that C5aR2 and C9 are expressed on microglia and astrocytes surrounding the regions of motor neuron death. These results indicate that the terminal complement activation pathway may play a role in the progression of MND, and is worthy of further investigation as a potential therapeutic target.

Alesha Heath (University of Western Australia)

**FREQUENCY DEPENDENT EFFECTS OF LOW INTENSITY RTMS IN A MURINE MODEL OF ALZHEIMER’S DISEASE**

Heath AM<sup>1,2</sup>, Sherrard RM<sup>1</sup>

1. Sorbonne Universités - UPMC Paris 6 & CNRS, UMR 8256 Biological Adaptation and Ageing, Institut de Biologie Paris Seine, France. 2. Experimental and Regenerative Neuroscience, School of Biological Sciences, the University of Western Australia, Perth, Australia.

Repetitive transcranial magnetic stimulation (rTMS) has emerged as a promising technique to target cognitive decline in Alzheimer’s disease (AD), although there is some concern that improved AD scores may be a result of alleviated mood. While high intensity rTMS is the main focus of current research, low intensity stimulation delivered diffusely to the whole brain may also have beneficial effects. Focal low intensity rTMS (LI-rTMS) also affects intracellular calcium, BDNF and dendritic branching, indicating this form of magnetic stimulation may be appropriate for targeting specific aspects of neuroplasticity. We treated the PS1M146V knock in mouse model of AD and C57BL6/1 age-matched controls with LI-rTMS (12mT) focused over the hippocampus. After two weeks of stimulation we found positive effects of the excitatory frequencies (BHFS and 10Hz) in restoring spatial memory deficits and dendritic spine abnormalities in both six and 12 month mice. Whereas the two inhibitory frequencies had different effects, with 1Hz stimulation inducing partial improvements and cTBS having either no effect or in some cases worsening these measures. In contrast, none of the stimulation protocols improved the loss of neurogenesis or long-term BDNF levels in AD mice. However, importantly they also did not improve the anxious phenotype of the mice, suggesting that the behavioural effect was due to better cognition, not just less anxiety. These results suggest excitatory LI-rTMS applied to the hippocampus could provide a targeted treatment to the loss of synaptic function underlying the cognitive impairment in Alzheimer’s Disease.

Thomas Hedl (Macquarie University)

**PROTEOMIC CHARACTERISATION OF TDP-43 INCLUSION PATHOLOGY IN MND**

Hedl T<sup>1</sup>, Gul H, Mehta P, Le S, Berning B, Shahheydari H, Lee A, Walker AK

1. Centre for MND Research, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, New South Wales, Australia.

Motor neuron disease (MND) is a fatal neurodegenerative disorder caused by progressive degeneration of upper and lower motor neurons. The deterioration and death of motor neurons results in gradual paralysis that culminates in respiratory failure. Transactive response DNA-binding protein 43 (TDP-43) is a regulator of DNA and RNA metabolism that is expressed predominantly in the nucleus. In MND, a transition occurs that results in primarily cytoplasmic TDP-43 aggregation, forming protein inclusions. Cytoplasmic TDP-43 aggregation, post-translational modification and inclusion formation are implicated in more than 97% of cases. However, the protein interactors of aggregated TDP-43, and the contents of TDP-43-positive neuronal inclusions that may be involved in disease pathogenesis, have been poorly characterised. We expressed wildtype, cytoplasm-targeted, RNA-binding deficient and post-translational modification mimic mutant forms of human TDP-43 in mouse motor neuron-like (NSC-34) and human neuroblastoma (SH-SYSY) cell.
lines, and isolated aggregated proteins using sequential biochemical extraction with ultracentrifugation. Through our high-efficacy fractionation method, we have selected for proteins of low detergent solubility, a characteristic trait for aggregated TDP-43 and disease inclusions found in MND patient brain and spinal cord tissues. Coupled with high-throughput shotgun mass spectrometry, we have identified a broad range of potential interactors within these inclusions that may be implicated in pathology formation. These results reflect the discovery stage of a pipeline aiming to elucidate disease mechanisms by identifying both the biochemical perturbations that occur upon TDP-43 aggregation as well as potential endogenous anti-protein aggregation mechanisms that could be harnessed for disease therapy.

Alison Hogan (Macquarie University)

**NOVEL ZEBRAFISH MODELS OF MOTOR NEURON DISEASE BASED ON EXPRESSION OF A DISEASE-LINKED MUTATION IN CCNF.**


*Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney*

Motor Neuron Disease (MND) is a rapidly progressive, invariably fatal neurodegenerative disease characterised by the death of upper and lower motor neurons. Approximately 10% of MND patients have a known family history of the disease and causative mutations in multiple genes have been identified. MND-linked mutations in *CCNF* have recently been reported, however the pathogenic mechanisms associated with these mutations are yet to be established.

To investigate possible disease mechanisms, we have developed a transient zebrafish model based on an MND-linked mutation in *CCNF*. Characterisation of the transient mutant model demonstrated increased caspase-3 activity and increased cell death in the spinal cord in addition to a motor neuron axonopathy. This axonopathy was characterised by shortened primary motor axons and an increased incidence of aberrant axonal branching. Importantly, we demonstrated a significant correlation between the severity of the mutant *CCNF*-induced axonopathy and a reduced motor response to a light stimulus (photomotor response) in these models. These findings have identified a robust phenotype that will be used in preclinical investigations of potential therapeutics and indicates that zebrafish will be a useful tool to model the pathogenesis of *CCNF*-linked motor neuron degeneration.

Phillip Janowicz (Queensland Brain Institute)

**ENHANCING TARGET ENGAGEMENT OF TAU USING ANTIBODY ENGINEERING AND SCANNING ULTRASOUND FOR THE TREATMENT OF ALZHEIMER'S DISEASE**

**Janowicz PW, Götz J, Nisbet RN**

*Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, St Lucia Campus, Brisbane, Queensland 4072, Australia.*

Alzheimer’s disease (AD) is an extremely costly neurodegenerative disease characterised by extracellular amyloid plaques and intraneuronal neurofibrillary tangles. The main component of neurofibrillary tangles is the protein tau in a hyperphosphorylated state. Tau is an attractive therapeutic target due to its pathology being a strong correlate of cognitive decline in AD; however the blood brain barrier and neuronal membrane are formidable obstacles for therapeutics. We have previously increased the delivery of an anti-tau antibody, in a single chain variable fragment (scFv) format, into the brains of mice by combining antibody administration with microbubbles and non-invasive scanning focused ultrasound (SUS). Following on from this study, here we compare brain uptake of the anti-tau scFv, delivered in combination with SUS, to that of a larger fragment antigen binding (Fab) format and full-sized antibody isotypes IgG1 and IgG2a, to elucidate the importance of antibody size, binding-affinity and Fc-mediated receptor binding for neuronal uptake and tau engagement. We show that SUS increases the brain concentration of all antibody formats, and that IgG delivery is significantly enhanced compared to the smaller formats, suggesting that isotype size and format affects therapeutic delivery into the brain. The results of this study may affect the design of future immunotherapeutics targeting intraneuronal proteins, and validates the combination of scanning ultrasound to deliver IgG and smaller antibody peptides across the blood brain barrier.

Brooke Keating (University of New South Wales, Sydney)

**REGULATORY T CELLS AND INTERLEUKIN-35 SUPPRESS PAIN BEHAVIOURS AND NEUROINFLAMMATION IN EAE, AN ANIMAL MODEL OF MULTIPLE SCLEROSIS**

**Keating BA, Duffy SS, Perera CJ, Lees JG, Makker PGS, Tonkin RS, Carrive P, Moalem-Taylor**

*Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney.*
Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Pain represents an extremely common and difficult to treat symptom in MS, and is widely believed to have a central neuroinflammatory basis. Regulatory T (Treg) cells, and the Treg cell cytokine interleukin (IL)-35, have potent anti-inflammatory roles and control both innate and adaptive immune responses. We aimed to test the effects of adoptive transfer of regulatory T cells, and recombinant IL-35 therapy, on pain behaviours and neuroinflammation in mice with experimental autoimmune encephalomyelitis (EAE). For adoptive transfer experiments, FoxP3+ Treg cells were isolated from the periphery of DEREG mice with chronic EAE and activated in vitro before injection. Mechanical allodynia was assessed using von Frey filaments applied to the whisker pad and spontaneous pain was measured using the mouse grimace scale. Neuroinflammation was analysed using a combination of flow cytometry and immunohistochemistry. Intraperitoneal adoptive transfer of Treg cells prior to disease induction suppressed EAE severity, mechanical allodynia and facial grimacing. Intrathecal adoptive transfer of Treg cells at disease onset ameliorated mechanical allodynia without affecting disease severity, and was associated with decreased astrogliosis in the trigeminal nuclei of EAE mice. Intrathecal IL-35 therapy reduced EAE severity, facial allodynia and facial grimacing when commenced at disease onset, and was associated with increased IL-10 expression in the CNS, and decreased monocyte infiltration into the trigeminal nuclei of EAE mice. Overall, these findings suggest that both Treg cells and IL-35 suppress pain behaviours in EAE through modulation of neuroinflammation.

Shu Liu (Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College)

INCREASED EXPRESSION OF GLYCINE TRANSPORTER-1 IN MAJOR DEPRESSION DISORDER

Tingfu Du, Jianbo Xiu, Yan Shen, Qi Xu

1. National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences & Neuroscience Center, Chinese Academy of Medical Sciences & Peking Union Medical College. 2. Institute of Medical Biology, Chinese Academy of Medical Sciences and Peking Union Medical College, Kunming, 650118, China.

Major depressive disorder (MDD) is a common, chronic and recurrent mental disease, which affects approximately 17% of the population, and places a heavy burden on the family and society. However, the specific molecular mechanisms underlying the pathophysiology of MDD have not been identified. Here we use expression array combination with methylation chip, we found that the expression level of SLC6A9 is increased in MDD patients possibly due to its hypomethylation in peripheral blood samples from MDD patients (n=40) and controls (n=20). The increased expression of SLC6A9 was confirmed in another MDD patients(n=60) as well as matched controls (n=60) by qPCR. Glycine transporter-1 (SLC6A9) can regulate extracellular glycine concentrations in the central nervous system, so it plays an important role in maintaining a balance between inhibitory and excitatory neurotransmission. Some reports have shown that alterations in glycine-mediated neurotransmission contribute to the pathologies of mental disorders, such as schizophrenia and major depressive disorder. Several researchers consider that glycine transporter-1 may be an alternative therapeutic target for the treatment of these mental disorders. The results suggest that SLC6A9 may contribute to the pathogenesis of MDD.

Tanya Mcdonald (University Of Queensland)

RAGE ACTIVATION DRIVES DISEASE PROGRESSION IN THE SOD1G93A MOUSE MODEL OF MOTOR NEURON DISEASE

McDonald TM1, Woodruff TM1, Lee JD1, 2
1 School of Biomedical Science, University of Queensland, St Lucia, Australia
2 Centre for Clinical Research, University of Queensland, Herston, Australia

There is increasing evidence that neuroinflammation drives the progression of many neurodegenerative diseases. The receptor for advance glycation end-products (RAGE) has been shown to regulate both innate and adaptive immune responses in different pathologies associated with neuroinflammation. Previously SOD1G93A mice treated with a RAGE decoy ligand were shown to have reduced motor deficits suggesting RAGE itself may have a pathogenic function. However, the specific contribution of RAGE signalling to MND progression is still unknown. The current study therefore aimed to determine the precise function of RAGE in the disease progression of MND using SOD1G93A mice genetically deficient in RAGE. Initially, lumbar spinal cords from SOD1G93A and their wild-type littermates were obtained at 4 different disease stages, and expression of RAGE examined using qPCR. Behavioural tests were then conducted on SOD1G93A and SOD1G93A x RAGE mice to observe any differences in motor symptoms, along with body weight and...
Australasian Neuroscience Society Annual Scientific Meeting 2017
International Convention Centre, Sydney, December 3rd – 6th 2017

**BACE1 ACTIVITY MODULATES THE CELL SURFACE PROTEOME OF NEURONS**

Jasenka Njavro (German Center for Neurodegenerative Diseases (dZNE))

Proteolysis is a mechanism to control the levels and functions of cell surface membrane proteins. One of the contributing proteases is the β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1), which cleaves off the ectodomain of its mostly single-span transmembrane protein substrates. BACE1 is a central drug target in Alzheimer’s disease (AD), but has multiple substrates besides APP that possess different functions in neurobiology. This raises the concern that therapeutic BACE1 inhibition may result in mechanism-based side-effects due to reduced cleavage of its substrates and their subsequent accumulation on the cell surface. Yet, the consequences of BACE1 inhibition on the neuronal cell surface proteome are only partly known. Therefore, primary neurons were treated with a BACE inhibitor, and cell surface proteins were labeled using click chemistry-mediated biotinylation. Label-free proteomics identified over 30 membrane proteins to be enriched at the neuronal surface. This included both BACE1 substrates and surprisingly also membrane proteins which are unlikely to be BACE1 substrates, such as tetraspanins. A small subset of BACE1 substrates, APLP1, SEZ6/L, CHL1 and contactin-2, was strongly enriched – 2.5 to 7-fold – at the neuronal surface upon BACE inhibition. Other known substrates, including L1, were mildly enriched – less than 2-fold. Several proteins were further validated by immunoblot and in BACE1-deficient mouse brains. Taken together, this study demonstrates that BACE1 cleavage is a mechanism to control the surface proteome of neurons, regulating the abundance of a subset of its substrates, but also indirectly altering the amount of many other membrane proteins.

**ARE MOTOR NEURON ABNORMALITIES CORRELATED WITH IMPAIRED MOTOR FUNCTION IN SOD1-EXPRESSING ZEBRAFISH?**

Robinson KJ1, Watchon M2, Yuan KC1, Hogan AL1, Shahheydari H1, Bazzi R1, Don EK1, Cole NJ and Laird AS2.

 Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterised by progressive loss of motor neurons in the central nervous system. Several ALS-causing gene mutations have been identified, including mutation to superoxide dismutase 1 (SOD1) which is implicated in both familial and sporadic cases of ALS. Previous studies in zebrafish (Danio rerio) have shown overexpression of mutated human SOD1 causes shortening and aberrant branching of spinal axons, however the impact of SOD1 on motor function remains elusive. Our aim was to determine if shortened axon length was correlated with impaired motor function in SOD1 expressing zebrafish. Transgenic zebrafish embryos expressing BFP in motor axons were injected with either human wild-type (WT) SOD1 or mutant (MT) SOD1 mRNA at the single cell stage of fertilisation. At 48 hours post fertilisation, a photomotor response was triggered and movement in response to light was recorded as distance travelled. Larvae were then anaesthetised and imaged, allowing measurement of axons. Our results showed that MT SOD1 injected larvae had significantly shorter axons (p = 0.002) and travelled a significantly shorter distance (p <0.001) in response to a flash of light when compared with WT SOD1 injected and non-injected larvae. Furthermore, there was a significant correlation between distance travelled and axon length (R² = 0.357, p <0.001) albeit weak. These data present the first correlative investigation of axonal length and motor function in SOD1-expressing zebrafish and indicate that decreased axonal outgrowth likely has effects on motor behaviour.

Neha Soni (University of Queensland)
CHARACTERISATION OF CONTROLLED CORTICAL IMPACT INJURY IN ADOLESCENT MICE USING MRI AND HISTOLOGY

Soni N1,2, Mohamed AZ2, Kurniawan Nyoman D3, Nasrallah F2,3, Borges K1.
1. School of Biomedical Sciences, Department of Pharmacology, The University of Queensland, Brisbane, Queensland, Australia. 2. Queensland Brain Institute, The University of Queensland, Brisbane, Queensland, Australia. 3. The Centre for Advanced Imaging, The University of Queensland, Brisbane, Queensland, Australia.

In this study we characterised controlled cortical impact (CCI) injury in adolescent CD1 mice (4 weeks old) using MRI along with histology to investigate grey matter changes in response to traumatic brain injury. We performed in vivo T2 weighted scans and DTI (Diffusion tensor imaging) scans immediately after 5 h of injury and on 1 day, 3, 7, 14 and 30 days (n=6 mice each time point). After each scan the animals were sacrificed for histological studies. In histological studies we performed cresyl violet staining for lesion volume estimation that was compared with the T2 structural data. GFAP immunohistochemistry was used to measure the extent of gliosis. Histological studies demonstrated a significant increase in lesion volume at early time points with the maximum volume seen on day 3 after injury (p<0.05). The lesion volume decreased thereafter. A similar progression of the lesion volume changes was observed from T2 weighted structural data. A significant decrease in the axial and radial diffusivity in the injured cortex was found 5 h to 3 days after injury (p<0.05). These early changes are best explained by high immune cell density and cytotoxic edema. The axial and radial diffusivity in the injured site were restored after one week and were significantly increased at 2 weeks and thereafter (p<0.05). The later changes appear to be caused by structural rearrangement of the hippocampal formation and in some mice gliosis.

Annabel Sorby-adams (The University of Adelaide)

TAKE THE PRESSURE DOWN: A NOVEL AGENT FOR THE TREATMENT OF CEREBRAL OEDEMA AND ELEVATED INTRACRANIAL PRESSURE FOLLOWING STROKE

1. Adelaide Medical School and Adelaide Centre for Neuroscience Research, The University of Adelaide, Adelaide, SA, Australia. 2. Sansom Institute for Health Research, University of South Australia, Adelaide, SA, Australia. 3. The University of Queensland, Brisbane, Queensland, Australia.

Cerebral oedema and elevated intracranial pressure (ICP) are the leading cause of death in the first week following stroke. Despite this, current treatments are limited and fail to address underlying mechanisms, highlighting the need for development of targeted treatments. Recently, neurogenic inflammation and associated release of substance P (SP) following stroke has been linked to the development of profound cerebral oedema. SP elicits its effects by binding the NK1 tachykinin receptor (NK1R), with administration of an NK1R-antagonist ameliorating cerebral oedema following stroke in rodent models. When screening novel agents, it is also essential to use clinically relevant large animal models to improve the likelihood of successful clinical translation. The current study thus examined the efficacy of NK1R-antagonist treatment in reducing cerebral oedema and ICP in an ovine stroke model. Merino-sheep (9M;13F) were anaesthetised-and-subject to 2hrs transient MCAo, then allocated into the following treatment regimes: early treatment (1mg/kg NK1R-antagonist at 28, 33, 52, 57, 76, 81hrs post-stroke; n=6), delayed treatment (1mg/kg NK1R-antagonist at 124 and 129hrs post-stroke; n=6) saline vehicle (n=6) or sham surgery (n=4). At 6d post stroke ICP was measured for 4hrs, followed by FLAIR MRI to assess cerebral oedema. Following stroke, ICP was significantly decreased following NK1R-antagonist administration in both the early (p<0.01) and late (p<0.0001) treatment regimes compared to vehicle. Profound cerebral oedema was observed in vehicle treated animals at 6d, in keeping with the elevated ICP. These findings provide substantial evidence that NK1R-antagonist treatment is efficacious for the treatment cerebral oedema and elevated ICP following stroke.

Stephen Stefanou (The University Of Queensland)

PROFILING C5A RECEPTOR EXPRESSION IN A MOUSE MODEL OF MOTOR NEURON DISEASE USING A NOVEL GFP-CSAR1 REPORTER KNOCK-IN MOUSE

Stephen Stefanou1, Marc J. Ruitenberg1,2,3, John D. Lee1,4, Trent M. Woodruff1
1. School of Biomedical Sciences, the University of Queensland, QLD. 2. Queensland Brain Institute, the University of Queensland, QLD. 3. Trauma, Critical Care and Recovery, Brisbane Diamantina Health Partners, The University of Queensland, QLD. 4. Centre for Clinical Research, the University of Queensland, QLD.
Motor neuron disease (MND) is a terminal neurodegenerative disease that causes selective and irreversible damage to the body’s upper and lower motor neurons. Whilst the underlying disease pathogenesis is yet to be determined, mounting evidence has implicated injurious immune responses, including over-activation of the complement system, in MND. In particular, the signalling axis between complement activation component C5a and its classical receptor C5aR1 has been shown to drive disease progression in MND rodent models. Whilst C5aR1 expression has been widely documented on most rodent and human inflammatory cells, a comprehensive profiling of its expression and changes throughout different disease stages in MND models is lacking, in part due to lack of murine specific antibodies. Here, using a novel GFP-C5aR1 reporter knock-in strain backcrossed with the SOD1<sup>G93A</sup> model of MND, the present study quantified the expression and cellular localisation of C5aR1 in blood and lumbar spinal cord tissue at three disease stages; onset (OS), mid-symptomatic (MS) and end-stage (ES). We demonstrated that C5aR1 levels were unaltered in peripheral neutrophils and M1 pro-inflammatory monocytes, while its expression was increased on eosinophil and anti-inflammatory M2 monocytes during disease progression. We also confirmed that C5aR1-GFP expression was localised to microglia/macrophages in wild-type mice, with increasing expression in diseased SOD1<sup>G93A</sup> mice. These results demonstrate that C5aR1 is present on specific circulating immune populations, and inflammatory microglia/macrophages, which may play a role in the disease pathogenesis. Inhibiting C5aR1 may therefore be a therapeutic strategy to reduce inflammation and treat MND progression.

Anuradha Tennakoon (The University Of Adelaide)

GLYCOLIC ACID AND D-LACTATE PROTECT AGAINST PARAQUAT TOXICITY IN MICE

Straßl T<sup>1</sup>, Levin J<sup>1</sup>, Dieterich M<sup>1,3</sup>, Giese A<sup>2</sup>, Pan-Montoto F<sup>1,3</sup>

1. Department of Neurology, University Hospital, Ludwig-Maximilians-University Munich, Marchioninistraße 15, 81377 Munich, Germany.
2. Center of Neuropathology, Ludwig-Maximilians-University Munich, Feodor-Lynen-Straße 23, 81377 Munich, Germany.

Glycolic acid (GA) and D-lactate (DL) are products of the Parkinson’s disease related glyoxalase DJ-1. Loss of function mutations in DJ-1 are associated with early forms of the disease and linked to alterations in multiple cellular processes. We recently showed that GA and DL are important to support mitochondrial function and protect against paraquat toxicity in vitro (Toyoda et al. 2014). In this study we tested the neuroprotective effect of GA and DL by treating wild-type mice with vehicle, paraquat, paraquat and GA, or paraquat and DL for three weeks each. Motor function was analysed before and after treatment with the help of an accelerating rotarod test (0.3 rpm/sec). Brain sections were stained by immunofluorescence with an anti-tyrosine hydroxylase antibody. Stereological analysis was performed using a fluorescence microscope and the Stereo Investigator Software (mbf Bioscience) to estimate the amount of dopaminergic neurons in the substantia nigra pars compacta. Our results show that paraquat treatment induces motor dysfunction together with a significant loss of dopaminergic neurons in the substantia nigra when compared to control littermates. Co-treatment with GA or DL completely rescued this effect. Mice treated with paraquat and GA or paraquat and DL showed no motor dysfunctions and a normal number of dopaminergic neurons in the substantia nigra compared to control littermates without any signs of toxicity. These results suggest that the products of DJ-1 play an important role in the protection against environmental factors and are potential candidates to be used in the treatment of Parkinson’s disease.

Anuradha Tennakoon (The University Of Adelaide)

CHANGES IN BRAINSTEM CYTOKINES IN NORMAL AGEING AND MOTOR NEURONE DISEASE.

Tennakoon AS<sup>1</sup>, Johnson IP<sup>1</sup>, Katharesan V<sup>1</sup>

1. The Motor Neurone Laboratory, The University of Adelaide, Adelaide, South Australia.

Age-related increases in inflammatory status, as measured by elevated levels of pro-inflammatory cytokines, have been implicated in the development of age-related neurodegenerative diseases including Motor Neurone Disease (MND). However, recent rat studies have revealed that elevated inflammation associated with healthy ageing may promote motoneuronal survival. To clarify these apparently contradictory roles of inflammation in MND, changes in 27 cytokines have been compared here in healthy ageing and in MND. The 27 cytokines (IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17A, TNF-α, IFN-γ, FGF, G-CSF, GM-CSF, IFN-γ, MCP-1, MIP-1α, MIP-1β, Eotaxin, PDGF-β, RANTES and VEGF) were analysed using multiplex technology in fresh frozen post-mortem brainstems of MND patients (60-68 years, n=6), and compared with those of ageing controls (48-86 years, n=6) and young adult controls (20-33 years, n=6). Immunocytochemistry was used to co-localise selected cytokines to neuroglia. Levels of IL-1β and IP-10...
were higher in brainstems of ageing controls compared to young adult controls (p=0.017 and p=0.020 respectively). Moreover, MIP-1β levels were higher in brainstems of ageing controls compared to young adult brainstems (p=0.006) and decreased in MND brainstems compared to that of ageing controls (p=0.023). Immunocytochemistry showed that astrocytes were the source of MIP-1β. There is evidence from animal studies that MIP-1β is neuroprotective. The increased levels of MIP-1β with normal ageing may therefore be neuroprotective, whereas lower levels in MND may be associated with age-related motor neuronal degeneration. This suggests that modification of levels of specific cytokines may be a therapeutic strategy in MND.

Reka Petra Toth (Macquarie University)

CELLULAR PATHOGENIC MECHANISMS LINKED TO TBK-1 AND OPTINEURIN IN AMYOTROPHIC LATERAL SCLEROSIS

Reka P. Toth, Adam K. Walker, Julie Atkin.
Biomedical Department, Macquarie University, North Ryde, NSW, Australia

Motor Neuron disease (MND) is a rapidly progressing, fatal neurodegenerative disease caused by the loss of motor neurons. A pathological hallmark of MND is the accumulation of misfolded protein aggregates in degenerating motor neurons. Multiple studies have suggested that autophagy dysfunction in MND is associated with the accumulation of misfolded protein aggregates. TBK-1 is a kinase with a central function in autophagy, via activation of autophagy receptors, including optineurin. Importantly, TBK-1 and optineurin are mutated in MND patients. However, the mechanisms by which mutant optineurin and TBK-1 affect autophagy in MND is unknown. The aim of this study was to investigate how MND-associated mutant optineurin interacts with TBK-1, and whether MND-mutant TBK-1 disrupts autophagy. NSC-34, a mouse motoneuron-like cell line, was transfected with different EGFP-optineurin constructs. Wildtype optineurin-EGFP colocalized with endogenous TBK-1 and formed typical vesicular structures in 86.2% of NSC-34 cells, unlike the MND-optineurin mutants (E478G, Q398X), which did not localise with vesicles. Moreover, co-expression of LC3-dsRed and optineurin revealed that TBK-1 was recruited to autophagosomes in an optineurin-dependent manner, which was abrogated in the presence of MND-mutant optineurin. Co-immunoprecipitation of TBK-1 and MND-mutant optineurin showed intact binding, indicating that loss of recruitment was not due abrogated physical interaction between optineurin and TBK-1. Overexpression of MND-mutant TBK-1 (R308Q, R357Q, E696K) in HEK293 cells resulted in lower LC3-II levels compared to wildtype TBK-1, indicating decreased autophagosome formation. These data indicate that both MND-associated optineurin and TBK-1 can dysregulate autophagy, further verifying the importance of intact autophagy machinery in MND.

Chitra Vinnakota (The University Of Auckland)

EXTRASYNAPTIC ALPHA 5 TYPE GABAA RECEPTORS AS THERAPEUTIC TARGETS FOR ALZHEIMER’S DISEASE

Vinnakota C1, Govindpani K1, Calvo-Flores Guzman B1, Tate WP2, Waldvogel HJ1, Faull RLM2, Kwakow A1
1. Centre for Brain Research, University of Auckland, Auckland, New Zealand.
2. Department of Biochemistry, University of Otago, Dunedin, Otago, New Zealand.

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia in elderly patients. Current medications only provide temporary, symptomatic relief to some patients; no cures or disease modifying therapies have yet been discovered for AD. g-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the brain and plays a key role in regulating neuronal excitability. There is some evidence that the GABAergic system undergoes remodelling in AD and thus might represent an important therapeutic target. The compound, 3-(5-Methylisoxazol-3-yl)-6-[(1-methyl-1,2,3-triazol-4-yl)methoxy]-1,2,4-triazolo[3,4-a]phthalazine acts as an inverse agonist of GABAA receptors containing the α5 subunit and has displayed cognition enhancing properties in previous studies. Further investigations are warranted to determine the compound’s mechanisms of action. This study aimed to characterise the effects of the compound on amyloid beta (Ab1-42)-induced molecular and cellular changes in an in vitro AD model. Mouse primary hippocampal cultures were exposed to either Ab1-42, the compound and Ab1-42 or vehicle and changes in cell viability were assessed. Treatment with 1nM Ab1-42 caused 46% cell death. However, Ab1-42-induced cell loss was reduced to 32% (p<0.05, compared to the vehicle treated group) following 6h treatment with 100nM of the compound. Lower concentrations of the compound (0.3nM, 3nM, 30nM) were not effective at increasing cell viability. In summary, this compound might hold neuroprotective potential and represent a new therapeutic avenue for treating AD. (223 words).

This work was supported by the Aotearoa Foundation; Centre for Brain Research, University of Auckland, the Health Research Council of New Zealand; Brain Research New Zealand; Auckalnd Medical Foundation; Otago Medical School
and the Department of Physiology, University of Otago.
Kaitlin Wolfe (University of Otago)

ANTI-INFLAMMATORY CHARACTERISTICS EXHIBITED BY HETEROCYCLIC CYCLOHEXANONE CURCUMIN DERIVATIVES AGAINST LPS-INDUCED INFLAMMATION

Wolfe KM1,2, McGregor Al2,3, Kerr DS1,3.
1. Department of Pharmacology and Toxicology, School of Medical Sciences, University of Otago, Dunedin, NZ. 2. School of Pharmacy, University of Otago, Dunedin, NZ. 3. Brain Health Research Centre, University of Otago, Dunedin, NZ.

Curcumin, a natural polyphenol, has been studied thoroughly for its various medicinal properties. The instability and fast metabolism of curcumin has necessitated the development of curcumin derivatives. A series of heterocyclic cyclohexanone curcumin derivatives (RL compounds) have been created to improve the solubility and pharmacokinetic limitations of curcumin. This study aimed to evaluate toxicity and anti-inflammatory effects of 5 RL compounds (RL66, RL71, RL91, RL118, and RL121) against LPS-induced inflammation by measuring extracellular field potentials in hippocampal slices. Hippocampal slices were randomly allocated into 3 experimental groups: LPS, LPS with RL, and untreated controls. Hippocampal slices were incubated in LPS for a minimum of 3 hours before undertaking electrophysiology assessment. Input/output and paired pulse paradigms were used to assess neuronal excitability, response threshold, maximal neuronal response, and local circuit inhibition in CA1 region. Slices that were co-incubated with RL66 and RL121 showed significantly reduced LPS-induced damage (p<0.05). RL121 showed significant improvement of slice health greater than control slices at both 5uM and 20uM doses, indicating possible neuroprotective potential. The results of this study show that RL66 and RL121 have promising anti-inflammatory characteristics that could be beneficial against brain inflammation.

Vanessa Helena Brait (The Florey Institute of Neuroscience and Mental Health)

DOES STROKE INDUCE REMOTE BRAIN ATROPHY AND COGNITIVE DEFICITS IN MICE?

Brait VH1, Wright DK1, O’Brien KR1, Johnston LA1,2, Thompson LH1, Jackman KA1, Nithianantharajah J1, Brodtmann A1.
1. The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Victoria, Australia. 2. Department of Biomedical Engineering, University of Melbourne, Parkville, Victoria, Australia.

There is a strong link between ischemic brain injury and dementia. In the clinic, remote brain atrophy after ischemic stroke has been observed to be associated with cognitive dysfunction. We aimed to test this clinical observation in animal models, by investigating whether remote brain atrophy and cognitive deficits occur after stroke in mice employing the middle cerebral artery occlusion (MCAO) model. Male C57Bl/6J mice were exposed to a 30-minute intraluminal filament-induced MCAO. T2-weighted MRI scans (Bruker Biospin 4.7T) were performed at baseline, and at 1, 4, 12, 24, 36 and 48 weeks post-stroke. Regions of interest were manually delineated. Cognitive changes were also assessed using a rodent touchscreen paradigm. Our results show significant atrophy in the ipsilateral cortex at 4, 12, 24, 36 and 48 weeks post-stroke compared with sham-operated mice. We also see significant atrophy in the ipsilateral hippocampus at all time-points from 4 weeks post-stroke compared with sham-operated mice, but only when the hippocampus was directly affected by the infarct. Our findings suggest no overt changes in volume in the contralateral cortex or hippocampus post-MCAO. Cognitive impairments were seen early post-stroke and persisted over time.

Paul Dawson (Mater Research Institute, The University Of Queensland)

MgSO4 THERAPY FOR PRETERM INFANTS DOES NOT PREVENT ALL CASES OF CEREBRAL PALSY. ARE SULPHATE MAINTENANCE GENES THE MISSING LINK?

Dawson PA1, Langford R1, Hurrion E1,2
1. Mater Research Institute, The University of Queensland, Woolloongabba, Australia. 2. Mater Mothers’ Hospital, South Brisbane, Australia

The neuroprotective benefit of antenatal MgSO4 for preterm infants is currently attributed to the magnesium. Even though the mechanism is not fully understood, the potential contribution of sulfate has not been considered. Since sulfate is important for modulating brain development, we propose that sulfate deficiency is detrimental to normal neurodevelopment. This is relevant to very/extremely preterm infants that lack the capacity to generate sulfate and become sulfamate deficient. However, some babies become sulphate deficient even though their mothers received MgSO4.
therapy. These findings suggest that sulphate levels in the neonate vary not only due to MgSO₄ exposure, but also to genetic factors that limit sulphate supply from mother to infant. We recruited 36 preterm infants (24-31 wk gestation) and their mothers, and sequenced the genes that are important for placental sulphate transfer (SLC13A4) and maintenance of maternal circulating sulphate levels (SLC13A1 and SLC26A1). We identified 9 non-synonymous variants in SLC13A4, SLC13A1 and SLC26A1, including: 2 loss-of-function variants (N174S and R12X) in SLC13A1; 6 variants in SLC26A1 (Q556R, G368S, L348P, R340C, A277T, A272E); and one variant in SLC13A4 (P451S). We show a trend for decreased (approximately 30%, P=0.0511) plasma sulphate level in infants whose mothers have variants (N174S, R12X and L348P) in the renal SLC13A1 and SLC26A1 genes, and when carrying P451S in the placental SLC13A4 gene. This is the first study to assess genetic variation in sulphate maintenance genes, which may explain why some preterm infants have low blood sulphate levels despite antenatal MgSO₄ administration.

Chao Deng (University of Wollongong)

ANTIPSYCHOTIC MODULATING NMDA RECEPTORS VIA GSK3β-CREB1/β-CATENIN SIGNALLING PATHWAYS IN THE NUCLEUS ACCUMBENS OF JUVENILE RATS

Lian J¹,², Huang X¹,², Deng C¹,².
1. School of Medicine, University of Wollongong, Wollongong, NSW, Australia. 2. Illawarra Health and Medical Research Institute, Wollongong, NSW, Australia.

The second-generation antipsychotic drug olanzapine is widely used to treat schizophrenia and other mental disorders. However, it is associated with adverse obesity and other metabolic disorders. Histamine H1 receptor (H1R) plays an important role in olanzapine-induced weight gain, while muscarinic M3 receptor (M3R) involves in the gluco-metabolic side-effects of olanzapine. The upregulation of sterol regulatory element-binding proteins (SREBP-1 and SREBP-2) are associated with olanzapine-induced abnormal lipid and cholesterol synthesis. The carbohydrate response element binding protein (ChREBP) contributes to improve insulin sensitivity in the liver. Therefore, this study investigated the effect of olanzapine treatment on gluco-lipid homeostasis and their potential mechanisms. To address these issues, female Sprague Dawley rats (201-225g) were treated with olanzapine (2 mg/kg . t.i.d.) for 2,3,4,5,7 and 9 weeks. Plasma glucose, insulin and lipid levels were measured. Hepatic protein levels of SREBP-1, SREBP-2, ChREBP, H1R and M3R were examined by Western Blots. The result shows that olanzapine significantly increased body weight in all treatment durations. Furthermore, olanzapine elevated glucose level in 2, 3, 4 weeks’ treatment, and insulin levels in 4,5 and 9 weeks’ treatment. The upregulation of cholesterol was observed after 2,5,7,9 olanzapine treatment. Olanzapine significantly increased body weight in all treatment periods. Therefore, this study provides evidence underlying the time-dependent effects of olanzapine on weight gain, glucose and lipid metabolic disturbances associated with the activation of H1R, M3R, SREBP-2 and ChREBP pathway in the liver.

Shelley Forrest (University Of Sydney)

CO-EXISTING LEWY BODY DISEASE AND CLINICAL PARKINSONISM IN FRONTOTEMPORAL LOBAR DEGENERATION

Forrest SL¹, Crockford DR¹, Sizemova A¹, McCann H², Shepherd CE²,³, Affleck AJ²,³, Kwok JB²,³,⁴, Kim WS²,³,⁴, McGinley CV¹, Piguet O²,³,⁵, Hodges JR²,³,⁵, Kril JJ¹, Halliday GM²,³,⁴.
1 Discipline of Pathology, Sydney Medical School, University of Sydney, Australia. 2. Neuroscience Research Australia, Sydney, Australia. 3. School of Medical Sciences, University of New South Wales, Australia. 4. Brain and Mind Centre and Sydney Medical School, University of Sydney, Australia. 5. Brain and Mind Centre and School of Psychology, University of Sydney, Australia.

To investigate the prevalence of multiple system atrophy (MSA) pathology and clinically relevant Lewy body disease (LBD) in frontotemporal lobar degeneration (FTLD) to determine if concomitant pathologies explain heterogeneity of clinical symptoms. The degree of heritability and the association of the microtubule associated protein tau (MAPT) genotype, a risk factor for parkinsonism phenotypes, were investigated. All FTLD cases with tau and TAR DNA binding protein (TDP)-immunopositive inclusions held by the Sydney Brain Bank (n=126) were screened for co-existing MSA pathology and LBD (Braak ≥ stage IV). Using Goldman pedigree classification criteria, the degree of heritability was examined in cases with co-existing pathologies. Nine cases had co-existing LBD and were associated with different pathological subtypes including Pick’s disease (n=2), corticobasal degeneration (n=2), progressive supranuclear palsy (n=2) and TDP-Type A (n=3). Concomitant MSA pathology was not identified. FTLD-TDP cases with co-existing LBD had mutations in progranulin (n=2) or an abnormal C9orf72 repeat expansion (n=1). All FTLD-tau cases were sporadic. The
H1H1 MAPT haplotype was found in all cases that could be genotyped (n=5 of 9). Seven cases presented with a predominant dementia disorder, four of which developed parkinsonism. Two cases presented with a movement disorder and developed dementia in their disease course. Age at symptom onset (62±11 years) and disease duration (8±5 years) in FTLD cases with co-existing LBD did not differ from pure FTLD or LBD cases. Co-existing LBD in FTLD comprises a small proportion of cases but has implications for clinical and neuropathological diagnoses, and biomarker identification.

Michael Lardelli (The University Of Adelaide)

RNA-Seq ANALYSIS OF ZEBRAFISH FAMILIAL ALZHEIMER’S DISEASE (fAD) MUTATION-LIKE MODEL BRAINS SUPPORTS A SYSTEM REGULATORY “INVERSION” INTO AN ALZHEIMER’S DISEASE-LIKE STATE

Hin N1,*, Newman N1,*, Pederson S2, Adelson D2, Lardelli M1
University of Adelaide, School of Biological Sciences, Centre for Molecular Pathology1 and Bioinformatics Hub2, (*equal first authors)

Berchtold et al. 2014 (doi: 10.1016/j.neurobiolaging.2014.03.031) discovered that many genes with relatively increased expression in mild cognitive impairment (MCI) brains show, contrarily, decreased expression in Alzheimer’s disease (AD) brains and vice versa. Other studies have supported increased activity in early MCI brains before these become hypometabolic AD brains (e.g. Ashraf et al. 2015, doi: 10.1007/s00259-014-2919-z). Thus the decades-long progression of brains into AD may not follow a linear path. Instead, brains may “invert” into AD. This phenomenon may have confounded our attempts to understand AD pathogenesis. For genetic analysis in vertebrates, zebrafish offer particular advantages for reducing genetic and environmental noise. Families of over 100 siblings can be raised in a common environment. We have created the first models of dominant fAD-like mutations in endogenous zebrafish genes. We exploited large zebrafish families to make detailed transcriptomic analyses of adult brains from young (6 month) and older, infertile (24 month) heterozygous mutants compared to wild type siblings. This revealed a striking pattern of gene expression inversion: genes (predominantly) relatively upregulated in young mutant brains versus wild type brains are subsequently downregulated in older mutant brains versus wild type brains. Expression of FKBPS (associated with decreased MAPT degradation) was notably inverted. Gene Ontology analysis suggests the genes with inverted expression are important in circadian rhythm, PI3K and insulin receptor signalling, stress responses, and transcriptional regulation. Our results support that: 1) Aged fAD brain changes are not linearly consistent with prodromal changes, 2) the AD brain inverts into a discrete, stable transcriptomic state.

Shohreh Majd (Flinders University)

CHRONIC BETA ESTRADIOL AND NOREPINEPHRINE TREATMENT OF DIFFERENTIATED SH-SY5Y CELLS ENHANCES TAU PHOSPHORYLATION AT SERINE396 VIA AMPK BUT NOT MTOR SIGNALING PATHWAY

The incidence of Alzheimer’s disease (AD) in women is significantly higher than men. A substantial elevation in physiological and psychological stress during menopause has been demonstrated, but the impact of stress on AD development during menopause is unclear. Previous studies proposed a role for stress-related hormones such as norepinephrine (NE) in AD progression, while a controversial role for 17-β-estradiol (E2) in modulating tau phosphorylation (p-tau) was suggested. Chronic exposure to E2 is believed to reduce NE release, however, the link between these two hormones and AD at cellular level is remained unknown. Here, we examined whether NE and E2 treatment of differentiated SH-SY5Y cells affected p-tau (Ser396). The involvement of Adenosine Monophosphate Kinase Protein Kinase (AMPK) and target of rapamycin (mTOR) as the possible mechanisms, underlying this effect was also investigated. Subsequent to SH-SY5Y differentiation to mature neurons, we treated the cells with NE, E2, NE plus E2 in presence and absence of Compound C and Rapamycin. Our western blot and immunofluorescent findings showed that exposure to NE and E2 separately, and in combination enhanced p-tau (Ser396), (Ser202)/tau but not (Ser202/Thr205)/tau. Blocking AMPK by Compound C reduced p-tau (Ser396) and (Ser202), while GSK-3β and PP2A activities were remained unchanged. We also found that blocking mTOR by Rapamycin did not change increased p-tau (Ser396) and (Ser202) due to NE+E2 treatment. Collectively, our results suggested that tau hyperphosphorylation due to chronic exposure to NE/E2 was mediated by AMPK, the main energy regulator of the cells during stress with no significant involvement of m-TOR, GSK-3β and PP2A.

Shohreh Majd (Flinders University)

MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS BASED MECHANISM OF CONCURRENT ACTIVATION OF AMPK AND MTOR IN ALZHEIMER’S DISEASE

Australasian Neuroscience Society Annual Scientific Meeting 2017
International Convention Centre, Sydney, December 3rd – 6th 2017
An increasing number of evidence suggests a metabolism dysfunction hypothesis for Alzheimer’s disease (AD). Dysregulation of cellular metabolic axis of adenosine monophosphate kinase protein kinase (AMPK) and mammalian target of rapamycin (mTOR) is widely present in cerebrovascular pathologies, type 2 diabetes and brain ischaemic events such as traumatic brain injury and ischaemic stroke, some of the leading causes for AD. On the other hand the progressive mitochondrial dysfunction due to aging which is associated with abnormal neuronal energy metabolism directly affects AMPK/mTOR signaling pathway. Here we analyzed the presence of activated AMPK and mTOR in different regions of the postmortem AD brains (n=7) and normal subjects (n=5). AMPK was highly phosphorylated (p-AMPK) in most of the neurons in our AD subjects. In spite of AMPK inhibitory control on mTOR, we found the concurrent phosphorylation of mTOR (p-mTOR) in AD patients. Both p-AMPK and p-mTOR showed a high colocalization with phosphorylated tau (p-tau), one of the main hallmarks of AD. Mitochondrial antioxidant enzymes of superoxide dismutase 2 (SOD2), peroxiredoxin 1 (p1) and peroxiredoxin 4 (p4) were substantially decreased in p-AMPK and p-mTOR positive cells, while higher levels of DNA and protein oxidation presented in the same cells. Collectively, we conclude that AMPK and mTOR metabolic axis is highly activated in AD brains. While the inhibitory link between AMPK and mTOR seems to be disrupted, we suggest the oxidative stress as the common underlying mechanism for concurrent activation of AMPK and mTOR in AD.

Shohreh Majd (Flinders University)

COMPOUND C ENHANCES TAU PHOSPHORYLATION AT SERINE396 VIA PI3K ACTIVATION IN AN AMPK AND RAPAMYCIN INDEPENDENT WAY IN DIFFERENTIATED SH-SY5Y CELLS

Aggregation of hyperphosphorylated tau (p-tau) in the form of neurofibrillary tangles (NFT) is a main hallmark for Alzheimer’s disease (AD). Activation of cellular metabolic axis, made of adenosine monophosphate kinase protein kinase (AMPK) and mammalian target of rapamycin (mTOR) have been implicated in generating tau pathology of AD. Thus, blocking either of these two proteins or both, are suggested as the future therapeutic approaches for AD. How and to what level these approaches could be applied, however are not entirely clear. By using Compound C (CC) in this study we showed a substantial decrease in mTOR activity in a rapamycin-independent way without blocking AMPK which increased p-tau (Ser396) but not p-tau (Ser422) in differentiated SH-SY5Y neuroblastoma cells. This elevation was blocked when the cells were treated with 15 µM of LY294002, a specific phosphoinositide 3 kinase (PI3K) inhibitor, suggesting the involvement of PI3K pathway in CC-mediated tau hyperphosphorylation at Ser396. For all groups the activity levels of glycogen synthase kinase-3β (GSK-3β), cyclin-dependent kinase-5 (cdk5) and protein phosphatase 2A (PP2A), the other main kinases and phosphatase responsible for tau phosphorylation/ dephosphorylation remained unchanged. Collectively, our results demonstrate that rapamycin-independent blocking of mTOR enhances p-tau (Ser396) in a PI3K-dependent way, suggesting the careful consideration of future therapeutic approaches for AD which will be based on mTOR inhibition.

Sarah McCann (The University of Edinburgh)

NETWORK META-ANALYSIS FOR PRECLINICAL STUDIES: ANALYSIS OF ANAESTHETIC NEUROPROTECTION IN RODENT MODELS OF ISCHAEMIC STROKE

McCann SK1, Sena ES1, Walker AM2, Archer, DP2.
1. Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK. 2. Department of Anesthesiology, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada.

General anaesthesia may be required for patients undergoing ‘clot-retrieval’ therapy during ischaemic stroke, but little guidance is available on the most appropriate anaesthetic to administer. We evaluated published studies reporting anaesthetic administration in rodent models of stroke to identify the relative impacts of different anaesthetics on neurologic outcome. Meta-analysis is an evidence-synthesis technique routinely used in clinical research to support evidence-based healthcare decisions, but similar uses remain underdeveloped and underutilised in preclinical research. Current approaches are limited to pairwise comparisons (e.g. treatment vs. control). Here, we have applied network meta-analysis, which allows comparison of multiple agents at the same time. This is especially useful in preclinical research where many experimental treatments are often investigated for a condition. 80 publications examining the effects of 16 anaesthetics in rodent models of stroke were identified through systematic review. We performed a random-effects network meta-analysis using the netmeta package in R. The highest ranking anaesthetic in the network was Sevoflurane, which improved neurologic outcome by 37.9% (95%CI 29.2–46.6). There was significant heterogeneity in the network (Q=466.6, p<0.0001) which originated from both within-designs (studies comparing the same treatment and control groups) and between-designs (different treatment and control groups).
DECLINE IN NEUROMUSCULAR SYNAPTIC ADHESION MAY BE ASSOCIATED WITH EARLY MUSCLE WEAKNESS IN PATIENTS SUFFERING FROM MOTOR NEURON DISEASE


1. School of Biomedical Sciences, The University of Queensland, Brisbane, Australia. 2. Kenneth G. Jamieson Department of Neurosurgery, Royal Brisbane and Women’s Hospital Herston, Brisbane, Australia. 3. Neurology Department, Royal Brisbane & Women’s Hospital Herston, Brisbane, Australia. 4. University of Queensland Center for Clinical Research, Herston, Brisbane, Australia. 5. Queensland Brain Institute, The University of Queensland, Brisbane, Australia. 5. Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane Australia.

Motor neuron disease (MND) is a neurodegenerative disease that involves the degeneration of the neuromuscular junction (NMJ) and the progressive death of upper and lower motor neurons within the central nervous system. Whether there is a primary role for muscle in pathogenesis is controversial. Our preliminary work in MND model mice (SOD1G93A and TDP43G38S1K) revealed deficiencies in NMJ adhesion molecules such as the synaptic laminins alpha-4 and -5, prior to muscle weakness and motor neuron death. The aim of this study was to examine the expression and localisation of synaptic laminins alpha-4, -5 and beta-2 at NMJs in asymptomatic leg muscles from early diagnosed MND patients compared to non-MND donors. Vastus Lateralis (leg) muscle biopsies from MND (sporadic and familial; n = 3-5) patients and non-MND donors (n = 3-5) were obtained and processed for immuno-histology for the expression and localisation of synaptic laminins with respect to postsynaptic acetylcholine receptors (AChRs). Results showed altered localization and decreased expression of laminins alpha-4, -5, and beta-2 at NMJs from MND muscle compared to non-MND muscle. These observations support the idea that declines in NMJ adhesion molecules results in the instability of the NMJ, which turn contributes to the loss of neuromuscular connections in MND.

Francisco Pan-montijo (University Hospital Munich)
ORAL ROTENONE TREATMENT DOES NOT INDUCE PARKINSON’S DISEASE PATHOLOGY PROGRESSION IN THE ABSENCE OF ALPHA-SYNUCLEIN IN MICE.

Dening Y1,2, Straßl T1,3, Ruf V2, Schmidt F2, Levin J1, Herms J2, Dieterich M1,3, Giese A2, Pan-Montejo F1,3
1. Department of Neurology, University Hospital, Ludwig-Maximilians University Munich, Marchioninstraße 15, 81377 Munich, Germany.
2. Center of Neuropathology, Ludwig-Maximilians University Munich, Feodor-Lynen-Straße 23, 81377 Munich, Germany.
3. SyNergy - Cluster of Systems Neurology, Munich, Germany

According to Braak’s hypothesis, PD pathology starts in the enteric nervous system (ENS) and the olfactory bulb and progresses through the nervous system until it reaches the brain. We have previously shown that the local action of rotenone on the ENS induces the appearance of PD-like pathology and its progression to the substantia nigra (SN) in mice and that this progression is dependent on the integrity of the connecting nerves. In vitro and in vivo studies suggest that this progression could be based on the transsynaptic transport of alpha-synuclein (ASYN) and a seeding effect of ASYN on the host neuron. In order to test these hypotheses, we treated ASYN knock-out mice with oral administered rotenone for 2 and 4 months respectively. Motor function was analyzed using a rotarod test and the number of dopaminergic neurons in the SN counted using a stereological software on immunofluorescent sections. Our results show that, in the absence of endogenous ASYN, rotenone treatment does not induce motor dysfunction or the degeneration of the dopaminergic neurons in the substantia nigra. Using in vitro primary dopaminergic neurons, we show that the toxicity of extracellular ASYN oligomers on dopaminergic neurons in vitro is independent of the presence of endogenous ASYN and due to the interaction between ASYN and mitochondria. Our results confirm that endogenous ASYN is crucial for the progression of the disease through the nervous system but suggest that this effect is triggered by a direct interaction of endocytosed ASYN with the host’s mitochondria and not a seeding-effect.

Steven Petratos (Monash University)

NOGO RECEPTOR 1 DELETION IN AXONS HALTS AXONOPATHY AND DEMYELINATION DURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Lee JY1, Thomas S1, Kim MJ1, Aui PM1, Harvey A2, Strittmatter SM1, Petratos S1.
1 Central Clinical School, Department of Medicine, Monash University, Prahran, Victoria, 3004, Australia; 2 The University of Western Australia, Crawley, Western Australia, 6009; 3 Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, CT 06536, USA

In this study we investigated how neuronal deletion of ngr1, may prevent axonal degeneration during experimental autoimmune encephalomyelitis (EAE). Conditional deletion of ngr1 in axons was produced by intraocular injection of AAV2 encoding Cre (AAV2-iCre-eGFP) in ngr1flx/flx mice. Conversely, re-introduction of NgR1 in axons was produced by intraocular injection of AAV2 encoding full-length mouse NgR1 (AAV2-NgR1-eGFP) in ngr1−/− mice. Animals were induced with EAE and culled at the peak stage of disease. The degree of axonal degeneration and demyelination were assessed by immunohistochemistry. Molecular mechanisms of axonal degeneration were verified by western immunoblotting. Axonal degeneration is limited in AAV2-iCre-eGFP injected ngr1flx/flx (mean ± SEM: 21.53 ± 0.7954% morphologically identifiable degenerative axons). Whereas, significant axonal damage is found in AAV2-NgR1-eGFP injected ngr1−/− optic nerves during EAE (19.25 ± 1.298%) when compared with AAV2-eGFP transduction [controls] (mean ± SEM: 7.734 ± 0.3381%). As a corollary, the preservation of myelin integrity was a prominent feature in AAV2-iCre-eGFP injected ngr1flx/flx, whereas significant demyelination was found in AAV2-NgR1-eGFP injected ngr1−/− optic nerves (% of demyelination normalized to naive AAV2-eGFP transduced optic nerves of ngr1flx/flx mice: ~ 42.38% in AAV2-eGFP-iCre versus 63.39% AAV2-eGFP injected optic nerves of ngr1−/− mice at the peak stage of EAE). Furthermore, the interaction between the axonal motor protein, kinesin-1 (KIF5) and collapsin response mediator protein 2 (CRMP-2) was reduced in AAV2-NgR1-eGFP injected ngr1−/− optic nerves. Our data suggest that NgR1 governs axonal degeneration in the context of EAE through phosphorylation of CRMP-2, abrogating axonal vesicular transport and inducing demyelination.

Abdur Rahman (College of Life Sciences, Kuwait University)

SYNERGISTIC NEUROTOXIC EFFECTS OF LEAD AND QUINOLINIC ACID ON CULTURED RAT EMBRYONIC HIPPOCAMPAL CELLS: PROTECTION BY MEMANTINE

Rahman A1, AlQenaie S1, Rao MS2, Guillemin G1
1Department of Food Science and Nutrition, College of Life Sciences, Kuwait University
Quinolinic acid (QA), an excitotoxic metabolite of the kynurenine pathway of tryptophan metabolism, is produced in response to inflammation and oxidative stress. Lead (Pb) a neurotoxic heavy metal, causes oxidative stress and thus may produce neurotoxicity by increasing QA production. In this study we investigated the in vitro synergistic neurotoxic effects of Pb and QA, and whether these effects could be abrogated by the NMDA receptor antagonist, memantine. We treated primary cultures of embryonic hippocampal cells from Wistar rats with different concentrations of Pb and/or QA without and with memantine. Cell viability was determined by MTT and crystal violet assays. Cell cycle, apoptosis and necrosis were analyzed by flow cytometry. For cell cycle analysis, cells were stained with propidium iodide (PI); for apoptosis and necrosis analysis cells were stained with Annexin-V and PI. Number of neurons and astrocytes were determined by immunostaining the cultures with β3-Tubulin (Tuj1) and glial fibrillary acidic protein (GFAP) respectively. Pb at 20 µg/dL and QA at 500 nM showed significant neurotoxic effects, as evidenced by decreased cell viability, increased apoptosis and mitosis, and decrease in the number of both astrocytes and neurons. At lower doses of both Pb and QA, significant synergistic neurotoxic effects were observed. Memantine (500 nM) was largely protective against the neurotoxic effects of both Pb and QA. These results suggest that the neurotoxic effects of both Pb and QA involve NMDA receptor activation and that memantine may be protective against Pb- and QA-induced neurotoxicity.

John Van Horn (Usc Mark And Mary Stevens Neuroimaging And Informatics Institute)

NEUROIMAGING-BASED CLASSIFICATION OF MCI FOLLOWING TBI DURING YOUTH


Laboratory of Neuroimaging, Mark & Mary Stevens Neuroimaging and Informatics Institute, Keck School of Medicine of USC, University of Southern California, Los Angeles, CA USA

Despite reasonable recovery and cognitive reserve [1], traumatic brain injury (TBI) experienced in youth may result in later life cognitive decline and dementia [2-4]. Here, we present the case of an older patient with a history of pediatric TBI, whose own MRI and DTI data were systematically compared to data from a large-scale collection of older healthy subjects and those having cognitive impairment and dementia. “Patient IH”, is a 67 year old female, hit by a car during childhood, experiencing severe head injury. Successful recovery ensued followed by exemplary adult life of philanthropy and community service. She now reports memory loss and other cognitive deficits. The ADNI-2 neuroimaging protocol was performed on a GE 3 Tesla MRI scanner. Imaging include T1-SPGR (1mm³) and 64-direction diffusion tensor imaging...
Traumatic brain injury (TBI) induces a robust neuroinflammatory response, which is never or poorly resolved. Non-resolving inflammation after TBI is believed to potentiate secondary injury and neurodegenerative changes, and also overall cognitive impairment. Intravenous immunoglobulin (IVIG), has been proposed as a potential therapy to modulate the harmful neuroinflammatory response following neurotraumatic events (Brennan et al. 2016); however, whether IVIG therapy can also improve the cognitive outcome from TBI remains largely unknown. To investigate this, we subjected C57Bl/6J mice to a moderate unilateral controlled cortical impact or sham surgery, administered either IVIG or proline vehicle (subcutaneous injection 30 min, 4 days, and 8 days after surgery), and subsequently examined spatial memory.

We found that IVIG therapy significantly improved Y-maze performance 12 days post-TBI (92% increase in time spent in novel arm, P = 0.021; 167.6% increase in ratio of novel:training arm, P = 0.043), with IVIG-treated TBI mice being comparable to sham controls (P > 0.99). We also explored whether IVIG therapy influenced the neurogenic response in the hippocampus following TBI. We found that IVIG therapy resulted in significantly greater numbers of DCX+ immature neurons (54% increase, P = 0.006) and supported the generation of newborn DCX+/BrdU+ immature neurons after TBI (48% increase, P = 0.015; BrdU injected bi-daily 1, 2, and 3 days post-TBI). Collectively, our data suggests that IVIG therapy improves the cognitive outcome and promotes hippocampal neurogenesis after TBI, presenting a putative therapy to reduce cognitive deficits arising from such brain injuries.
ALS/MND and FTD are characterised pathologically by accumulation of TAR DNA binding protein of 43 kDa (TDP-43) in the cytoplasm of affected neurons. To identify instigators of disease pathogenesis, we have studied neuronal cells expressing normal and mutated forms of TDP-43 in addition to a doxycycline-inducible transgenic mouse model in which cytoplasmically-targeted TDP-43 is expressed in neurons of the brain and spinal cord. These mice develop pathological disease features (accumulation of insoluble phosphorylated TDP-43, neuron loss, muscle atrophy and denervation) in conjunction with a progressive motor phenotype reminiscent of ALS (limb weakness, loss of coordinated movement, weight loss, and paresis leading to death). We performed quantitative label-free mass spectrometry (SWATH-MS) of soluble and insoluble proteins and targeted qPCR array analyses of RNA extracted from both neuronal cell cultures and brains and spinal cords of the TDP-43 transgenic mice at various disease stages. We have thereby identified a suite of biochemical disease signatures including dramatic up-regulation of multiple transcripts and statistically significant (>1.5-fold) increases and decreases in proteins related to numerous disease-relevant pathways, which were confirmed using target-specific qPCR, immunoblotting and fluorescence confocal microscopy. Importantly, many of the identified changes occurred prior to neurodegeneration. These findings provide a basis for continued mechanistic study of new potential modulators of TDP-43 pathology and neurodegeneration, for future development of disease treatments.

Alina Arulsamy (University Of Adelaide)

TIME COURSE OF FUNCTIONAL OUTCOMES POST-TRAUMATIC BRAIN INJURY: A POSSIBLE LINK TO DEMENTIA

Arulsamy A1, Corrigan F1, Collins-Praino LE1.
1. Translational Neuropathology Lab, Discipline of Anatomy and Pathology, Adelaide Medical School, University of Adelaide, Adelaide, Australia.

Although traumatic brain injury (TBI) is a risk factor for the development of dementia, the time-course of functional impairment post-injury and whether this differs depending on the severity of the original impact is not yet known. The current study investigated functional impairments following either mild TBI (mTBI), repetitive mild TBI (rmTBI) and moderate-severe TBI (msTBI) induced with the Marmarou model of impact-acceleration. A behavioural battery was conducted at 7 days, 1, 3 or 6 months post-injury (n=14-30/group), with preliminary findings at 12 months post-injury (n=2-6 per group). Assessment of general locomotor activity in the open field found no significant differences relative to sham animals in any of the TBI groups at any time-point post-injury. Anxiety was measured by time spent in the open arm of the elevated plus maze. At one month post-injury mTBI animals spent significantly more time in the open arm, which returned to sham level by 3 months, before increasing again at 6 months post-injury (p<0.05). No significant differences in relation to shams were seen in the rmTBI animals at any timepoint, whilst the TBI group also showed increased anxiety, but only at 6 months post-injury. Preliminary assessment of executive function at 12 months post-injury by the 5 choice continuous performance test (5C-CPT) suggests that rmTBI and msTBI have poor accuracy and high omission in the task compared to sham and mTBI animals. This indicates that the type of functional impairments seen post-injury are dependent on the nature of the original insult and can develop over time.

Britt Berning (Macquarie University)

FRAGMENTATION OF GOLGI APPARATUS OCCURS EARLY IN DISEASE IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

Berning BA1, Mehta P1, Riddell W1, Le S1, Krisp C1, Molloy M2, Lee A1, Atkin JD1, Walker AK1
1Centre for MND Research, Department of Biomedical Sciences, Macquarie University, Sydney, Australia.
2Australian Proteome Analysis Facility, Macquarie University, Sydney, Australia.

Aggregation of TAR DNA-binding protein of 43 kDa (TDP-43) in the cytoplasm of neurons is a hallmark of amyotrophic lateral sclerosis (ALS). Our transgenic mouse model uses doxycycline-suppressible expression of human TDP-43 containing a mutated nuclear localisation sequence under a neuron-specific promoter. This model recapitulates various aspects of ALS including neuronal cytoplasmic TDP-43 pathology in brain and spinal cord, and progressive motor dysfunction. The Golgi apparatus is a sub-cellular organelle involved in protein modification and intracellular transport. Previous studies showed Golgi fragmentation in motor neurons of post-mortem ALS patient tissue. To investigate whether this occurs early in ALS development we examined brains and spinal cords of transgenic mice and their non-transgenic littermate controls at different stages of disease (pre-symptomatic, symptom onset, mid- and end-stage). SWATH mass spectrometry revealed changes in levels of numerous proteins related to function of the Golgi apparatus. In transgenic mice, confocal microscopy and Golgi-immunolabelling demonstrated fragmentation into smaller stacks in neurons expressing cytoplasmic human TDP-43 in motor cortex and spinal cord. This was particularly evident in large...
alpha motor neurons of the spinal cord ventral horn, which are vulnerable to degeneration in ALS. Fragmentation preceded symptoms of motor impairment and development of TDP-43 pathology and overt neurodegeneration. Controls showed no evidence of TDP-43 inclusions and the Golgi apparatus remained intact. Breakdown of the Golgi complex may be an early indicator of disease. Preventing the disassembly of the Golgi complex by pharmacological targeting of Golgi-related proteins therefore represents a promising avenue for the development of ALS therapeutics.

Ling Chen (The University of Hong Kong)

MITOCHONDRIAL DYNAMICS AND OXIDATIVE STATUS IN CENTRAL NERVOUS SYSTEM OF LEIGH SYNDROME NDUFS4 KNOCKOUT MICE

Ling Chen1,2, Qizhou Lian1,2,3
1. Department of Medicine, the University of Hong Kong, Hong Kong SAR, P. R. China; 2. Shenzhen Institutes of Research and Innovation, the University of Hong Kong, P. R. China; 3. School of Biomedical Sciences, the University of Hong Kong, Hong Kong SAR, P. R. China;

Objective: Leigh syndrome (LS) is a progressive neurodegenerative disease mostly occurring in infancy or in young children. It is caused by mitochondrial dysfunction due to genetic defects in the complex I subunit Ndufs4. Here, we characterized the phenotypes related to mitochondrial oxidative stress and mitochondrial morphology of Ndufs4 knockout (KO) and wildtype (WT) mice in vitro and in vivo.

Methods and Results: We isolated and cultured primary neural progenitor cells (NPCs) derived from E12 Ndufs4 KO and WT embryos. Compared to wild type NPCs, elevated numbers of fragmented mitochondria, accompanied by an increase in reactive oxygen species (ROS) production were observed in KO NPCs (n = 6 per group; P < 0.001). We further analyzed the histological changes in vivo at day 50. We found that inflammation, measured by Iba-1 and GFAP immunostaining, and ROS levels assessed by DHE staining, was remarkably enhanced in the region of cerebellum of KO mice compared with WT littermates (n = 5 per group; P < 0.05). By electron microscope ultrastructure analysis, we determined that the olfactory bulb of KO mice (day 50) displayed disorganized mitochondrial cristae and vacuolar degeneration inside the organelle, whereas age-matched WT mice mitochondria retained normal morphology.

Conclusion: These results implicate aberrant mitochondrial dynamics and mitochondrial oxidative stress in LS Ndufs4 KO mice, suggesting that intervention studies aimed at reversing the improper dynamics and excessive oxidative stress are warranted.

Dominik Draxler (Monash University, Australian Centre for Blood Diseases)

SELF-REACTIVITY AND IMMUNOSUPPRESSION POST TRAUMATIC BRAIN INJURY: THE ROLE OF THE PLASMINOGEN ACTIVATION SYSTEM

Dominik F. Draxler (1), Maria Daglas (1), Anushka Fernando (1), Gryselda Hanafi (1), Fiona McCutcheon (1), Adam Galle (1), Heidi Ho (1) Amanda E Au (1), Magdalena Plebanski (2), Maithili Sashindranath (1), Robert L. Medcalf (1)
1) Molecular Neurotrauma and Haemostasis, Australian Centre for Blood Diseases, Monash University, Melbourne VIC, Australia
2) Department of Immunology, Monash University, Melbourne VIC, Australia

Traumatic brain injury (TBI) has been linked to an increased risk for CNS-related autoimmune diseases. Dendritic cells (DCs) are potent antigen presenting cells whose role in TBI-related self-reactivity and immunosuppression is largely unknown. Previous work published by our laboratory revealed that co-incubation of DCs with the fibrinolytic protease plasmin impedes the capacity of DCs to induce an immune response. In this project, we investigated the involvement of plasmin in promoting immunosuppression following TBI. Moreover, we assessed whether inhibition of plasmin generation in vivo using tranexamic acid (TXA), as currently tested in clinical trials on trauma, would induce a more robust immune response following TBI. Wild-type (WT) and plasminogen-deficient (PLG-KO) mice were subjected to TBI and administered either saline or TXA. At different time points post-TBI, cervical lymph nodes (cLN) were evaluated for temporal immunological changes. We detected significantly attenuated activation and reduced migration of cDCs to the cLN in WT mice compared with PLG-KO mice one week post-TBI. Only in PLG-KO mice cells from the cLN showed significant reactivity against CNS antigens. Treatment with TXA resulted in increased cellularity in the cLN 1 week post-TBI compared with saline, suggesting enhanced immune activation when plasmin formation is inhibited. However, cellular reactivity against CNS antigens was not significantly modulated by TXA treatment. These results are consistent with the notion of a direct immunosuppressive role of plasmin following TBI via modulation of DC function, yet suggest safety of TXA treatment in TBI with respect to enhancement of self-reactivity.
**THE COMPLEMENT C5A RECEPTOR, CSAR2, PLAYS A PATHOLOGICAL ROLE IN MOUSE MODELS OF NEURODEGENERATIVE DISEASE**

Rui Li, John D Lee, Samantha Levin, Richard Gordon, Trent M Woodruff

1School of Biomedical Sciences, the University of Queensland, Brisbane, Australia

Parkinson’s disease (PD) and Huntington’s disease (HD) are intractable neurodegenerative diseases characterised by progressive motor and cognitive deficits. Recent studies indicate an important role for the complement activation product, C5a, in these diseases, as blockade of C5a-C5aR1 signalling protects against disease progression in mouse models of PD and HD. However, C5a also binds to a second receptor, C5aR2, which has not been as well studied. The aim of this study was therefore to explore the functional role of C5aR2 during neurodegeneration, utilizing a R6/1 transgenic mouse model of HD, and a 6-OHDA neurotoxin-based model of PD. We first identified robust upregulation of C5aR2 in both HD and PD mouse brains. Next, to identify any potential functions for C5aR2, we generated R6/1 mice deficient in C5aR2 (R6/1 x C5aR2-/-), and injected 6-OHDA intrastriatally in C5aR2-/- and wild-type mice. Several behavioural tests were conducted, in a blinded fashion, to assess motor and cognitive function at key disease stages. We found that 6-OHDA injected C5aR2-/- mice had improved performance in both balance beam and amphetamine-induced rotation tests compared to wild-type mice. Similarly, genetic deletion of C5aR2 in R6/1 transgenic mice resulted in improved motor function and cognitive performance in rotarod, balance beam, grip strength and Y-maze tests. This was associated with reductions in glial activation markers in brain regions undergoing degeneration. These studies therefore collectively demonstrate a pathogenic role for C5aR2 in mouse models of HD and PD, and identify a potentially novel complement therapeutic target to mitigate neuropathology in these diseases.

**SPATIOTEMPORAL RELATIONSHIPS BETWEEN PATHOLOGICAL CHANGES AND MICROGLIAL SUBTYPES IN DIFFERENTIALLY AFFECTED AREAS OF THE ALZHEIMER’S DISEASE BRAIN.**

Paasila PJ1, Davies D2, Goldsbury CS2, Sutherland GT1

1Discipline of Pathology, Charles Perkins Centre, Sydney Medical School, University of Sydney, Sydney; 2Brain & Mind Research Institute, Sydney Medical School, University of Sydney, Sydney.

The pathogenesis of sporadic Alzheimer’s disease (AD) is largely unknown. Belatedly affected areas of the AD brain at post-mortem and areas with AD pathology in preclinical individuals may provide opportunities to model the natural history of AD. The evolving roles for microglia in the healthy brain suggests that their dysfunction could have been previously underestimated in AD pathogenesis. This immunohistochemical investigation sought to quantify tau and beta-amyloid pathology and microglial morphological subtypes in three increasingly-affected areas of the AD brain: the primary visual cortex (PVC), the superior frontal gyrus (SFG) and the inferior temporal gyrus (ITG). Four subtypes of microglia were observed and described as ramified, hyper-ramified, de-ramified and fragmented. Some AD had large numbers of fragmented microglia, while other cases contained very few, irrespective of the region explored. In contrast there was a strong inverse correlation between fragmented microglia and brain pH with lower brain pH values reflecting greater agonal period effects. A direct correlation was also seen between de-ramified microglia and age. Immunofluorescent microscopy was used to look at local microglia subtypes around diffuse, cored and neuritic plaques and tangles. In some cases, fragmented microglia co-localised with cored plaques but there were no clear relationships with tau pathology. This study shows microglial morphologies differ widely between individual AD cases suggesting potential heterogeneity in the role of microglial dysfunction in the pathogenesis of AD.

**IMPAIRED BEHAVIOURAL FLEXIBILITY AFTER REWARD DEVALUATION IN PEOPLE WITH OBSESSIVE-COMPULSIVE DISORDER: VENTROMEDIAL PREFRONTAL CORTEX HYPOACTIVITY AND CORTICOSTRIATAL DISCONNECTION.**

Perkes IE1-6, Morris RW1,5,6, QUAIL S1, Hazell PL2-3, Balleine B W1,6

1. Brain & Mind Centre, The University of Sydney, NSW, Australia. 2. Discipline of Psychiatry, The University of Sydney, NSW, Australia. 3. Sydney Local Health District, NSW, Australia. 4. New South Wales Institute of Psychiatry, NSW, Australia. 5. Australian Research Council, Centre for Cognition and its Disorders. 6. School of Psychology, UNSW, NSW, Australia.
Obsessive-compulsive disorder (OCD) is common, disabling, and current treatments are inadequate for half of people with OCD. Understanding pathophysiology will enable development of better treatments. Painful skin damage resulting from handwashing fails to reduce obsession-prompted compulsive handwashing in people with OCD. OCD imaging studies repeatedly implicate the caudate.

Repetitive behaviour despite changing outcomes characterises OCD, autism, eating disorders, substance use, schizophrenia, and dementia. With variable clinical phenotypic operationalisation, we do not know the neural substrates of repetitive behaviour within or between clinical diagnoses. Outcome devaluation, a cross-species test of behavioural flexibility, provides physiological and anatomical specificity. Anatomical correlates of behaviours targeting valued outcomes after devaluation include the caudate and medial prefrontal cortex (PFC).

We found adolescents (n=21) with OCD, compared with controls (N=20), were less able to achieve preferred outcomes after devaluation. That impairment was associated with ventromedial PFC (vmPFC) hypoactivity relative to controls. vmPFC-seeded DTI analysis found reduced tract strength to the caudate in adolescents with OCD. There was also a positive correlation between vmPFC-caudate tract strength and difference between valued and devalued actions. The current finding in adolescents with OCD contributes to transdiagnostic research of behavioural flexibility in brain disorders. Behavioural insensitivity to outcome devaluation tests, and associated vmPFC changes, are also present in adults with schizophrenia. We now need to understand points of difference that give rise to various phenotypic expressions.

Paulo Pinares-garcia (Hudson Institute of Medical Research)

**PRE-CLINICAL TESTING OF NIGRAL SRY INHIBITION IN THE CHRONIC ROTENONE RAT MODEL OF PARKINSON’S DISEASE**

Pinares-Garcia PC1,2, Loke H3, Thyagarajan DT3, Harley VR1,2, Lee j1,2

1Brain and Gender, Hudson Institute of Medical Research, Clayton, VIC, 2Department of Anatomy and Developmental Biology Monash University, Clayton, VIC, 3Department of Neuroscience, Monash Medical Centre, Clayton, VIC

Whilst the cause of dopamine (DA) cell loss in Parkinson’s disease (PD) is unknown, the male-sex is a strong risk factor. The incidence of PD is 2-fold higher in males, and disease progression more rapid in males than females. Aside from the protective actions of sex hormones, growing evidence suggests that sex-specific genes contribute to this male-bias in PD. We previously showed that the Y-chromosome gene, SRY, co-localises with DA neurons, where it regulates DA biosynthesis and motor function in males. Here, we investigated the regulation and function of nigral SRY in the chronic rotenone rat model of PD, which closely resembles the progressive behavioural decline and pathology of clinical PD. Repeated daily intraperitoneal rotenone injections (2.75mg/kg) for 10 days induced motor deficits in the rearing test (P<0.05 vs day 0, one-way ANOVA) and distance travelled (P<0.01 vs day 0) and velocity (P<0.05 vs day 0) in the open-field test. Rotenone-induced motor deficits were associated with increased nigral Sry mRNA expression at day 10 (+263%, P<0.05 vs day 0, one-way ANOVA). Remarkably, reducing Sry expression in male rats, via repeated nigral Sry antisense oligonucleotide infusions, at 4 weeks post rotenone treatment prevented the progression of motor deficits in the rearing test (P<0.001 vs sense-control, two-way ANOVA). These data indicate that dysregulation of SRY directs a novel genetic mechanism of nigral cell death in males, and that inhibition of nigral SRY may be a novel therapeutic target to slow the progression of PD in males.

Shalini Rao (Florey Institute of Neuroscience And Mental Health)

**CHARACTERISING THE EFFECT OF IRON MODULATION ON TAU**

RAO SS, FINKLESTEIN DI, ADLARD PA

*The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria, Australia*

**Introduction:** There is an emerging link between the accumulation of iron in the brain and abnormal tau pathology in a number of neurodegenerative disorders, such as Alzheimer’s disease (AD). Iron chelators, such as deferiprone and deferoxamine, have been reported to suppress tau hyperphosphorylation and alleviate the phenotypes of various animal models. However, how these compounds affect tau and its phosphorylation is not well understood. **Method:** Mouse primary cortical neurons (DIV 21) were treated for 6hrs with either iron (II) sulfate (50µM), deferiprone (100µM) or deferoxamine (100µM) to manipulate cellular iron levels. **Results:** Here we show that the levels of phosphorylated tau (Ser396) are reduced by 50% (p=0.0024) in cells treated with iron chelators compared to controls. Interestingly deferoxamine reduced iron levels by 20% (p=0.0013), whereas deferiprone did not reduce iron levels compared to controls, as assessed by inductively coupled plasma mass spectrometry (ICP-MS). Western blot analysis revealed...
CHARACTERISATION OF CHANGES TO INHIBITORY NEURONS IN THE PIRIFORM CORTEX IN A KINDLING MODEL OF EPILEPSY

Robertson JJ¹, Bekkers JM¹
1. Eccles Institute of Neuroscience, John Curtin School of Medical Research, The Australian National University, Canberra, Australia

The piriform cortex has been shown to be important for generalisation of epileptic seizures. A small number of studies suggest that epilepsy causes cell death in the piriform cortex. However, the effect of epilepsy on specific types of inhibitory cells has not previously been investigated. This study aimed to determine the effect of electrical kindling on the major inhibitory cell types in the piriform cortex. Experiments used GAD67-GFP+ mice, which allowed us to identify inhibitory neurons by their expression of GFP. Mice were kindled using olfactory bulb electrical stimulation, and compared to control and sham-kindled mice. Animals were perfusion-fixed and 100µm thick slices prepared. Slices were immunohistochemically processed to detect markers, including parvalbumin, calbindin, vasoactive intestinal peptide and somatostatin, each of which is associated with a known type of interneuron. Sections were imaged using a Nikon confocal microscope and cells counted using a custom ImageJ macro. We found a 13% overall reduction in the number of inhibitory neurons in the piriform cortex in the kindled mice compared to the sham and control (p=0.009, n=22,614 neurons, n=216 slices, n=12 mice). We found a specific reduction in the number of bitufted cells, regular-spinging multipolar cells and layer 2 neurogliaform cells (p<0.05). However, the number of horizontal cells, fast-spinging multipolar cells and layer 1 and layer 3 neurogliaform cells remained unchanged (p>0.05). This study provides the first rigorous characterisation of the effect of epilepsy on different inhibitory cell types and may provide insight into the mechanism of seizure generalisation through the piriform cortex.

Jennifer Robertson (ANU)

A COMPARISON OF THE NEUROINFLAMMATORY RESPONSE AFTER SPINAL CORD INJURY IN ADULT AND INFANT RATS SHOWS SIGNIFICANT DIFFERENCES IN NATURE AND MAGNITUDE

Sutherland T., O'Brien B, Ricafrente A., Gorrie C.
School of Life Sciences, University of Technology Sydney, Australia

Traumatic spinal cord injury (SCI) results in tissue damage causing both motor and sensory dysfunction below the level of the lesion. The immune and inflammatory response plays a significant role in the progression of this injury. We have previously used immunohistochemistry to show a decreased inflammatory response following SCI in neonatal rats at both acute and chronic time points. In the current study a mild contusion SCI was induced using a NYU impactor in adult (10 weeks) and infant (P7-9) Sprague-Dawley rats and compared to sham surgery controls (n=98). The spinal cord was removed fresh 1hr, 24hrs and 1wk post-injury and assessed using flow cytometry, to quantitate different phenotypes of macrophages, neutrophils and T-lymphocytes within the injured tissue, and multiplex cytokine ELIZA. This model showed significant differences in the nature and progression of the cellular and molecular inflammatory response between infants and adults. This manifested as greater leukocyte numbers in the injured adult cord than the infants (P< 0.05), and higher M1-like than M2-like macrophage percentages of the total leukocyte population (P< 0.05); a trend that was reversed in the infants. Pro-inflammatory cytokine expression was higher and more sustained in the adults than the infants, while the infants showed a steady increase in IL-4 and IL-13 as well as sustained IL-10 expression. This re-enforces our previous studies suggesting the inflammatory response is significantly different in developing and mature spinal cords; the infant response appears more balanced and potentially more beneficial to injury resolution than that displayed by the adults.

Tesfaye Wolde Tefera (The University of Queensland)

NEURONAL GLUCOSE METABOLISM IS IMPAIRED WHILE ASTROCYTIC TCA CYCLING IS UNAFFECTED IN THE

deferiprone treated cells had higher protein levels of phosphorylated glycogen synthase kinase 38 (p-GSK3β at Ser9, inactive form) and protein phosphatase 2A (PP2A), subunit b (the regulatory subunit), which are a key tau kinase and phosphatase, respectively. Conclusion: We have identified a potential mechanism by which iron chelators reduce the phosphorylation of tau and are currently validating these findings in a mouse model of tauopathy (rTg(tauP301L)4510). This will be an important step in understanding the potential role of iron modulation in the treatment of tauopathies, which includes conditions such as Progressive supranuclear palsy, Frontotemporal dementia and AD.

Niko, Bekkers JM, O'Brien B, Ricafrente A., Gorrie C.
Eccles Institute of Neuroscience, John Curtin School of Medical Research, The Australian National University, Canberra, Australia

This will be an important step in understanding the potential role of iron modulation in the treatment of tauopathies, which includes conditions such as Progressive supranuclear palsy, Frontotemporal dementia and AD.
SUPEROXIDE DISMUTASE 1 (HSOD1G93A) MOUSE MODEL

Tefera TW1, Borges K1.
1School of Biomedical Sciences, Department of Pharmacology, The University of Queensland, Brisbane, Queensland, Australia.

Although alterations in energy metabolism are known to modify ALS progression, the specific mechanisms leading to energy deficit and subsequent metabolic failure in this disease are not comprehensively understood. We investigated [1-13C]glucose and [1,2-13C]acetate metabolism in cortex and spinal cord extracts of wild-type and hSOD1G93A mice at symptomatic stages using HPLC, 1H and 13C NMR spectroscopy. Levels of various glycolytic derived metabolites were reduced in cortex, namely [3-13C]lactate, total lactate, [3-13C]alanine and total alanine by 20 to 53% at onset and 14 to 69% at mid stage (p<0.05). Similarly, in the hSOD1G93A mice spinal cord, [3-13C]lactate and total lactate were reduced by 23 to 55% (p<0.05) at both time points. At mid stage, the labelling in [4-13C]glutamate, [4-13C]glutamine, [2-13C]GABA and [3-13C]aspartate from [1-13C]glucose in hSOD1G93A mice cortex was diminished by 44 to 54% (p<0.05). In the spinal cord, the levels of [4-13C]glutamate, [2-13C]GABA and [2-13C]aspartate (p<0.05) were reduced by 37 to 41% at onset. Also, at mid stage reduced labeling was found in [4-13C]glutamate, [4-13C]glutamine, [2-13C]GABA and [2-13C]aspartate (p<0.01) and [3-13C]aspartate by 53 to 73% (p<0.05). These changes could be explained by impairments in the glycolytic pathway and reductions in glucose uptake. Astrocytic metabolism of 13C-acetate was unchanged in both cortex and spinal cord at both time points as evidenced by unaltered incorporation of 13C in [4,5-13C]glutamate and [4,5-13C]glutamine from [1,2-13C]GABA. In conclusion, glucose metabolism is compromised in cortex and spinal cord of glutamatergic and GABAergic neurons, while astrocytic TCA cycling appears to be normal at symptomatic stages of ALS.

Lillian Toomey (The University of Western Australia)

A NOVEL ION CHANNEL INHIBITOR COMBINATION ATTENUATES SECONDARY DEGENERATION TO PRESERVE VISUAL FUNCTION FOLLOWING PARTIAL OPTIC NERVE INJURY

Toomey LM1, Bartlett CA1, Rodger J1, Fitzgerald M2.
1. Experimental and Regenerative Neurosciences, School of Biological Sciences, The University of Western Australia, Perth WA 6009, Australia. 2. Curtin Health Innovation Research Institute, Curtin University and the Perron Institute for Neurological and Translational Science, Sarich Neuroscience Research Institute, QEII Medical Centre, Nedlands WA 6009, Australia.

Following injury to the central nervous system, initially undamaged tissue surrounding the injury site is vulnerable to secondary degeneration and cell death, associated with excess intracellular Ca2+ influx, and oxidative stress. Previous studies have shown that a combinatorial treatment of three ion channel inhibitors, lomerizine, YM872 and oxATP, limit secondary degeneration in a partial optic nerve transaction model by inhibiting voltage-gated calcium channels, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors and P2X7 receptors respectively. However, oxATP cannot cross the blood brain barrier. Brilliant Blue G (BBG), an alternative P2X7 receptor antagonist, can penetrate through the blood brain barrier, however has not been previously delivered in combination with lomerizine and YM872. To directly compare the efficacy of BBG to oxATP in the context of this therapeutic combination, adult, female PVG rats underwent a partial optic nerve transaction and were given oral lomerizine, together with intrathecal delivery of YM872 with either oxATP or BBG. Extent of visual function was assessed three days following injury using the optokinetic nystagmus test. Both drug combinations resulted in significantly more smooth pursuits than vehicle (p £ 0.05). Furthermore, those given the combination with BBG, rather than oxATP, spent significantly more time engaging in tracking behaviour than the vehicle group (p £ 0.05). Therefore, BBG may be a more effective alternative to oxATP when administered in combination with lomerizine and YM872. Ongoing immunohistochemical analysis examining oxidative stress and cellular subpopulations in optic nerve vulnerable to secondary degeneration will likely add mechanistic insight.

Christin Weissleder (Neuroscience Research Australia)

ALTERED INSULIN LIKE GROWTH FACTOR EXPRESSION IS LINKED TO REDUCED NEUROGENIC CAPACITY IN PSYCHIATRIC DISORDERS

Weissleder C1,2, Webster MJ3 and Shannon Weickert C1,2
1 Schizophrenia Research Laboratory, Neuroscience Research Australia, Sydney, NSW, Australia
2 School of Psychiatry, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia
3 Laboratory of Brain Research, Stanley Medical Research Institute, Maryland, USA
Deficits in inhibitory interneurons in psychiatric diseases may originate from abnormalities during their neurogenesis in the human subependymal zone (SEZ). Recent studies show decreased expression of the stem cell marker GFAPδ and of the neuronal progenitor marker ASCL1 in the adult SEZ in schizophrenia and bipolar disorder; however, mechanisms that underlie these deficits remain unknown. Insulin like growth factor (IGF) signalling regulates adult neurogenesis and altered expression of IGF family members may contribute to reduced neurogenesis in psychiatric diseases. Gene expression of IGF1, IGF1 receptor (IGF1R), insulin receptor (INSR) and high-affinity IGF binding proteins (IGFBPs) 2-5 were measured by quantitative polymerase chain reaction from 33 schizophrenia, 32 bipolar disorder and 33 controls. Multiple regression analyses determined whether IGF family members predict GFAPδ and ASCL1 expression in the SEZ. GFAPδ mRNA levels were decreased in both schizophrenia (37%, p=0.0001) and bipolar disorder (32%, p=0.006) compared to controls. IGF1R and IGFBP2 mRNA levels were decreased in schizophrenia compared to controls (12%, p=0.008 and 16%, p=0.016, respectively). Significant predictors for GFAPδ expression were IGF1, IGFBP2 and IGFBP5 mRNAs in schizophrenia (R²=0.48, p=0.001, in association with INSR mRNA) and IGFBP5 mRNA in bipolar disorder (R²=0.40, p=0.001). Significant predictors for ASCL1 expression were INSR mRNA in schizophrenia (R²=0.40, p=0.001, in association with IGF1, IGFBP2 and IGFBP3 mRNAs) and INSR, IGFBP3 and IGFBP5 mRNAs in bipolar disorder (R²=0.55, p=0.0001). The deficits in IGF family member expression indicate that altered IGF signaling may occur in the human SEZ and contribute to neuropathological abnormalities found in psychiatric disorders.

Surabhi Bhatia (Brain and Mind Centre, University of Sydney)

**APOLIPOPROTEIN D, A PROTECTIVE ANTIOXIDANT PROTEIN IN MODELS OF ALZHEIMER’S DISEASE**

Bhatia S1, Kim WS2, Halliday GM1,2
1. Brain and Mind Centre, Sydney Medical School, The University of Sydney, Australia. 2. School of Medical Sciences, University of New South Wales & Neuroscience Research Australia, Australia.

Apolipoprotein D (apoD) is a highly conserved lipocalin widely expressed in central and peripheral nervous system. Its expression is known to increase with oxidative stress, during aging and in various neural diseases such as Alzheimer’s Disease (AD), schizophrenia and Parkinson’s disease. The aim of this work is to study the role of apoD in protecting against oxidative stress and its potential role in AD. Using apoD overexpressing cell line (U87), we demonstrated that apoD is able to protect against hydrogen peroxide (H₂O₂) induced oxidative stress. We also analysed the expression of various genes of the eicosanoid mediated inflammatory pathway and found that the presence of apoD significantly downregulates the expression of the inflammatory genes PTGS1, PTGS2 and thromboxane synthase 1 (TXBAS1). We also found that apoD protects against the H₂O₂ induced increase in amyloid precursor protein (APP), the precursor of toxic amyloid beta peptides found in amyloid pathology. Thus, apoD may be able to exert its neuroprotective function in AD by inhibiting inflammation and the synthesis of APP.

Diba Ahmadi Rastegar (Usyd - Brain And Mind Centre)

**FLUORESCENCE TAGGING OF ENDOGENOUS LRRK2 IN MICE, AND HUMAN IPS CELLS**

Ahmadi Rastegar D1, Keshiya S1, Halliday GM1, Dzamko N1
13. Faculty of Medicine, Central Clinical School, University of Sydney

Missense mutations in leucine-rich repeat kinase 2 (LRRK2) predispose to Parkinson’s disease (PD). There are at least 6 pathogenic LRRK2 mutations, which in vitro all function to increase the catalytic kinase activity of the LRRK2 protein. How increased LRRK2 kinase activity predisposes to PD however, is unclear. LRRK2 kinase activity is thought to regulate the association of LRRK2 with intracellular membranes, and LRRK2 has been implicated in autophagy, mitochondrial and lysosomal function. LRRK2 also phosphorylates membrane bound Rab family proteins and may play an important role in protein trafficking. However, which membranes localize LRRK2 is unclear, as antibodies capable of recognizing endogenous LRRK2 in a native confirmation have not been robustly validated. Moreover, this has also complicated detection of LRRK2 in heterogeneous tissues, such as fixed brain tissue, where the relative expression of LRRK2 in neurons versus glia is still unknown. To overcome the current lack of antibodies capable of detecting LRRK2 under native conditions without relying on over-expression models, we are employing CRISPR/Cas9 genome editing to generate mice with the mKate2 fluorophore fused to endogenous LRRK2, and human induced pluripotent stem cells with the eGFP fluorophore fused to endogenous LRRK2. We outline our CRISPR methodology and initial results with these important new tools for LRRK2 research.

Eurwin Suryana (Brain and Mind Centre)
Levels of copper and its transporter copper transport protein 1 (Ctr1), are markedly reduced in vulnerable catecholaminergic neurons in early Parkinson’s disease, and this deficiency is hypothesised to contribute to progressive neuronal death. Thus copper represents a potential therapeutic target as it is associated with both catecholamine synthesis and hypothesised to contribute to neuronal protection. This project aimed to investigate the effects of decreased copper on brain dopamine levels and metabolism. Ctr1-knockdown mice and wild type littermates were used in the MPTP-toxin model of Parkinson’s disease (40mg/kg or 60mg/kg MPTP, delivered 4 times in one day). Twenty-one days post-lesion, mice were culled and brains micro-dissected to measure copper and dopamine levels using inductively coupled plasma-mass spectrometry and high-performance liquid chromatography respectively. Ctr1-knockdown mice exhibited significantly lower copper levels in the cortex (p<0.0001), cerebellum (p<0.0001), hippocampus (p=0.0004), and substantia nigra (p<0.0001), compared with wild type littermates. Dopamine levels were significantly reduced in wild type mice following either 40mg/kg (p=0.02) or 60mg/kg (p<0.001) MPTP compared to wild type controls. Ctr1-knockdown mice treated with 60mg/kg MPTP had significantly lower dopamine levels compared to saline treated Ctr1-knockdown mice (p<0.001). Dopamine levels, and rate of metabolism, were equivalent in wild type and knockdown mice but, as expected, dopamine metabolism was increased in animals following MPTP lesioning. However, no correlations were found between substantia nigra copper levels and striatal dopamine levels with, or without MPTP treatment. These results indicate that dopamine levels and rate of metabolism are independent of reduced brain copper in healthy animals.
Carlie Cullen (Menzies Institute for Medical Research, University of Tasmania)

INHIBITING PRIMARY CILIUM ASSEMBLY IN OLIGODENDROCYTE PROGENITOR CELLS PREVENTS NEW OLIGODENDROCYTE ADDITION IN THE ADULT BRAIN.

Megan O’Rourke*1, Carlie L Cullen*1, Robert Gasperini2 and Kaylene Young1
1Menzies Institute for Medical Research and 2 the School of Medicine, University of Tasmania

Oligodendrocyte progenitor cells (OPCs) are a proliferative cell type that makes up ~5% of the cells in the adult human brain. The primary ciliun is an organelle known to regulate the proliferation of a number of cell types by facilitating sonic hedgehog (Shh) signalling. Despite the fact that oligodendrogenesis is highly regulated by Shh signalling, the presence of primary cilia on these cells has not been reported. We have shown that OPCs assemble primary cilia both in vitro and in vivo. To examine the relationship between the primary cilia and OPC behaviour, we used PdgfraCreERT2::Kif3afl/fl mice to conditionally delete a gene essential for cilia assembly (Kif3a) from OPCs, and administered EdU to identify dividing cells. Over a 5 day period, 65.3% ± 4% of OPCs normally divide in the corpus callosum. However, following the deletion of Kif3a, only 43.8 ± 3% of OPCs divided in the same time period (P<0.05). Furthermore, oligodendrogenesis was reduced by more than 50% within 30 days of Kif3a ablation (P<0.001). As the prevention of oligodendrogenesis, by inducing OPC death, has been linked with anxiety and depression, we performed behavioural assays to determine whether preventing primary cilia assembly would have a similar affect. We did not observe an anxiety or depressive like phenotype in our mice, suggesting that only the loss of OPCs and not a loss of oligodendrogenesis produces this phenotype.

Claire Shepherd (Neuroscience Research Australia)

CYTOTOXIC T CELLS ARE SIGNIFICANTLY INCREASED IN SUBTYPES OF FRONTOTEMPORAL LOBAR DEGENERATION

Lack AT1, Halliday GM2, Kril JJ3, Shepherd CE1.
1. Neuroscience Research Australia, NSW Australia.
2. Brain and Mind Centre, Sydney Medical School, University of Sydney, NSW, Australia.
3. Discipline of Pathology, Sydney Medical School, University of Sydney, NSW, Australia.

Inflammation has been observed in brain tissue of individuals with frontotemporal lobar degeneration (FTLD), and recent genetic, blood and in vivo transgenic mice research has highlighted a role for immunity in the etiology of the disease, most notably T lymphocyte activation and regulation. Quantitative CD4 and CD8 immunohistochemistry was performed on formalin-fixed, paraffin-embedded sections of the inferior temporal cortex of 10 FTLD cases with tau pathology (FTLD-tau), 10 FTLD cases with TDP-43 pathology (FTLD-TDP), 10 FTLD cases with mutations in progranulin, 8 Alzheimer’s disease (AD) cases and 10 controls. No significant difference in the number of CD4-positive cells was seen in any disease group. No significant difference in the number of CD8 immunoreactive cells was seen in FTLD-TDP (p=0.4) or AD (p=0.3) cases compared to controls. In contrast, a significantly increased number of CD8-positive cells was observed in progranulin (p=<0.003) and FTLD-tau cases (p=<0.008) compared to controls. Furthermore, CD8 immunoreactive cells were increased in progranulin cases compared to FTLD-tau (p=<0.03).

This is the first study to investigate the role of T lymphocytes in FTLD and the results suggest a role for cytotoxic T cells in the disease process. These changes appear to be specific to cases with underlying FTLD-tau pathology and progranulin mutations, which have been shown to affect synaptic pruning in mouse models of the disease. The relationship between these changes and the pathological hallmarks of the disease requires further investigation but associations with TDP and tau deposition per se are unlikely.

Jenny Thai (University Of Melbourne)

ARTEMIN-INDUCED INTRACELLULAR CALCIUM TRANSIENTS IN GFR ALPHA-3 EXPRESSING NON-MYELINATING SCHWANN CELLS IN THE BONE MARROW

Thai J, Hao M, Stamp LA, and Ivanusic J
Department of Anatomy and Neuroscience, The University of Melbourne, Parkville, Australia.
Artemin is a neurotrophic growth factor known to play an important role in the development and maintenance of
sympathetic and nociceptive sensory neurons. Here we report a close association between nerve terminals and non-neuronal cells that express the artemin receptor (GFRα3) in the bone marrow. Immunohistochemical studies revealed that these cells expressed markers of non-myelinating Schwann cells (GFAP, p75 NTR, nestin). Further analysis of bone marrow sections of Wnt1-reporter mice demonstrated that the cells originate from the neural crest. We examined the effects of extracellular artemin on these cells using Wnt1-cre; ROSA26-GCaMP3 transgenic mice, where all the GFRα3+ cells in the bone marrow expressed the genetically encoded calcium indicator, GCaMP3. We observed that artemin induced rapid intracellular calcium increases in cultured non-myelinating Schwann cells isolated from the bone marrow, indicating a possible functional role of GFRα3 on these cells. Non-myelinating Schwann cells are known to have a role in the maintenance and survival of axonal projections, and in maintaining hematopoietic stem cell quiescence in the bone marrow. Our findings suggest a role for artemin/GFRα3 signalling in the development, regeneration and/or function of neurons that innervate bone marrow, and/or in the maintenance of the hematopoietic niche.

Faheem Ullah Western (Sydney University Campbelltown)

EVALUATION OF “MERIVA” CURCUMIN AS AN ANTI-INFLAMMATORY DRUG ON CHRONIC NEUROINFLAMMATION IN THE GFAP-IL6 MOUSE MODEL.

Faheem Ullah1, Víctor Pérez-Fernández2, Andy Liang3, Alejandra Rangel2, Morven Cameron3, Erika Gyengesi1, 2, Gerald Müench1, 2

14. Department of Pharmacology, School of Medicine, Western Sydney University, Campbelltown, NSW, Australia
1. Molecular Medicine Research Group, Western Sydney University, Campbelltown, NSW, Australia
2. Department of Anatomy and Cell Biology, School of Medicine, Western Sydney University, Campbelltown, NSW, Australia

Chronic neuroinflammation is a promising therapeutic target in degenerative diseases. Heterozygous GFAP-IL6 mice have been used in this study as a mouse model for chronic neuroinflammation in which the murine IL-6 gene is expressed in astroglia under the transcriptional control of GFAP promoter. GFAP-IL6 mice display chronic glial activation in both brain and retina, starting from 3 months of age. We hypothesize that targeting neuroinflammation with a highly bioavailable curcumin, a prominent cytokine-suppressive anti-inflammatory drug, will decrease microglial and astroglial activation, and consequently prevent neurodegenerative diseases.

GFAP-IL6 mice were fed the high bioavailability curcumin Meriva® 140 mg/kg bw/day and 70 mg/kg bw/day (Indena’s Phytosome®) for 1 month. Mice were subsequently perfused and immunofluorescent staining was performed. The total numbers of Iba-1 and GFAP positive cells were estimated using the Optical Fractionator (MBF Biosciences) in the hippocampus and the cerebellum. Immunofluorescence results revealed a significant decrease in the number of positively stained microglial cells in hippocampus. Both doses of Meriva curcumin significantly decreased Iba-1 positive microglia in cerebellum region, but it slightly reduced in hippocampus compared to control. Similarly, both doses of Meriva curcumin insignificantly decreased GFAP positive astrocytes in hippocampus of fed GFAP-IL6 cohort compared to the regular diet fed cohort. In retina, only the 140 mg/kg bw/day dose showed a significant reduction in Iba-1.

This data supports the hypothesis that high bioavailable curcumin preparation such as Meriva® has the potential to ameliorate the detrimental effects of chronic neuroinflammation in diseases such as Alzheimer’s, dementia, chronic neuropathies and retinal inflammation.

E. Myfanwy Cohen (Heart Research Institute)

LPS CAUSES GREATER UPREGULATION OF IL-1b IN THE HYPOTHALAMUS COMPARED TO CORTICAL OR CARDIOVASCULAR CONTROL SITES.

Cohen EM1, 2, Abbott SBG1, 2, Kavurma MM1, 2, Pilowsky PM1, 2.

15. The Heart Research Institute, Sydney, Australia
1. The University of Sydney, Australia

Microglia, the innate immune cells of the brain, participate in the homeostasis of neurons and display regional differences in activity. We gave lipopolysaccharide (LPS) intraperitoneally to activate the peripheral immune system and trigger a systemic inflammatory response, then extracted various regions of fresh brain tissue. We found that, compared to vehicle controls, intraperitoneal LPS caused an approximately twofold upregulation of IL-1b expression in the hypothalamus after two hours. This upregulation was greater than that observed in the cortex, RVLM, or NTS. These results show that the hypothalamus is more sensitive to systemic inflammation than other cardiovascular control areas. Parts of the...
Hypothalamus are open to circulation, while other sites are behind the blood-brain barrier. This shows that the central inflammatory response can be differentially regulated by microglia.

Mitchell Cummins (University Of Newcastle)

**BLOOD-CNS BARRIERS AND AGEING**

Mitchell J Cummins¹, Doug W Smith¹
¹ School of Biomedical Sciences and Pharmacy, Preclinical Neurobiology Program, Priority Research Centre for Brain and Mental Health Research, The University of Newcastle, and Hunter Medical Research Institute

The blood-brain and blood-spinal cord barriers protect the neural environment. Ageing is thought to alter barrier permeability, and we hypothesised ageing changes barrier gene expression. We used RNA-seq, laser microdissection, qPCR, and barrier permeability assays to assess the effects of ageing on the CNS barriers in C57Bl6 mice. RNA-seq was carried out for the frontal cortex and spinal cord, and differential gene expression (DEG) between young and old groups determined. DEGs were compared to previously characterised cell-specific gene lists for endothelial (EC), pericyte, and astrocyte cells as well as other barrier relevant genes. A number of EC (cortex 19%, spinal cord 19%), pericyte (20%, 40%), and astrocyte (23%, 27%) specific genes were differentially expressed, whilst genes in the barrier gene list were also changed (18%, 32%). Spinal cord was generally more affected by ageing.

A subset of DEGs was then investigated by qPCR using RNA extracted from frontal cortex and cervical spinal cord grey matter homogenates. Of the 29 genes investigated, only 2 spinal cord genes were significantly changed. Next, immunolabelled brain microvessels containing endothelial cells and pericytes were microdissected from frontal cortex and cervical spinal cord grey matter, and gene expression determined by qPCR. Only one of the 12 genes investigated was differentially expressed with age in the spinal cord.

Barrier function was assessed by the sodium-fluoroscein assay. Spinal cord permeability decreased with age. No significant change was observed for cortex.

Overall, our data indicate barrier function in the spinal cord but not the cortex is affected by ageing.

Muwoong Kim (Kyung Hee University)

**HYDROGEN SULFIDE TRIGGERS WALLERIAN DEGENERATION THROUGH ACTIVATING CYCLIN D1.**

Muwoong Kim, Youngbuhm Huh, Junyang Jung#
Department of Anatomy and neurobiology, College of Medicine, Kyung Hee University, Seoul, 02453.

Hydrogen sulfide (H2S) is well known, widely used gas transmitter along with nitric oxide (NO), carbon monoxide (CO). H2S production is regulated by 3 enzymes, cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (MST). Previously, we had showed that one strategy of peripheral nervous system for responding its damage is H2S emission through elevating CSE level for triggering Wallerian degeneration (WD). During WD, peripheral nerve starts to break down its structural components to distal part from damaged region with help of Schwann cell autophagy as well as macrophage. We detect significant drop in mRNA level related to inflammatory, proliferative and antioxidant related signaling when treating H2S inhibitor, N-ethylmaleimide (NEM). We have seen that among diverse cyclin, cyclin D1 (CCND1), one of essential molecule for Schwann cell proliferation and could be essential for WD. We have revealed that CCND1 inhibitor applied sciatic nerve ex-vivo culture shows significantly intact striped feature, inhibition of both myelin fragmentation and axonal degeneration compared to positive control. In addition, we demonstrate that CCND1 signaling might be upstream of diverse Schwann cell de-differentiation and proliferation genes. These results mean that CCND1 could be deeply related molecule for WD progression and its role, Schwann cell proliferation might induce its de-differentiation. In conclusion, H2S regulates WD through CCND1 mediated proliferation among other diverse cyclins.

Murielle Kluge (University Of Newcastle)

**NON-RESPONSIVE MICROGLIA PHENOTYPE SPECIFIC TO SITES OF SECONDARY NEURODEGENERATION AFTER STROKE**

Murielle Kluge¹, Laura Notdurft², Lin Kooi Ong¹,³, Laura Notdurft², Lin Kooi Ong¹,³, Sarah J. Johnson⁵, Michael Nilsson¹,³,⁴, Frederick R. Walker¹,³,⁴
1. School of Biomedical Sciences and Pharmacy and the Priority Research Centre Stroke and Brain Injury, University of Newcastle, Callaghan, NSW, Australia. 2. Department of Neuroscience, University of Groningen, Groningen, Netherlands. 3. Hunter Medical Research Institute, New Lambton Heights, NSW, Australia. 4. NHMRC Centre of Research Excellence Stroke Rehabilitation and Brain Recovery, Heidelberg, VIC, Australia 5. School of Electrical Engineering and Computer Science, University of Newcastle, Callaghan, NSW, Australia.
In addition to the initial infarct, stroke also leads to secondary damage and neuronal loss in remote areas of the brain, which were synaptically connected to the primary site of injury. This secondary neurodegenerative process (SND) is accompanied by intense microglia activation; however, the exact role and function of microglia at those sites remain yet to be fully defined. Microglia are highly dynamic and known to move their fine process tips towards synaptic structures and sites of local damage via the purinergic receptor P₂Y₁₂. In this study we use live multi-photon imaging and fixed tissue analysis to investigate microglia phenotypes and their dynamic properties in a mouse model of photo-thrombotic stroke. We show that directed process movement towards local damage or ATP release is lost in areas of SND whilst microglia at the infarct site maintain their process responsiveness. Loss of process extension is connected to distinct changes in microglia morphology, their expression of P₂Y₁₂ and is correlated to the appearance and location of neuronal damage. Microglia alterations specific to sites of SND develop around 7 days after the initial stroke and persist for up to 2 months post-stroke. Whilst the observed microglia phenotypes at SND are very consistent, microglia at the infract sites display a range of heterogeneous morphologies. Our findings describe a homogeneous non-responsive microglia phenotype appearing specifically at sites of SND, suggesting that microglia responses and inflammatory processes involved in SND differ substantially from those at the primary infarction.

Kaveh Moradi (The Florey Institute Of Neuroscience And Mental Health)

INFUSION OF GROWTH FACTORS INTO THE DEMYELINATED BRAIN MODULATES THE REGENERATION OF OLIGODENDROCYTES FROM NEURAL PROGENITOR CELLS

Moradi K1,2, Xing YL1, Chuang BHA1, Mitew S1, Merson TD1
1. Australian Regenerative Medicine Institute, Monash University, Clayton, Victoria
2. Florey Institute of Neuroscience and Mental Health, Parkville, Victoria

In multiple sclerosis (MS), chronic demyelination initiated by immune-mediated destruction of myelin, leads to axonal damage and neuronal cell death, resulting in a progressive decline in neurological function. The development of interventions that potentiate remyelination could hold promise as a novel treatment strategy for MS. To this end, our group has recently demonstrated that neural progenitor cells (NPCs) residing in the subventricular zone (SVZ) of the adult mouse brain are capable of generating large numbers of oligodendrocytes and contributing significantly to remyelination in response to CNS demyelination. However aging takes its toll on the regenerative potential of these cells and reduces their contribution to remyelination. In this study, we investigated whether the delivery of growth factors into the brains of aged mice could potentiate the oligodendrogenic potential of NPCs. Using osmotic minipumps, we infused epidermal growth factor (EGF) and/or heparin-binding EGF-like growth factor (HB-EGF) into the cerebrospinal fluid of aged mice for two weeks following demyelination (n=8 mice per group). We utilised Nestin-CreERT2; Rosa26-LSL-eYFP mice to enable us to map the fate of NPCs. Our data reveal that, compared to vehicle infused controls, growth factor recipients generated significantly more NPC-derived oligodendrocytes following demyelination. Our results shed light on the beneficial effects of EGF and HB-EGF for increasing the contribution of NPCs to remyelination and indicate their therapeutic potential to combat negative effects of aging effects upon remyelination efficacy.

Anja Schomann (University Of Melbourne)

ALTERED COMPOSITION OF SYNAPTOSOMES IN MICE LACKING SEZ6 FAMILY PROTEINS

Schomann A1, Teng KS-L1, Kuhn P-H1,2,3, Pigoni M1,2,3, Müller SA1,2, Nash AN1, Munro KM1, Jobling AI1, Lichtenthaler SF1,2,3,4, Gunnersen JM1
1. Department of Anatomy and Neuroscience, School of Biomedical Sciences, The University of Melbourne, Parkville, Melbourne, Australia. 2. Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Munich, Germany. 3. Neuroproteomics, Klinikum rechts der Isar and Institute for Advanced Study, Technische Universität München, Munich, Germany. 4. Munich Cluster for Systems Neurology (SyNergy), Munich, Germany.

Microglia are resident macrophages of the central nervous system (CNS). They play key roles in homeostasis as well as in neuroinflammation and, hence, display very different states involving different interactions with their environment. During post-natal development, microglia help shape synaptic connectivity; consequently, aberrant microglial activation can have devastating consequences. For example, synapse loss in early Alzheimer’s disease is triggered by microglial complement receptor (CR) 3 activation by neuronal Complement C3. Similar to complement proteins, members of the Sez6 family contain Complement Control Protein (CCP) domains and altered synapse development and maintenance are observed in mice in which the three Sez6 family members are lacking. To investigate whether the synaptic proteome composition...
DISRUPTING MICROGLIA DURING EARLY POSTNATAL LIFE IMPAIRS BRAIN DEVELOPMENT

Soch A, De Luca SN, Sominsky L, Spencer SJ.

Microglia are the primary immune cells of the brain and are involved in early life neuronal development. Disruption of this process is thought to result in developmental deficits, but the influence of microglia at specific developmental stages remains unknown. Through specific conditional ablation of microglia, the role of these cells in brain development can be observed.

Using CRISPR-CAS9 technology, we have developed a transgenic rat with a diphtheria toxin receptor (DTR) gene inserted into the CX3CR1 receptor, found on microglia and monocytes. Upon application of diphtheria toxin (DT), we can specifically deplete microglia and monocytes. DT given to CX3CR1-DTR adult rats almost completely ablates microglia, with full microglial repopulation of the brain after approximately 2 weeks. Here we hypothesized that microglial ablation during early life development, beginning at postnatal day 7 or postnatal day 14 would lead to long term changes in brain development. We have found that in CX3CR1-DTR pups, administration of DT at these time points can impair microglial development, even after microglial repopulation of the brain. Thus, one to two weeks after microglial ablation, microglial numbers in the hippocampus returned to normal. However, gene expression of CX3CR1, the fractalkine receptor responsible for microglial-neuronal interactions, as well as inflammatory (NFκB, CXCL10) and stress (CRHR1) markers remained altered in response to early life microglial ablation. Our findings suggest that microglial ablation during development has the potential to affect the establishment of microglial-neuronal interactions.

Dario Valdinocci (Menzies Health Institute Queensland, Griffith University, Gold Coast, Australia)

EPOTHILONE D INHIBITS MICROGLIA-MEDIATED SPREAD OF ALPHA-SYNUCLEIN AGGREGATES

Valdinocci D, Grant G, Dickson TC, Pountney DL.

Multiple System Atrophy (MSA) is characterized by neuroinflammation and widespread α-synuclein oligodendroglial cytoplasmic inclusions. Immunofluorescence of MSA brain (n = 4) tissue revealed microglia bearing α-synuclein inclusions distal from affected oligodendrocytes. Experiments were conducted using microglial-like differentiated human THP-1 cells to determine if microglia mediate α-synuclein spread. Monomeric or aggregated α-synuclein was immobilized in the centre of glass coverslips and treated (48 hr; n = 3) with either cell free medium, undifferentiated THP-1 cells or microglial-like THP-1 cells differentiated with phorbol-12-myristate-13-acetate. Quantitative immunofluorescence revealed a significant decrease in immobilized α-synuclein density with differentiated THP-1 cells compared to cell free medium (p=0.016; 50+/−17%) and undifferentiated THP-1 (p=0.032, 46%+/−18%), but no significant differences with α-synuclein monomer. This corresponded to a significant proportion (55+/−15%) of differentiated THP-1 cells bearing cytoplasmic α-synuclein distal from the immobilized protein; rare with undifferentiated THP-1 cells or α-synuclein monomer. Cells were co-treated with the microtubule depolymerisation inhibitor, Epothilone D (EpoD), to determine if microglial migration could be impeded. Co-treatment with 10µM EpoD resulted in a significant increase (p=0.018) in residual immobilized α-synuclein after incubation with differentiated THP-1 cells compared to cells not co-treated with EpoD. There was also a significant (p=0.040) reduction in cells at the coverslip edge bearing α-synuclein inclusion bodies, although the overall proportion of α-synuclein positive cells was unaffected by EpoD, indicating that the drug inhibits cell migration rather than phagocytosis. These results suggest that microglia may play an important role in α-synuclein spread in MSA that could be inhibited therapeutically by EpoD.
ENVIRONMENTAL ENRICHMENT INFLUENCES OLIGODENDROGLIAL DYNAMICS IN THE ADULT CNS

Nicholson M1, Liu B1, Wood R1, Hannan A2, Gonsalvez D1, Murray SS1,2, Xiao J1,2
1. Department of Anatomy and Neuroscience, The University of Melbourne, Melbourne, Australia. 2. Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Melbourne, Australia.

Myelin formation plays a critical role in adult brain function and plasticity. However, it is unclear exactly how brain activity...
influences myelination in the adult central nervous system (CNS). Environmental Enrichment (EE) provides novel and complex stimulation to enhance sensory, cognitive and motor experience. EE has been shown to induce experience-dependent neurogenesis and exert benefit in a variety of brain disorders. Here, we investigated whether modulating brain activity with EE influences the plasticity of adult oligodendrocytes (OLs), by determining oligodendroglial cell dynamics and the extent of myelin formation in key white matter tracts of the CNS.

Adult C57Bl/6 mice (n=6) were housed under EE or standard-housing (SH) conditions for 6 weeks, during which they were given EdU in the drinking water to label newly generated cells. Motor function was assessed using Digigait. Brain and spinal cord were collected for histological analysis.

In the corpus callosum, we found significantly fewer total EdU+ cells (p=0.004) and significantly fewer EdU+/Olig2+ newly generated OLs (p=0.004) in EE mice compared to SH controls. There was, however, a trend towards greater numbers of total Olig2+ OLs in the EE animals, suggesting the EE paradigm promoted the differentiation of progenitor cells over proliferation. No significant differences were found in the spinal cord. Digigait analysis of gait function revealed a significant improvement in gait symmetry after EE (p=0.05), suggesting better motor coordination.

Together, our data indicate that EE influences the behaviour of “adult-born” OLs, potentially via promoting their maturation, which correlates with better motor coordination.

Late Submissions – to be added post conference.